

THE FACTORS THAT INFLUENCE THE
REESTABLISHMENT OF PODOCARPUS TOTARA
(TOTARA) AND DACRYCARPUS DACRYDIODES
(KAHIKATEA) IN A FRESHWATER NEW ZEALAND
WETLAND

BY

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Abstract

Wetlands are productive transitional lands between terrestrial and aquatic systems. They provide social, economic and cultural values, while providing valuable services such as carbon storage, water purification, flood abatement and biodiversity support. While wetlands only cover ~3% of the globe, they contribute up to 40% of these global renewable ecosystem services. Worldwide degradation of wetlands through urbanisation, conversion to agriculture and flood management schemes has resulted in a 50% loss of the world's original wetlands, with New Zealand being one of the most extreme examples of this with >90% of the original extent of wetlands being lost.

Wetlands unique hydrology results in distinct plant zonation and community composition and seedling survival is the primary factor that influences stand structure and community composition. However, restoring degraded wetlands is challenging because the alterations to the hydrology through filling or diverting water will impact the effect of physical, chemical and biotic environmental variables on native plant establishment. The use of facilitation in restoration through successional planting using nurse trees, and arbuscular mycorrhizal fungi (AMF) is common in restoration, however research into the effectiveness of these techniques in wetland systems is lacking.

This thesis is comprised of two studies with aims to determine the factors that had the biggest impact on the survival and growth of kahikatea and totara in Wairio wetland and inform future restoration. Wairio wetland has large isolated remnant kahikatea trees, so my first study focused on how these established trees and a connection to fungal hyphae networks influenced the survival and growth of newly planted saplings. One sapling of each species was planted at the dripline of the remnant tree, and another sapling of each species was planted 2 metres from that point. Out of the 16 remnant kahikatea trees used, 8 were 'disturbance' plots where saplings were planted in 35 μ m mesh bags that excluded roots but fungal hyphae could penetrate, in slotted pots which were turned every 3 months. In the 8 remaining 'undisturbed' plots, saplings were planted into the ground. I assessed the influence of distance and disturbance on sapling survival and growth using a chi-square test of independence and general linear models.

Results showed that kahikatea trees survived better than totara overall. The survival of totara was significantly reliant on a close proximity to the remnant kahikatea, and a connection with mycorrhizal networks. Kahikatea had greater biomass than totara, however they suffered strong conspecific competition with the remnant trees, with kahikatea saplings planted at the dripline having 51.28g greater biomass with regular disturbance of AMF mycelium. Kahikatea trees are light demanding species, and therefore growing under the canopy of a parent tree has a negative impact on kahikatea saplings growth.

Knowing that kahikatea and totara trees respond differently to nurse trees and AMF, my second study focused on how nurse effects and AMF association changes with chemical, physical and environmental stressors. The survival and growth of 5-year-old kahikatea and totara trees, with or without a nurse across 10 blocks in Wairio wetland were analysed. At each tree, soil moisture and root available nutrients were measured and soil cores were taken to determine gravimetric soil moisture, reduction-oxidation reaction (redox), pH, soil carbon content and I counted the presence of AMF spores in two size classes. I assessed tree survival and growth against these variables using a chi-squared test of independence and general linear models. The results of this study showed that kahikatea survived better than totara trees in the very wet blocks, where no other species survived. Totara trees survived in the upland sites of the wetland and had better growth than kahikatea trees. Moreover, totara trees grew 24cm taller in the presence of a nurse tree, and were strongly positively associated with spore number. Nurse trees further benefitted totara trees by increasing plant available soil nitrate and potassium by almost double.

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List of terms and abbreviations

AMF – arbuscular mycorrhizal fungi

CMN/MN – common mycorrhizal network/mycorrhizal network

Redox – reduction-oxidation reaction

PRSTM probes – plant root simulator probes

NO₃-N – nitrate

N – nitrogen

K – potassium

List of species mentioned in thesis

Bush daisy – *Olearia virgata*

Cabbage tree – *Cordyline australis*

Kahikatea – *Dacrydium dacrydioides*

Karamu – *Coprosma robusta*

Kohuhu – *Pittosporum tenuifolium*

Kowhai – *Sophora tetraptera*

Lacebark – *Hoheria sp.*

Manuka – *Leptospermum scoparium*

Mingimingi – *Coprosma propinqua*

Ribbonwood – *Plagianthus regius*

Rimu – *Dacrydium cupressinum*

Totara – *Podocarpus totara*

Wineberry – *Aristotelia serrata*

I GENERAL INTRODUCTION

Wetland ecosystems

Freshwater wetlands are transitional lands between terrestrial and aquatic systems (McGlone, 2009; Zedler & Kercher, 2005). They are home to diverse assemblages of species (Brinson & Malvarez, 2002; Ogle, 1989) and provide a wide array of social, economic, environmental and cultural values (Clarkson et al., 2013). In wetlands, strong topographical and hydrological gradients combine to create a diversity of habitats that can lead to high levels of beta diversity (Bauer et al., 2003; Clarkson et al., 2013; Pennington & Walters, 2006). Wetlands vary from freshwater to saline systems and differ in the vegetation communities they support. Wetlands are commonly divided into four categories (Ausseil *et al.*, 2011), bogs are acidic, nutrient low, peat filled wetlands that receive the majority of their water from precipitation, fens are peat forming wetlands that receive nutrients from surrounding mineral soils and groundwater movement, resulting in higher nutrient availability and therefore more diverse plant and animal communities. Marshes are frequently or consistently inundated with water, they may be coastal or inland, freshwater or saline, but their nutrient rich with a neutral pH, leading to an abundance of biodiversity. Finally, swamps are wetlands dominated by woody plants, they experience saturated soils in the growing year, nutrient-rich soils and water tolerant trees (Cowardin *et al.*, 1979). Hydrological regimes cause the movement of abiotic and biotic resources in a source-sink manner, and these material and energy exchanges contribute to the highly valuable ecosystem services that wetlands provide (Clarkson et al., 2013). These ecosystem services are numerous and significant. While wetlands cover ~3% of the globe, they contribute up to 40% of global renewable ecosystem services (Zedler & Kercher, 2005). These include, such services as water purification, carbon storage, flood abatement and biodiversity support (Truu *et al.*, 2009; Zedler & Kercher, 2005). For water-related services such as this, processes such as soil formation and tree growth are slow in relation to human time frames, making service provision difficult to repair (Brauman *et al.*, 2007).

Degradation & restoration

Wetlands have been degraded worldwide through agriculture, infrastructure construction and flood management (Co-operation & Development, 1996; McGlone, 2009; Zedler & Kercher, 2005). New Zealand is one of the most extreme examples of wetland conversion (Mitsch & Gosselink, 2000), with >90% of the original extent of wetlands now lost (Ausseil *et al.*, 2008; McGlone, 2009). The primary cause of wetland degradation is for agricultural use, which results in draining and water diversion. Degradation of wetland leads to losses of biodiversity, as well as cultural and recreational values (Airey *et al.*, 2000) and is frequently associated with soil erosion and reduced water quality (Anton *et al.*, 2015; Johnson, 2012; McAlister & Tidswell, 2007). Water use by plants decreases both flood peaks and lows, seasonal effects are influenced by root depth, seasonal growth patterns and local climate interactions (Brauman *et al.*, 2007).

The value of a wetland is reflected through its economic, ecological and recreational significance (Brauman *et al.*, 2007). The value of wetlands is now widely acknowledged and many countries are evoking legislation to protect remaining wetlands and employing practices to restore degraded ones. Despite this, the growing human population will continue to require more land for housing and urban development leading to increased demand for land (Co-operation & Development, 1996), and their low landscape positions make wetlands particularly accessible for development. Urban development through the construction of roads and the draining of land is often irreversible, leading to an ecosystem that can never fully return to its previous state (Zedler, 2003). There will be a growing need for the restoration of wetlands in the future. However, wetlands are challenging to successfully restore because they are systems that are subject to periodic mechanical disturbance such as flooding. This results in wetlands being readily colonised by invasive species (Zedler & Kercher, 2004) and subject to altered nutrient availability (Casanova & Brock, 2000), reduction-oxygen (redox) potential and pH (Pennington & Walters, 2006). Restoration projects should therefore be well researched, planned and executed so to ensure the maximum success of restoration efforts, and current restoration sites should be used as models to inform future restoration projects.

The goal of wetland restoration projects is to restore prior values (e.g. cultural, recreational, spiritual, and aesthetic) and functions (e.g. water purification, carbon storage, flood abatement and biodiversity) to these ecosystems (Brinson & Malvarez, 2002; Johnson, 2012). To do this, there is often a focus on removing invasive or exotic species and reintroducing native species to create communities resembling those present prior to site degradation (Bannister *et al.*, 2013; Zedler & Kercher, 2005). In particular, the planting of native vegetation is a primary but often limiting goal of wetland restoration projects (Gomez-Aparicio, 2009). While the balance between native and exotic species is a key indicator of restoration success, the composition of restored plant communities is frequently dependent upon a host of biotic and abiotic factors such as the presence of mutualist, antagonist and facilitating species, and soil properties such as nutrient levels and pH (Anton *et al.*, 2015; Bannister *et al.*, 2013; van der Putten *et al.*, 2013).

Wetland hydrology

Wetlands are defined by the variation of the water levels that occur within them. This hydrology varies on both a temporal and spatial scale, ranging from almost entirely dry upland sites, to constantly submerged lowland sites (Pennington & Walters, 2006; Zedler & Kercher, 2005). This gradient creates distinct vegetation zones with species of similar ecological tolerances being assembled together, and these alternating wet and dry periods can stimulate or inhibit plant seed germination (Johnson, 2012). In the upland area of a wetland terrestrial species predominate and the level of the water table may never submerge vegetation or lead to significant aerobic stress. Further downslope, seasonal fluctuations in water levels periodically require plants to access oxygen and nutrients through alternative mechanisms such as symbioses with soil microbes and arbuscular mycorrhizal fungi (AMF), and plants may have adaptations that allow them to time germination and growth according to moisture conditions (Entry *et al.*, 2002; Pennington & Walters, 2006; Wilcox, 2004). Toe slope positions may be constantly submerged, aquatic plants and animals thrive and soil free oxygen concentrations are extremely low.

The natural fluctuation of water in a wetland is important because it can affect plant establishment and inhibit or stimulate growth. The water regime of a wetland is described by the depth, frequency, duration, timing and predictability of flooding. The complexity and high biodiversity of wetlands is partly due to the range of ecosystems that this spatial and temporal water gradient creates (Caldwell *et al.*, 2011), allowing different species with different water, oxygen and symbiont requirements to coexist simultaneously or sequentially over the growing season (Bohrer *et al.*, 2004). This complex balance of water levels associated with the time of year and species present can make any alteration to the water levels a threat to the integrity of the area (Casanova & Brock, 2000). The water regime may lead to prolonged flooding or desiccation that can eliminate certain species while favouring others (Miller, 2000; Wilcox, 2004). Although this is a natural process, an artificial change to the water regime through water diversion or draining can disturb the plant composition and encourage the growth of invasive species while eliminating natives (Zedler & Kercher, 2005). Once draining has occurred and the ecosystem has changed for a significant period of time – multiple growing seasons for example – reversing the effects of disturbance becomes increasingly difficult (Zedler, 2003) because it is not only the water levels that have been changed, but the plant species and soil properties that will affect plant establishment and growth (van der Putten *et al.*, 2013).

The loss (oxidation) or gain (reduction) of electrons due to changes in the water regime is referred to as oxidation-reduction (redox) processes and will alter in the soil depending on the hydrology in a given area (Miller, 2000; Pennington & Walters, 2006; Szogi *et al.*, 2004), which is an indicator of root zone oxygen availability in plant communities (Pennington & Walters, 2006). The redox potential of soil is often measured in millivolts (mV), with dry upland soils having mV levels above +400, and saturated or flooded soils ranging from -400 to +400 mV (Pennington & Walters, 2006). Redox measurements are correlated to plant stress, affecting rates of photosynthesis and plant survival (Pennington & Walters, 2006; Truu *et al.*, 2009). Redox is a major factor that determines wetland vegetation zones and will influence the spatial distribution of beneficial soil microbial communities including (AMF) (Brown & Bledsoe, 1996). Degradation through drainage shifts soil chemistry from reductive to oxidative, and this can be damaging to microorganisms, plants and soil animals (Zedler & Kercher, 2005).

Hydrology and redox potential of soil determines the spatial distribution of aerobic and anaerobic metabolic pathways, which are major determinants of soil pH (Paul *et al.*, 1996). Many microbially mediated processes such as nitrification, are extremely sensitive to changes in pH (Truu *et al.*, 2009). Because many wetlands worldwide have been drained for agriculture (McGlone, 2009), wetland restoration sites are often surrounded by agricultural land and run-off from these adjacent lands can increase nutrient levels in the wetland (Co-operation & Development, 1996). Agricultural runoff may include inorganic nitrogen, phosphorous and potassium fertilisers, as well as farm effluents containing nutrients held in complex organic form. As these nutrients move into wetlands, cation exchange can alter pH as H⁺ ions are released from the surfaces of soil particles which can gradually lower the pH, but can also influence the nutrient status of the area and may cause eutrophication. This may favour invasive plant species over native species (Zedler & Kercher, 2005).

Plant species can also alter pH, through changes in carbon and nitrogen cycling (Truu *et al.*, 2009; van der Putten *et al.*, 2013). This may create a positive plant-soil feedback, which encourages the growth of the same species (Dickie *et al.*, 2013; van der Putten *et al.*, 2013). Such interactions can be advantageous when attempting to revegetate an area during restoration. Negative plant-soil feedbacks are also common, occurring when plants promote the growth of antagonistic soil microbes that reduce their fitness relative to that of their competitors (Bever *et al.*, 2012; Dickie *et al.*, 2013). The interactions between plants and microbial communities determine the nutrient status and pH of soils (Zak *et al.*, 2003). These interactions can be viewed as both a positive and negative point for restoration purposes because when invasive exotic species dominate an area, they may alter their environment to suit them and hinder the growth of native species, while the establishment of small numbers of native species may facilitate the recruitment of others (van der Putten *et al.*, 2013). Thus, establishing beneficial plant-soil feedbacks may be an important component of ecosystem restoration.

Nutrient levels

The nutrient status of a wetland can fluctuate regularly, usually as a result of seasonal weather events, surrounding land use and run off (Blyth *et al.*, 2013; Houlahan & Findlay,

2004). Worldwide degradation and restoration of wetlands has created pockets of protected and created wetlands that are often surrounded by lands that are still used for agriculture and industry. Urban runoff wastewaters can severely harm a wetlands ability to restore, through pollution and changes to plant and animal community composition (Day *et al.*, 2006).

The flooding and drying events common in ephemeral wetlands cause regular nutrient loss and gain (Casanova & Brock, 2000) and internal regulation and storage of nutrients are often vital mechanisms for plants in stressful environments. In addition, any long-term increases and decreases of vital nutrients may cause particular species to be favoured over others (Brinson & Malvarez, 2002).

Nitrogen (N) and phosphorous (P) play important roles in the nutrient cycling in most terrestrial and wetland plants. Nutrient loss is a major concern for crops, and the leaching of these nutrients in wetlands and other neighbouring sites, is a concern. Conversely, water-soluble mobile nutrients in the soil such as nitrate and sulphate are readily leached into plants root zone in wet soils (Cavagnaro *et al.*, 2015). However, the transitional properties of wetlands means that upland soils can be entirely dried in summer months (Miller, 2000), and therefore there is reduced mobility of these nutrients to wetland species (Liebersbach *et al.*, 2004). Immobile nutrients such as phosphorous and zinc are lost through erosive processes (Cavagnaro *et al.*, 2015) such as the degradation of sites.

Plants ability to reduce nutrient loss depends on adaptations and symbioses they possess. Senescing leaves for example, may absorb ~50% of the leaf N to be resorbed in growing parts of stored in tissue for the future growing season (Ławniczak, 2011). Plants may also increase their nutrient availability by using neighbouring trees that have a larger root biomass that can explore larger volumes of soil and redistributing water in dry months (Okland & Okland, 1996), thus mobilising water-soluble nutrients once again. In addition, plants often use AMF to further prevent nutrient loss (Bender *et al.*, 2015; Cavagnaro *et al.*, 2015). AMF can enhance plant growth by enlarging the root zone and intercepting nutrients more readily, while also preventing nutrient loss through rain-induced leaching events (Cavagnaro *et al.*, 2015). This can therefore improve plant P nutrition and transfer N from soil to plants and improve N nutrition in some cases (Ławniczak, 2011). It has been implied

that AMF have an important role in the increase of nutrient use sustainability and efficiency of plant-soil systems, although this is under researched.

Nurse effects

Restoring wetland ecosystems involves returning the plant community to a previous state. This ecosystem state is not always well understood but both remnant plant species that persist at the site and historical literature or photography can be helpful for determining species well suited to the environment. In forested wetlands, canopy dominant species often take decades to mature (Roquemore *et al.*, 2014; Zedler & Kercher, 2005) and thus restoration can be a very slow process. Some researchers have suggested a successional approach to forest wetland restoration (Valades, 2013). In New Zealand, this regime involves firstly planting fast growing 'pioneer' species that will rapidly create a native cover, such as wineberry (*Aristotelia serrata*) and cabbage tree (*Cordyline australis*). The dominant native cover then discourages exotic species. This may also alter the soil properties that favour exotic species by altering water levels, pH, nutrients and microbial communities. The second stage involves planting slow growing focal species, such as Totara (*Podocarpus totara*) and Rimu (*Dacrydium cupressinum*). The pioneer plants may facilitate these slower-growing podocarp species acting as 'nurse plants'. At different restoration sites, it's of vital importance that species are chosen that are already adapted to that environment, have historical distribution there, or are well adapted to the altered hydrological regime that has existed since the time of degradation. The concept of a 'nurse plant' is widely used in wetland restoration with success (Ehrenfeld & Toth, 1997; Padilla & Pugnaire, 2006; Valades, 2013; Zedler, 2000), but the processes and mechanisms behind this success is still poorly understood.

Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi belong to the Phylum Glomeromycota and have existed for more than 400 million years (Wang *et al.*, 2011). They are a vital part of many ecosystems, as they form a symbiotic relationship with plant roots. The AMF provides the plant with soil

nutrients, including phosphorous, nitrogen and minerals in exchange for photosynthates (Bauer *et al.*, 2003; Perry *et al.*, 1987; Treseder & Cross, 2006; van der Heijden *et al.*, 2015; Wang *et al.*, 2011). AMF can influence above-ground processes such as plant fitness and productivity through nutrient uptake, water conservation, pathogen resistance, insect herbivory and plant community diversity (Brundrett & Abbott, 2002; Wang *et al.*, 2011). Their importance is highlighted in stressful environments, such as arid and nutrient poor soils. In wetlands, AMF biomass decreases with increasing nutrients and increasing water levels (Entry *et al.*, 2002; Solaiman & Hirata, 1995).

AMF have been shown to have idiosyncratic effects on plant community composition in various terrestrial ecosystems and their presence and importance in wetlands has been controversial (Wolfe *et al.*, 2006). Previously AMF were assumed to be of negligible importance in wetlands because they are aerobic symbionts, and wetland soils are frequently anoxic (Turner *et al.*, 2000; Van Hoewyk *et al.*, 2001; Wolfe *et al.*, 2006). However, as research and methodological processes have improved, so has the understanding of the role of AMF in wetlands (Olsson, 1999; Olsson *et al.*, 1995). Multiple studies have now reported an abundance of AMF in most dicot wetland species (Weishampel & Bedford, 2006; Wolfe *et al.*, 2007; Wolfe *et al.*, 2006), with the highest levels found in the upper drier elevation gradients of the wetland, but AMF roots have been found 42cm below the soil surface where oxygen is not detectable (Bohrer *et al.*, 2004). AMF symbiosis is key for plant species in wetlands because they provide nutrients that would be otherwise unavailable to them, especially in these anoxic conditions. Periodic fluctuations in the water table may allow mycorrhizas to persist in wetlands, where their importance for plant P acquisition may be strongly seasonal (Miller, 2000). Wetland plant species may have extensive root aerenchyma which may be sufficient to meet plant root and AMF requirements for oxygen during periods of inundation (Van Hoewyk *et al.*, 2001). Indeed, AMF colonisation in salt marsh was found to differ among plant species but not across hydrological zones (Bauer *et al.*, 2003).

Wairio Wetlands

Wairio wetland is part of the largest wetland complex in the lower North Island (Ogle, 1989) that surrounds Lake Wairarapa ($41^{\circ} 16'30''S$, $175^{\circ} 08'30''E$) in New Zealand. This temperate inland freshwater wetland provides ecological, cultural, and recreational resources worth protecting (Ogle *et al.*, 1990), but this understanding has only recently come about.



Figure 1. Map of New Zealand highlighting and enlarging Lake Wairarapa and the surrounding area.

(Source: Google maps)

Two major events caused the degradation of Wairio Wetlands. Firstly, a severe 8.2 magnitude earthquake hit the Wairarapa region in 1855, the most extreme of its kind since European Colonisation. The result was a lifting of land on the shore of Lake Wairarapa by ~2 metres, causing drainage and drying of the area. Secondly, a 1947 flood prompted the establishment of the lower Wairarapa Development Scheme. The goal of this scheme was to alter the hydrological regime of the area to prevent future severe flooding events (Airey *et al.*, 2000). This scheme involved the diversion of water, construction of a floodway and the drainage of 1237ha of wetlands. While controversial at the time, the project proceeded and flooding of the area has now been reduced to <10 days per year. Today, Wairio wetland has only scattered remnant trees of kahikatea, cabbage trees, totara, ribbonwood, kowhai and lacebark (Airey *et al.*, 2000; McGlone, 2009; Miller *et al.*, 2004; Valades, 2013). A restoration project for Wairio wetland has been in progress since 2005 led by the Wairio Wetland Restoration Committee whose members include Ducks Unlimited, the Department of Conservation, Victoria University of Wellington and various community groups. The wetland has been divided into four stages according to when restoration efforts were initiated.

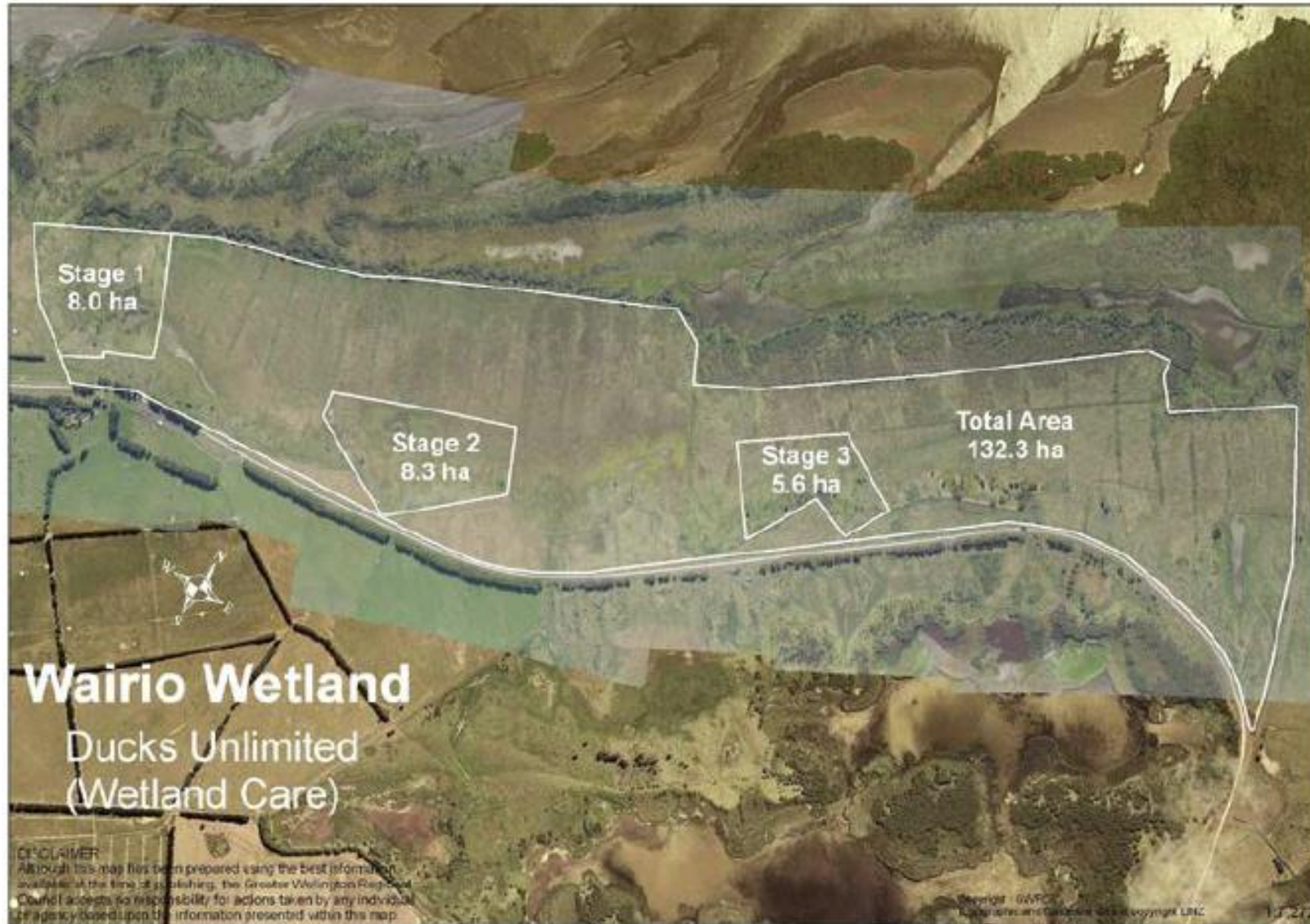


Figure 2. The three stages of Wairio wetland are shown. Stage 4 has been established to the right of stage 3 (not shown). (Source: Ducks Unlimited)

Restoration activities have included native tree planting, earthworks, weed control, pest management and fencing sections of the site to exclude livestock (Gillon, 2014). This history, the presence of remnant tree species and a research program that has been active at the site since 2008 make Wairio wetland an excellent model to explore the effects of soil physical biological properties on the establishment of positive plant-soil feedbacks. Promotion of these feedbacks may enhance restoration outcomes by promoting podocarp tree species survival, growth and ultimately facilitating natural regeneration.

The objective of this study was to understand how nurse effects impact the survival and growth of totara and kahikatea in Wairio wetland. The goal was to understand what environmental changes would impact the strength and direction of this interaction, and apply this to future restoration and management efforts and plans in the future.

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II THE INFLUENCE OF MYCORRHIZAL NETWORKS ON THE SURVIVAL AND GROWTH OF PODOCARP SPECIES IN A NEW ZEALAND WETLAND

Abstract

Root competition between overstory trees and seedlings has a major effect on sapling survival which is the primary factor influencing stand structure in forested sites. The impact of nurse species and mycorrhizal networks on sapling survival and growth, and the factors influencing the strength and direction of this interaction in wetland systems, is largely unknown. Within Wairio wetland, I planted one *Dacrycarpus dacrydioides* (kahikatea) and *Podocarpus totara* (totara) sapling at the dripline and one sapling of each species two metres out from the dripline of 16 remnant kahikatea trees. Eight of the remnant trees were 'disturbance plots' where saplings were planted in 35 μ m mesh bags and slotted plots that allow fungal hyphae penetration while excluding roots, these were turned every three months to break fungal hyphae connection, while the saplings in the 'undisturbed plots' were planted into the ground for a year. I assessed the influence of distance and disturbance on sapling survival and growth using a chi-squared test of independence and general linear models. After one year, survival was high overall (78.13%), and kahikatea saplings survived better than totara. Totara survival was heavily dependent on the facilitative effects of being close to remnant kahikatea, and in connection with common mycorrhizal networks. Kahikatea grew better than totara after one year, although at the dripline where root competition is most pronounced, kahikatea biomass was reduced by 51.28g unless the fungal hyphae connection to their roots was disturbed, suggesting a change in direction from competition to facilitation. This study shows that nurse species and mycorrhizal networks can have both a positive and negative effect on the survival and growth of saplings in a wetland, and the strength and direction of this interaction is species-specific.

Introduction

Now recognised as one of the most productive ecosystems in the world, wetlands were historically drained and converted to what was considered more productive and useful applications, especially agriculture (Brinson & Malvarez, 2002). Wetlands are now valued for the services they provide, such as carbon storage, water purification, erosion stabilisation, protection from flood damage and wildlife habitat (Clarkson et al., 2013). In New Zealand, wetlands historically covered 2.4 million hectares of land, covering almost 10% of the country (Johnson, 2012), since European settlement, agriculture and development has reduced this by 90% (McGlone, 2009), so that wetlands now occupy only 1% of their previous extent. Today, approximately 470 New Zealand vascular plants regularly occur in wetland environments (McGlone, 2009), though many species that were previously common or dominant in wetland environments have declined or become rare. Wetland restoration frequently involves the planting of native species, however, high rates of mortality of planted saplings often limit the re-establishment of wetland plant communities (Pennington & Walters, 2006). Exotic plant invasion can reduce plant biodiversity, which in turn reduces the animals that consume plants, such as herbivores and detritivores and their predators, leading to reduced regional biodiversity (Richardson *et al.*, 2015). For example, the drainage, deforestation, and cultivation of Wairio Wetland has severely reduced the number and species of trees and water birds in the area (Johnson, 2012).

Seedling survival is the primary factor influencing stand structure, nutrient cycling, species diversity and carbon storage for forested sites (Booth & Hoeksema, 2010). Root competition for soil resources between overstory trees and seedlings has a major influence of survival (Coomes & Grubb, 2000). This effect of having a neighbouring individual may be beneficial or detrimental, and the strength and direction of this interaction will vary with space, time, developmental stage and environmental stress level (Nunez et al., 2009). 'Nurse effects' are a type of plant-plant facilitation that can occur within any ecosystem, but are most commonly observed in harsh environments (Gomez-Aparicio et al., 2004). Nurse effects occur when one plant, the nurse plant, creates a more favourable habitat for a neighbouring plant, which may be conspecific or another species. Habitat modifications that are associated with nurse effects include improved access to water and nutrient acquisition and transfer, wind protection and soil property alterations (Nunez et al., 2009). Beneficial

interactions in the form of facilitation are regarded as one of the most important plant interactions that impacts plant community dynamics such as plant survival, fitness and growth (Arredondo-Núñez *et al.*, 2009; Nunez *et al.*, 2009), however nurse trees can have competitive effects on saplings planted in close proximity (Harms *et al.*, 2000; Lambers *et al.*, 2002).

Within forest systems, succession naturally occurs through the plant community changing over time starting with resilient plants colonising the ground, then shrubs and small trees will grow. This cover will create a more favourable environment by protecting seedlings from abiotic stressors and invasive species and allowing them to establish and create a young forest which will encourage further growth resulting in a mature forest.

This approach has been used in many terrestrial systems (van der Putten *et al.*, 2013), such as forest ecosystems (Bannister *et al.*, 2013; Valades, 2013) and wetlands (Airey *et al.*, 2000; Roquemore *et al.*, 2014). Successional planting can occur in two ways. Firstly, and more commonly, fast-growing native 'nurse species' are planted in a restoration area. They are often already present in the environment, or are found in similar ecosystems, and are usually quite resilient. They create a native-cover that provides protection from extreme weather events and they exclude non-native or exotic plants in the area, allowing seedlings to grow with reduced competition (Valades, 2013). Once the site achieves approximately 80% native cover through nurse species, the slower growing focal species can then be planted near the nurse species to freely grow. Alternatively, established 'remnant' plants at a site can be used as nurse species, this method is used less frequently as it is uncommon for target species to survive at degraded sites. Consequently, the effectiveness of 'remnant' nurses is poorly understood (van der Heijden & Horton, 2009), though given their ability to persist, they may provide relative strong nurse effects to new arrivals. Despite this, the age and size of the nurse trees in arid environments tend to be inversely related to their nurse effects on newcomers, with young and small trees having an overall positive effect, and old, large trees having a negative effect (Callaway & Walker, 1997; Padilla & Pugnaire, 2006; Tewksbury & Lloyd, 2001). Research on plant facilitation has been largely confined to dry shrubland environments (Gomez-Aparicio *et al.*, 2004; Nunez *et al.*, 2009; G. Siles *et al.*, 2010), with relatively little known of these effects in wetlands.

A mechanism for underground transfer of resources may be a result of underground networks forming between the two or more individuals called common mycorrhizal networks (CMNs) which are physical linkages among plant individuals via the mycelia of mycorrhizal fungi (Hoeksema, 2015). Up to 80% of all terrestrial plant species are mycorrhizal (Harley, 1989), however it is less clear how many of these plant species form CMNs and literature based in wetland systems is lacking (Dunham et al., 2003).

Although some literature suggests that older and larger nurses may not benefit seedlings, studies have shown that CMNs may mitigate the negative effects of trees on seedling survival, such as overstory competition and shade (Booth & Hoeksema, 2010; Simard et al., 1997). Furthermore, some evidence suggests that the presence of a neighbour, particularly an established tree may result in net facilitation of seedlings resulting in increased survival and growth (Newbery et al., 2000). Clearly, there is conflicting results and some have suggested this is due to species-specific facilitation, as well as species-specific competition leading to higher mortality rates due to lack of resources (McKee *et al.*, 2007; Gemma Siles *et al.*, 2010).

Another aspect of this interaction that is under researched is the impact of tree proximity and potential thresholds of distance that will impact nurse effects on target trees. Mycorrhizal fungi act as extensions of root systems in plants, and they can explore a larger volume of soil than plant roots alone (Garg & Chandel, 2011). Therefore, mycorrhizal networks have the ability to connect multiple plants at various distances from each other. As the majority of plants are mycorrhizal, there is a possibility that an increase in distance from one tree to another will lessen any positive or negative effects of a connection.

The pool of knowledge regarding CMNs effect on plant survival and growth is much larger in terrestrial systems, mainly due to the misconception that mycorrhizal fungi were unable to survive in anoxic conditions, however the presence of AMF in wetland systems is now accepted, the literature is still lacking.

Ephemeral wetlands such as Wairio Wetland, has is constantly fluctuating in its hydrology, which results in inundation in parts during winter/spring and drought in parts during summer. From the large pool of knowledge of mycorrhizal networks and plant facilitation understood in upland systems, we know that the positive effect of having a nurse will be

dictated by the species of both nurse and sapling plant, and on the proximity between them. In wetland ecosystems, inundation, drought and disturbance may change the balance of these interactions ultimately shifting plant interaction from positive to negative, or vice versa. Therefore, understanding the mechanisms of plant facilitation and competition in wetland environments is critical for successful restoration of these ecosystems.

The objective of this study was to assess if remnant wetland species *Dacrycarpus dacrydioides* trees have nurse effects on newly planted sapling trees of four wetland species – *Dacrycarpus dacrydioides*, *Podocarpus totara*, *Cordyline australis* and *Olearia virgata*. I used a mixed treatment of saplings planted at different distances from the remnant *Dacrycarpus dacrydioides* trees, with or without hyphae-exclusion bags and periodically disturbed fungal roots to disturb mycorrhizal connections.

I tested the following hypotheses;

1. Remnant kahikatea have nurse effects on planted sapling trees, enhancing survival and growth in their first year.
2. Nurse effects of remnant kahikatea will lessen with distance, and saplings planted at the dripline will have the highest survival and growth.
3. Disturbance will reduce nurse effects of remnant kahikatea on sapling trees, this will manifest as survival and growth of the saplings in their first year.

Materials and Methods

Study area

Lake Wairarapa (41° 16'30"S, 175° 08'30"E) is situated on the southern end of the North Island of New Zealand. Wairio wetland is part of the largest wetland complex of the lower north island. Wairio wetland is a temperate inland freshwater ephemeral wetland and is the site of an ecological restoration project. The wetland is divided into four stages, according to when restoration efforts were initiated. Stage 3 of Wairio wetland has had an active restoration project since 2005 which has included native tree planting, earthworks, weed control, pest mammal management and fencing to exclude livestock. Within the 100-hectare area of stage 3, there are approximately 25 remnant kahikatea trees and one large

totara tree. These are of historical importance because they represent the plant community composition prior to the degradation of the wetland forest. Remnant trees are found in drier, higher elevation areas, which reflects less frequent disturbance by inundation of these areas of the landscape. These trees have survived several degradation events, including clearing of the surrounding forest, draining of the wetland and grazing by cattle. Large trees are often left on agricultural land as shelter for cattle.



Figure 3. Stage three of Wairio wetland. The blue outline represents the fenced perimeters of the stage. Yellow stars represent the remnant kahikatea that have undisturbed saplings planted around them. Red stars represent remnant kahikatea that have disturbed saplings planted around them. (Image source: Google Maps)

Plot design and measurements

The initial plot establishment was led by Dr. Julie Deslippe, School of Biological Sciences, Victoria University of Wellington. It was executed with Victoria University students and volunteers. Study plots were established around 16 remnant kahikatea trees. The remnant trees varied in diameter at breast height from 41.3cm to 195.1cm, with a mean DBH of 102.4cm. Around each remnant kahikatea tree we planted sapling trees of four species: kahikatea, totara (*Podocarpus totara*), cabbage tree (*Cordyline australis*), and bush daisy (*Olearia virgata*). These species were selected because they were historically present in the area and had already been successfully re-established in stage 3 in an adjacent restoration planting experiment led by Dr. Stephen Hartley.

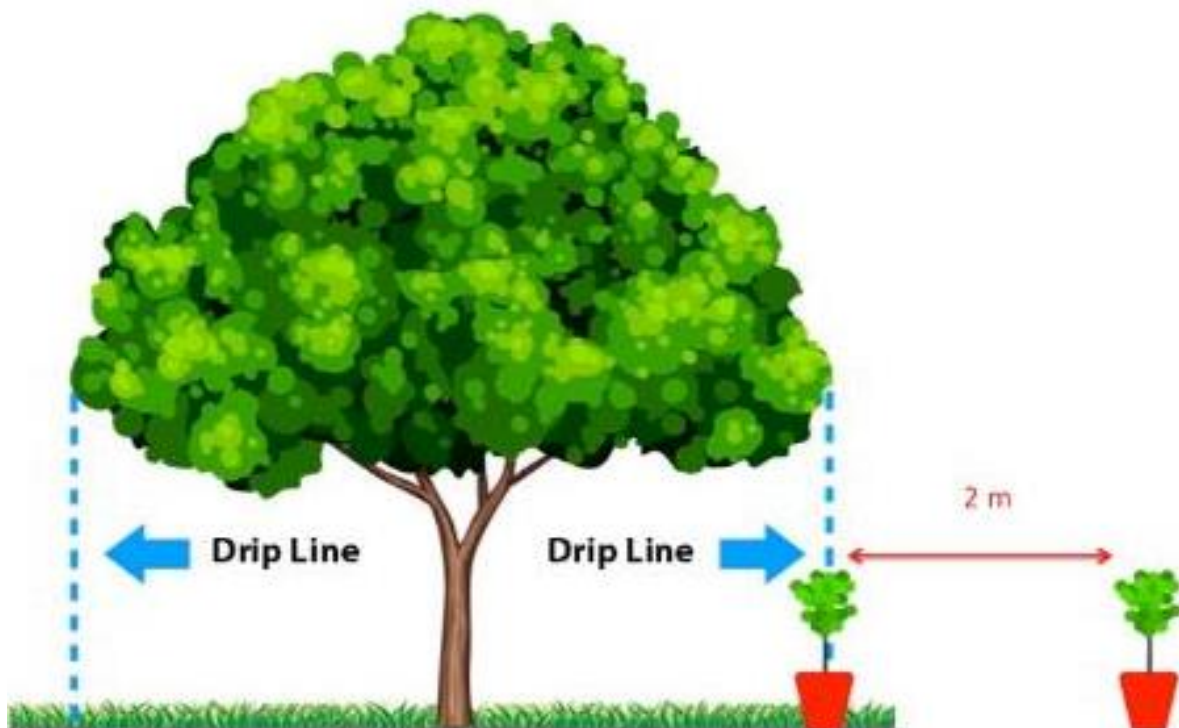


Figure 4. Diagram showing the experimental planting set up in the wetland. The large tree represents the remnant kahikatea tree, the blue dots represent the dripline of the tree where one individual sapling was planted, and two metres out from that sapling, an individual of the same species was planted. This occurred for all four species around each remnant tree in this study.

Eight plots were designated at random for the application of the disturbance treatment, while 8 plots were left 'undisturbed'. In 'disturbed' plots, planted saplings were inserted into mesh bags with a pore diameter of 35µm, which allowed fungal hyphae to penetrate but excluded plant roots. These bags were placed in slotted plastic pots and planted into the soil. The disturbance treatment consisted of breaking the mycelial network of sapling trees. To accomplish this, pots containing saplings were twisted clockwise by 90 degrees at 8 week intervals for one year (Figure 5). In the 'undisturbed' plots, saplings of totara, bush daisy and cabbage tree were planted directly into the soil, while kahikatea saplings were planted into mesh bags without slotted pots in order to prevent the formation of root grafts with the remnant kahikatea.

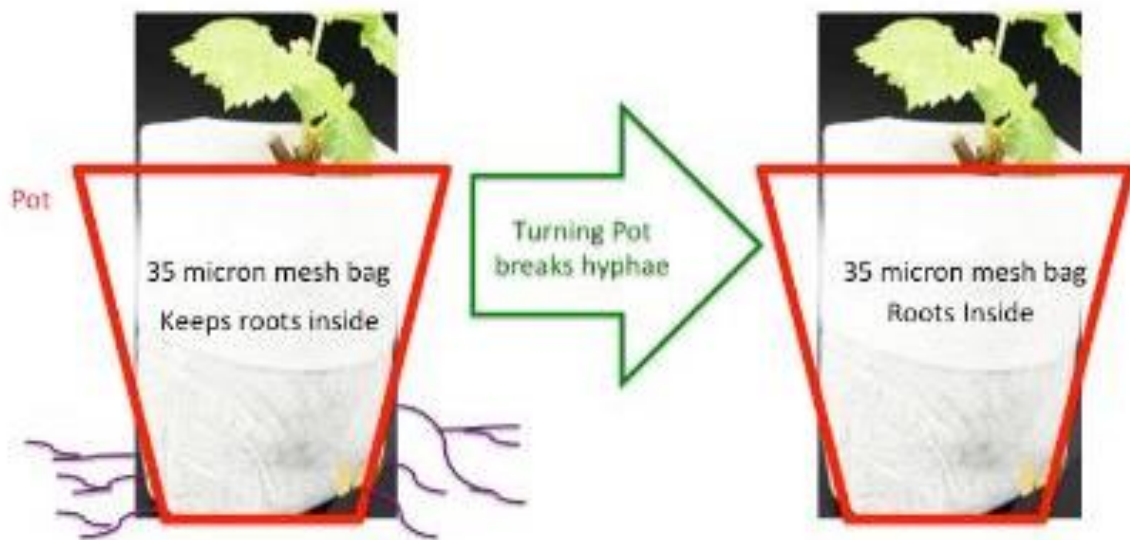


Figure 5. Diagram shows the mesh bag and pot set up for saplings in the disturbed plots. The left side depicts the fungal hyphae (purple) escaping the mesh bag and forming a network with surrounding plants and fungi, and the right side depicts the mesh bag once the pot is turned, and the connection is broken.

Within each plot, two saplings per species were planted. The first planted at the furthest extent of the drip line of the crown of the tree, a rough indication of the extent of the root zone. The second sapling of each species was planted 2 metres beyond the drip line, except in where the remnant kahikatea was <70cm DBH, in which case saplings were planted only 1 metre beyond the drip line. Figure 2 provides a schematic of the plot planting design.

Approximately every 3 months, we measured the survival and growth of all planted saplings. We also noted overall plant health and local environmental conditions that may have influenced survival and growth such as inundation, and competition by weeds. Saplings were measured from the crown of the roots to the top of the apical shoot with a wooden ruler and absolute growth (*Final height – initial height*) and relative growth rate:

$$R_h = \frac{(\log_e H_f - \log_e H_i)}{t_f - t_i}$$

where H1 and H2 are the heights of the trees at the time of initial planting (t_i) and five years later (t_f). Time was measured in years ($t_f - t_i = 1$) was calculated. Grasses and herbs were weeded from the immediate vicinity of all saplings at the time of measurements.

Destructive harvesting of saplings

Saplings were harvested as quickly as possible to avoid differences among plots due to length of treatment time, and seasonal factors. However, the spring and summer of 2015 was unseasonably warm which led to vigorous weed grasses (up to ~2 metres). These conditions made it difficult to locate the saplings in plots and increased harvesting time to 5 weeks.

Cutting the plant at ground level and placing it into a labelled sealable bag collected the aboveground biomass of each sapling. To collect belowground biomass of saplings in disturbed plots, pots and mesh bags containing the sapling roots were placed in large labelled, sealable bags. To collect belowground biomass of saplings in undisturbed plots, holes ~60cm across were dug around each sapling, and ~30cm deep. The shovel was used to move the soil and feel for resistance that may have indicated roots connecting to the soil. If there was resistance, the hole was made larger until total root biomass could be removed. Excess soil was shaken off before placing in a large rubbish bag with any loose roots.

Soil moisture content

At each visit site, soil moisture measurements were made in the immediate vicinity of each sapling. Soil moisture was measured with time domain reflectometry using a Delta-t ML3 Theta Probe Soil Moisture and Temperature Sensor at three different locations. If the soil moisture probe was unavailable for fieldwork, then a soil core was taken and gravimetric soil moisture was calculated using the oven drying method.

Cleaning root biomass

Root balls of each sapling were soaked in tap water for 10-30 minutes to loosen the soil. Root balls were then alternately washed using low-pressure running water and by gently agitating by hand in clean tap water. This process was repeated until the majority of soil particles were removed. Care was taken not to damage podocarp root nodules. Stones, woody debris and roots of non-target species were then removed manually with forceps. The roots were then further cleaned of soil particles by washing through a three-tier sieve. The remaining roots were left to air dry for 15-20 minutes, and then weighed to determine fresh weigh.

During the cleaning and examination of cabbage tree roots and mesh bags, it was discovered that the roots of cabbage trees had growth and broken through the stitching of the mesh bags. This meant that the effect of disturbance wasn't valid anymore. Upon examination of the root structures of the few bush daisy saplings collected, all were dead and didn't appear to have grown throughout the experimental period. For these reasons, bush daisy and cabbage tree were excluded from all analyses.

Measuring aboveground and belowground biomass

Aboveground and belowground biomass was calculated by placing the sapling in a labelled paper bag and oven drying at 60°C for 48 hours and being weighed (dry weight).

Statistical analysis

Chi-squared tests of independence were used to determine the effects of plant species, disturbance and distance, on sapling survival. General linear models (GLMs) were used to determine the relationships between absolute growth, aboveground biomass, belowground biomass, total biomass, biomass ratio (aboveground:belowground biomass), and species. Levene's tests were used to assess the homoscedasticity of each dataset. Where data violated the assumptions of the model, I applied a nonparametric Mann-Whitney U test. All analyses were done using STATISTICA version 22 software (IBM SPSS Statistics 22, Armonk NY).

Results

Survival

Overall survival of totara and kahikatea was high (78.13%, Table 1). Survival of saplings in disturbed plots were significantly affected by species, with totara survival being much lower than kahikatea. Survival of saplings planted 2 metres from the dripline of remnant kahikatea trees was significantly affected by species also, with totara having much lower survival at this distance, compared to kahikatea (Table 2).

Table 1. Survival of all saplings by species and treatment

Category	Alive	Dead	Total	% Survival
All trees	50	14	64	78.13
Kahikatea	29	3	32	90.62
Totara	21	11	32	65.63
Disturbed	25	7	32	78.13
Undisturbed	25	7	32	78.13
Dripline	27	5	32	84.38
2m from dripline	23	9	32	71.88

Table 2: Survival of kahikatea and totara saplings by treatment.

Grouping Variable	Total Survival	Treatment	Surviving Trees	Chi-squared test statistic	p-value	Significance
Kahikatea	29	Dripline 2m from dripline	15 14	0.368	0.544	N.S.
Totara	21	Dripline 2m from dripline	12 9	1.247	0.264	N.S.
Kahikatea	29	Disturbed Undisturbed	15 14	0.368	0.544	N.S.
Totara	21	Disturbed Undisturbed	10 11	0.139	0.71	N.S.
Disturbed	25	Kahikatea Totara	15 10	4.571	0.033	*
Undisturbed	25	Kahikatea Totara	14 11	1.646	0.2	N.S.
Disturbed	25	Dripline 2m from dripline	14 11	1.646	0.2	N.S.
Undisturbed	25	Dripline 2m from dripline	13 12	0.183	0.669	N.S.
Dripline	27	Kahikatea Totara	15 12	2.113	0.144	N.S.
2m from dripline	23	Kahikatea Totara	14 9	3.865	0.049	*
Dripline	27	Disturbed Undisturbed	14 13	0.237	0.626	N.S.
2m from dripline	23	Disturbed Undisturbed	11 12	0.155	0.694	N.S.

Biomass and growth

The biomass of saplings differed by species, with kahikatea having significantly greater aboveground biomass ($z = -4.587, p = 0.000$), belowground biomass ($z = -5.422, p = 0.000$) and total biomass ($z = -5.748, p = 0.000$) than totara (Figure 6). However, the ratio of aboveground:belowground biomass was similar among species. Likewise, kahikatea and totara did not differ in absolute growth or in RGR over the course of the 1-year experiment.

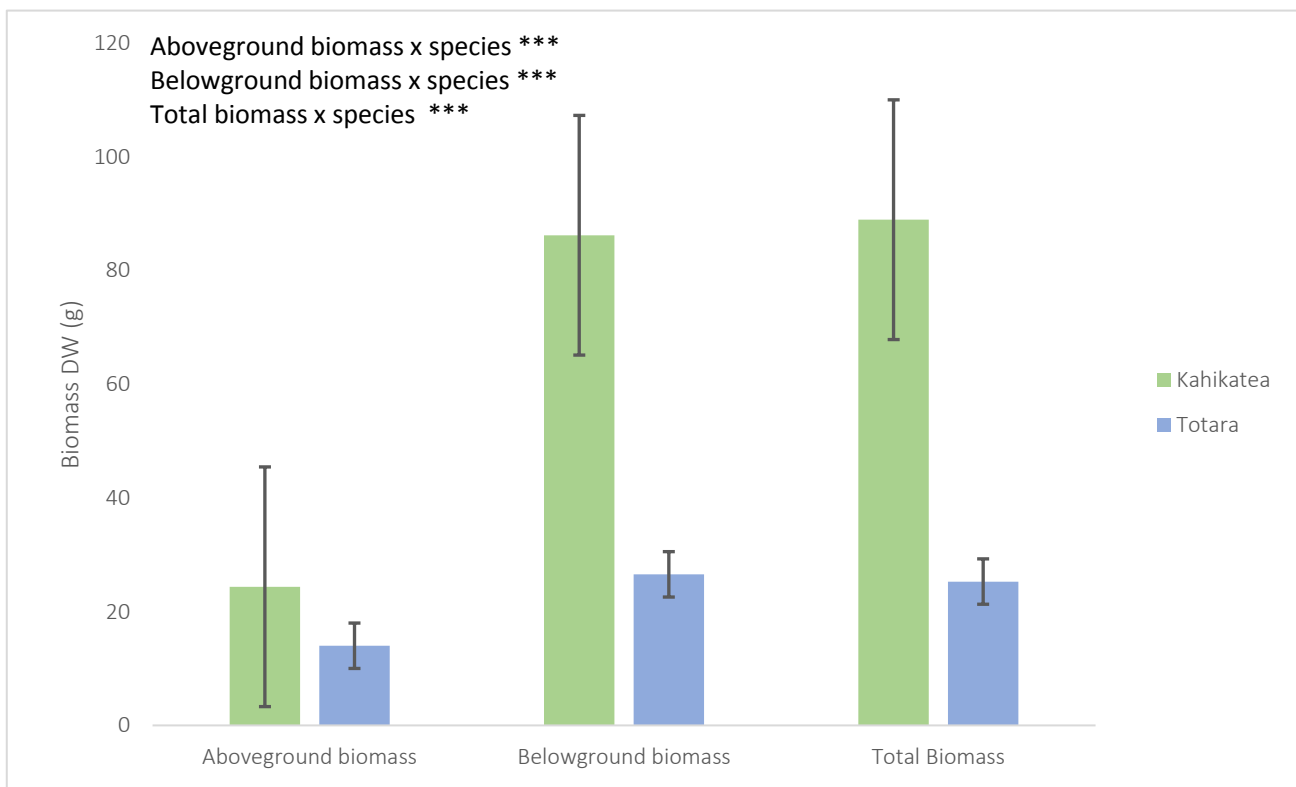


Figure 6. Kahikatea had significantly more aboveground, belowground and total biomass compared to totara, $p < 0.001$ for all three measurements.

While the aboveground, belowground and total biomass of totara was unaffected by the disturbance and distance regimes, I found that total biomass of kahikatea showed a marginally significant interaction between the distance and disturbance treatments ($f = 3.127, p\text{-value} = 0.088$). When mean total biomass is observed under these treatments, the biomass of kahikatea trees at the dripline is 51.28g greater when disturbed. Kahikatea trees planted 2 metres from the dripline were unaffected by disturbance (Figure 7).

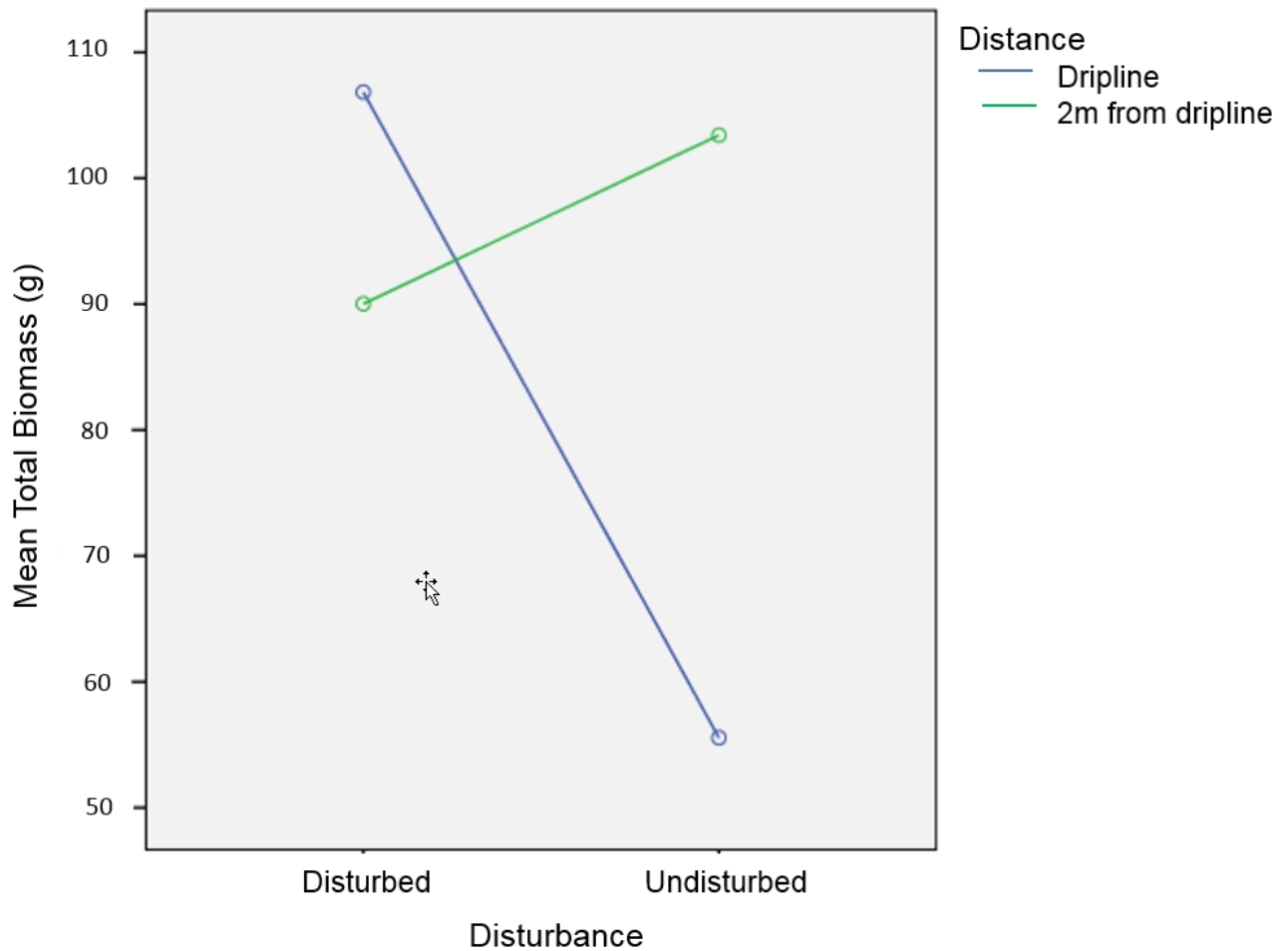


Figure 7. Mean total biomass of kahikatea trees was much higher at the dripline when they were disturbed, when undisturbed this drops dramatically. Total biomass of kahikatea trees 2 metres from the dripline was not impacted by disturbance.

Discussion

Survival

Survival of kahikatea and totara saplings in their first year of growth was relatively high in this study. Ernst and Brooks (2003) found that in a North American hardwood swamp, survival was 65% in severely disturbed forest areas. Additionally, a study in North American wetlands found that tree survival averaged 47% (Roquemore et al., 2014). Mortality of trees in Wairio wetland peaks in the first year of planting (Gillon, 2014), however literature suggests that it can take up to three years for survival to plateau (Ledgard & Henley, 2009; Valades, 2013), therefore it's important to look at the growth to understand the health of these trees, which may indicate whether their survival will stay at this high rate.

Furthermore, it's important to consider the size of the trees at planting, as research indicates that this can have a significant impact on the survival and growth of trees (Ernst & Brooks, 2003). Although more kahikatea saplings survived than totara saplings, this difference was not significant overall, and their survival may be correlated with their size which buffered them against the various stressors at Wairio such as inundation and wind, for example.

Disturbance affected the survival of kahikatea and totara differently. Kahikatea tolerated disturbance much better than totara overall, which suggests that totara saplings are more dependent on a connection to mycorrhizal networks than kahikatea saplings. Podocarp species roots are unique in the nodules that form and host AMF species. The roots of kahikatea trees have differing communities of AMF within these nodules that either get invaded individually from the soil or laterally from the root (Russell et al., 2002). If the fungal community on kahikatea roots differs from totara roots because of species, then we wouldn't see kahikatea survival suffering when disconnected from the network because this species-specific symbiosis will occur again through invasion in the soil. The connection to this diverse fungal community is likely very beneficial for the survival of totara saplings in the first stages of development and establishment in Wairio, through increased phosphate uptake and nitrogen fixation, both of which are both critical nutrients for plant survival and growth (Hermans *et al.*, 2006; Kaelke & Dawson, 2003). Additionally, there's evidence that the balance between competition and facilitation between remnant trees and the saplings differ depending on species (McKee et al., 2007; van Breugel et al., 2012), therefore totara saplings may rely on AMF to outweigh the negative competitive effects of close proximity to an interspecific tree.

Secondly, kahikatea and totara survived differently 2 metres away from the dripline of the remnant trees. Again, totara had much lower survival than kahikatea when planted at a greater distance from remnant nurse trees, where root competition with the remnant was expected to have been minimal but mycorrhizal networks may still play a facilitative role. This further supports the idea that totara is more reliant on nurse effects through mycorrhizal networks than was kahikatea.

Biomass and growth

As sapling species was the strongest predictor of overall survival, we investigated whether this was also true also for biomass and growth. Kahikatea were significantly larger than totara with greater aboveground, belowground and total biomass. Biomass of kahikatea was also more variable than for totara. Although there was this significant difference in the biomass between the two species, there was no difference in growth. This result suggests that growth was similar between kahikatea and totara during the 1 year of this experiment, but kahikatea was bigger at the time of planting. This difference in size is likely why there is a difference in survival between the two species, as some research finds that larger trees survive better, especially at the establishment phase of development (Harms et al., 1980; Valades, 2013). A large tree has more storage resources for carbohydrates that become useful during periods of water stress that will either prolong survival or initiate growth again if the stress ceases (Chapin et al., 1990). In flooded soils, larger trees may be more tolerant to flooding because they have important mechanisms for anaerobic respiration without the build-up of alcohol dehydrogenase (ADH), which is an anaerobic polypeptide that stops the normal synthesis of cellular proteins within a plant (Angelov et al., 1996). These processes include the formation of aerenchyma tissue and the development of adventitious roots that may reach more aerobic soils (Ernst & Brooks, 2003). Furthermore, the response of species-specific survival to root flooding and its impact on tree leaf photosynthesis varies greatly, as does the ability to recover from these events (Angelov et al., 1996).

The absolute growth and ratio of biomass was not effected by species in any treatment. Previous studies have shown that there can be relatively minor variation in growth of tree seedlings, possibly because in stressful environments, competition lessens and conversely, in favourable conditions positive effects of facilitation are lessened and shade cast from large neighbours can have a negative effect (Berkowitz et al., 1995). If this was the case in our study, we would expect to see this trend throughout all of our growth measurements, and we did not. It's more likely then, that the stressful conditions of the wetland caused the saplings to allocate more carbon from photosynthesis to increase its aboveground and belowground biomass to increase light acquisition and root anchorage, instead of growing tall as this may result in saplings being more prone to wind damage. Although grasses cover the wetland and may provide saplings with some wind protection, grass height is very

variable in Wairio and becomes reduced in the wet and windy seasons, and grows dramatically in summer months (S. Waring personal observation).

To further understand the net effects of a nurse tree, we tested whether biomass and growth were impacted by distance from nurse tree, or regular disturbance of mycorrhizal networks underground. We found a marginally significant relationship between total biomass of kahikatea saplings and distance and disturbance. When kahikatea saplings were planted at the dripline, their mean total biomass dropped on average by 51.28g when disturbed. This suggests that when kahikatea saplings are planted at the dripline, the root competition with remnant kahikatea trees is more pronounced, and this negative competitive effect on the biomass of kahikatea is reduced when the roots are disturbed. Furthermore, when kahikatea saplings are planted 2 metres from the dripline, there is less root competition with the remnant tree, and therefore the connection to mycorrhizal networks has a more positive facilitative effect on the total biomass of these trees, which decreases slightly when the roots are disturbed.

Previous studies have shown that distance from a nurse tree will lessen the amelioration of negative conditions and improved availability of resources (Castro et al., 2002; Dickie et al., 2005; Moro et al., 1997), however these studies show that the positive effects are through shade and protection, and do not included the treatment of disturbance to mycorrhizal networks which illustrates the important role they have in this process.

Other measures of biomass were not significantly affected by disturbance and distance treatments for kahikatea trees and biomass was not effected in any way for totara trees under the two treatments. This shows that although survival was heavily influenced by the presence of a nurse and the facilitative benefits via mycorrhizal networks, those that do survive are not reliant this connection for growth. Conversely, the survival of kahikatea trees was not affected by the presence of a nurse tree, but their growth and resource allocation was impacted if these connections were manipulated. These results show how variable the effects of having a nurse present are. What we have seen may be attributed to the strength and direction (positive or negative) of these nurse effects changing with seasons, or plant physiology (Callaway & Walker, 1997; Padilla & Pugnaire, 2006) as the year progressed.

Conclusion

In this study, I found that totara and kahikatea both respond drastically different to the presence of a nurse tree, and distance and root disturbance changed the strength and direction of this response for both species. Totara saplings overall have lower survival than kahikatea in Wairio wetland, and this effect was exacerbated when totara saplings were planted at a greater distance from remnant kahikatea trees, suggesting that the facilitative effects of being close to a large established tree outweighs the negative effects of root competition. Furthermore, totara survival is negatively impacted when the connection to mycorrhizal networks is periodically disturbed, further illustrating the reliance of totara on facilitative effects for survival. Kahikatea saplings also outperformed totara saplings in growth, with significantly more biomass after a year. Interestingly, I found that kahikatea saplings that were planted at the dripline of the remnant tree had significantly more biomass when regularly disturbed, showing that root competition negatively impacts kahikatea saplings growth. The effect of distance was not significant for kahikatea, although kahikatea saplings 2 metres from the dripline did slightly better when left undisturbed and connected to mycorrhizal networks. This shows that totara saplings will survive in Wairio wetland if in close proximity to a nurse tree and in connection with AM fungal hyphae, and kahikatea survives well overall, and grows well with increased distance from a neighbour.

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III THE INFLUENCE OF BIOTIC AND ABIOTIC FACTORS ON THE NURSE EFFECTS IMPACTING SURVIVAL AND GROWTH OF PODOCARP SPECIES IN A NEW ZEALAND WETLAND

Abstract

This study identified the factors that influence the survival and growth of two podocarp species in a New Zealand freshwater ephemeral wetland. Wairio wetland is part of a large, degraded wetland complex in the lower North Island of New Zealand. Agriculture, water diversion and draining have resulted in low survival and growth of trees planted at the site, limiting restoration efforts in recent years. Kahikatea (*Dacrycarpus dacrydioides*) and totara (*Podocarpus totara*) were planted into two planting treatments – with or without an established nurse tree of a different species. After one year, I assessed the survival and growth of all saplings, measured soil moisture and root available nutrients, and took soil samples at each plant. For each soil sample, I measured gravimetric soil moisture, redox, pH and soil carbon content and counted the presence of arbuscular mycorrhizal fungi (AMF) spores in two size classes. I assessed the influence of the soil physical, chemical and biological factors on sapling survival and growth using a chi-squared test of independence and general linear models. Moisture variability across the wetland had a significant impact on the survival, growth and distribution of both species in this study. Kahikatea survived better than totara, especially in very wet areas, but was slower growing. Totara survived better in the drier areas of the wetland, where grew, on average 24cm taller in the presence of a nurse tree. Root available NO₃-N and K to totara trees was significantly higher in the presence of a nurse tree. Large AMF spores were significantly negatively correlated with the growth of both species. Conversely, small AMF spores were significantly positively correlated with the growth of both species. Moisture had a negative impact on spore counts, and the distribution of totara trees in the dry areas of the wetland reflected a strong correlation between totara trees and AMF spores.

Introduction

Wetlands are ecologically important, biologically rich and unique habitats that support a vast range of habitats for plants, animals and soil microbes. Swamps are a category of wetland that are distinguished by the dominance of woody plants, including inundation-tolerant trees which inhabit the nutrient-rich and periodically saturated soils (Cowardin *et al.*, 1979). Swamps worldwide have been severely degraded (Brinson & Malvarez, 2002). Approximately 50% of the world's original wetlands have been lost through draining, filling, urban expansion and agriculture (Clarkson *et al.*, 2013). It is only recently that wetlands have been appreciated for the range of economic, social, environmental and cultural benefits they provide to people (Costanza *et al.*, 1997). These ecosystem services include shoreline protection, sustaining biodiversity, sequestering carbon, flood mitigation and maintaining water quality and supply (Caldwell *et al.*, 2011; Clarkson *et al.*, 2013). Among developed nations, New Zealand is one of the most extreme examples of wetland conversion, with >90% of the original extent of wetlands now lost (Mitsch & Gosselink, 2000). This situation has led to considerable public interest in restoring wetland ecosystems in New Zealand.

Urban development is often irreversible, land is drained and roads are built, resulting in an ecosystem that can never fully return to its former state. Wetlands are notoriously difficult to restore because wetland habitats are subject to frequent mechanical disturbance, such as flooding are readily colonised by invasive plant species (Zedler & Kercher, 2004). Moreover, frequent flooding alters nutrient availability to plants (Casanova & Brock, 2000), as well as the pH of the soil (Gutknecht *et al.*, 2006) and root oxygen availability (Pennington & Walters, 2006). Therein lies the difficulty of restoring a wetland and plant community to its original state to regain its functions. Water diversion, draining and filling is a common tool to convert wetland areas to agricultural, or more "functional land", however the natural fluctuating hydrology of the wetland is then irreversibly altered, and plants are often unable to then survive the wet and dry seasons (Kozlowski & Pallardy, 1997; Padilla & Pugnaire, 2006). The hydrology of a wetland not only changes on a temporal scale, but also a spatial scale. Within a wetland, the topography can vary drastically and commonly results in well-drained upland soils, and poorly drained lowland soils (Pennington & Walters, 2006) which determines plant community development and patterns of plant zonation (Casanova &

Brock, 2000). The alternating wet and dry periods can stimulate or inhibit plant seed germination, modify the oxygen availability and concentrations of nutrients in the soil. Therefore, changes to this natural cycle may result in plant mortality and reduced seedling establishment. Predicting and modifying this hydrological regime of a wetland is notoriously difficult, in particular once it's been altered through disturbance, but this variation is one of the most important factors in maintaining wetland functioning and diversity (Mitchell & Rogers, 1985).

Plant zonation and community structure is linked closely with soil properties that are directly affected by the water regime in a wetland. Oxygen availability varies greatly between upland and lowland areas of a wetland and is indexed by the redox potential, often expressed in millivolts (mV). This has a great physiological impact on variation in tree survival and growth in wetlands because areas of anoxic conditions prevent respiration by plant roots, soil microbes and fungal communities. The difference in trees ability to tolerate anoxia, the soil redox potential and pH can be used to predict the survival and growth of tree seedlings planted across wetlands (Faulkner & Patrick, 1992). Wetland systems are efficient water purifiers and store, trap or remove nutrients such as nitrogen, phosphorous and pollutants associated with land runoff or sewage effluent in soils, sediment and vegetation (Clarkson *et al.*, 2013). This nutrient input encourages plant growth supporting the high biodiversity of wetlands. However, when these nutrient inputs increase significantly through anthropogenic causes, nutrient levels may become toxic for plant species (Stepniewska *et al.*, 2010), or encourage the establishment of invasive or exotic plant species.

Successfully re-establishing native species in a degraded wetland can also be very challenging. Wetland restoration failures often occur because the planted vegetation isn't adapted to the altered hydrology at a given site (Caldwell *et al.*, 2011). Moreover, it is often insufficient to allow target species to re-establish (van der Putten *et al.*, 2013) because the removal of unwanted exotic plant species can be difficult (Zedler & Kercher, 2005). One approach is to establish a canopy of native plants, which then suppresses exotic graminoid and forb species. However, woody wetland plants often take decades to mature (Brauman *et al.*, 2007; Zedler & Kercher, 2005), and unless exotic plant species are regularly monitored and removed, reestablishment of a dominantly native plant community will not occur.

Therefore, a successional approach, which involves planting a fast growing and resilient (nurse) tree species, can be an effective tool for wetland revegetation (Valades, 2013). To use succession successfully, one must understand the underlying mechanisms and the factors that will influence the outcome of trees being in close proximity to each other. Facilitation is a plant-plant interaction that benefits at least one of the participants (Arredondo-Núñez *et al.*, 2009), and is a common interaction that occurs across a wide range of ecosystems (Choler *et al.*, 2001; Siles *et al.*, 2010). With a successional approach to replanting, the nurse species facilitates the growth of a target species, this occurs when a nurse interacts with the environment to create a more favourable microhabitat to its surroundings for germination, establishment and survival of other plants (Arredondo-Núñez *et al.*, 2009). This may be through altered soil conditions, protection from wind or sun, or by excluding exotic plants. This interaction can result in competition, facilitation or both simultaneously (Holmgren *et al.*, 1997), depending on seasons, stress type and level and plant species (Callaway & Walker, 1997). Trees in systems that are subject to regular stressors such as arid and alpine environments, often have positive facilitative outcomes from close tree proximity (Callaway *et al.*, 2002; James *et al.*, 2015). It's likely that trees in anoxic areas of a wetland would respond similarly to close proximity of a neighbouring tree. As with plant zonation from upland to lowland areas of the wetland, it can be expected that a similar pattern is seen from competition to facilitation, depending on the topography. Many wetland plants rely on AMF through increased acquisition of minerals and nutrients for survival and growth. AMF abundance and distribution through wetlands can be varied however as they are aerobic and are less abundant in wetter soils (Miller, 2000; Wang *et al.*, 2011), as well as being sensitive to pH and temperature (Entry *et al.*, 2002; Högborg *et al.*, 2007). Recent literature also suggests that this plant-fungal symbiosis ranges from mutualistic to parasitic depending on soil moisture, availability of soil nutrients, plant and fungal species among other factors (Klironomos, 2003; Solaiman & Hirata, 1995). However little is known of the role of AMF in plant facilitation within wetland environments.

The objective of this study was to assess the relative importance of factors that influence the survival and growth of two podocarp tree species in a temperate ephemeral wetland. To achieve this, I measured the survival and growth of *Dacrycarpus dacrydioides* (kahikatea) and *Podocarpus totara* (totara) saplings and a range of soil and environmental factors in two

different planting regimes at stage 3 of Wairio wetland. The planting regime treatment altered the presence of a neighbouring 'nurse' plant in the immediate vicinity of focal saplings. The nurse plant was always of a different species.

I tested the following hypotheses

Hypotheses;

1. The presence of a nurse tree (concurrent or focal-only planting treatment) will have a significant affect on kahikatea and totara survival and growth.
2. Increased soil moisture will negatively affect the growth of kahikatea and totara across the wetland.
3. The presence of a nurse tree (concurrent or focal-only planting treatment) will have a significant affect on the moisture, redox potential, pH, carbon and nutrient contents of soils, and on the abundance of spores of arbuscular mycorrhizal fungi in the immediate vicinity of focal saplings.

Materials and methods

Study area

Within stage 3 of Wairio wetland (41° 16'30"S, 175° 08'30"E), a large-scale study to assess the effects of tree planting techniques was established in 2011 by Dr. Stephen Hartley, School of Biological Sciences, Victoria University of Wellington. The study design consists of 10 randomised blocks of planted trees, in which treatments were applied. Each block was split into two halves – top soil scraped, and top soil not scraped. Within each half, there are three different planting treatments to assess the order of trees – 'advanced planting' plots, where non-target native trees were planted in advance of focal species so that they may potentially act as nurse trees, 'concurrent planting' plots, where non-focal and focal native trees were planted concurrently, and 'focal-only' plots, where focal species were planted alone. The focal species were: kahikatea, totara, cabbage tree (*Cordyline australis*), and bush daisy (*Olearia virgata*). The nurse trees that were planted in the 'advanced' and 'concurrent' plots were: karamu (*Coprosma robusta*), mingimingi (*Coprosma propinqua*), manuka

(*Leptosporum scoparium*) and kohuhu (*Pittasproum tenuiforum*). All trees were planted in 2011.

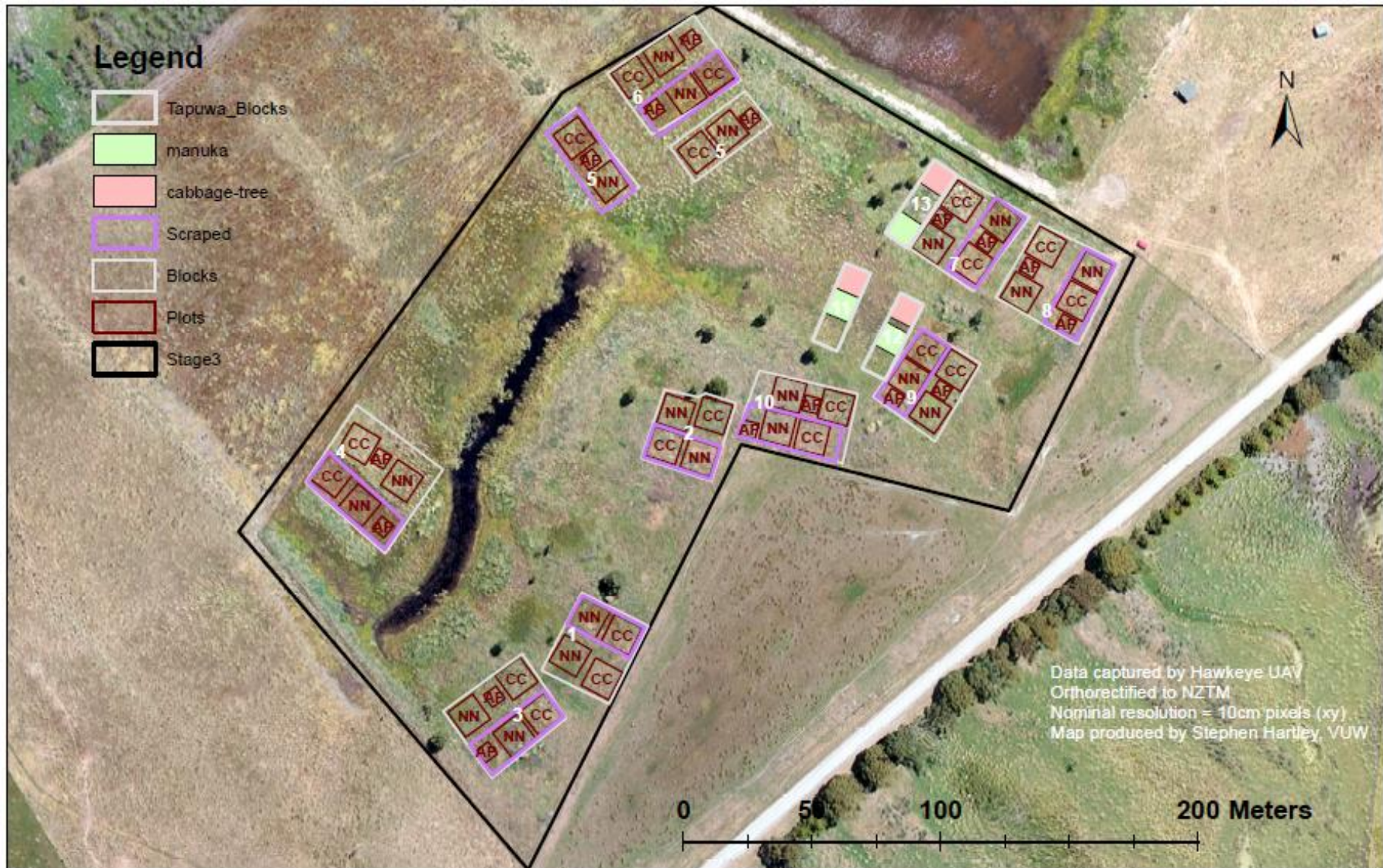


Figure 8. Aerial map of stage 3, Wairio wetland. The block numbers are shown in white, and each block is labelled with the treatment code. AP – advanced planting, CC – concurrent planting, NN – focal only. CC and NN are the treatments used in this study. The purple boxes surround the scraped side of each block, and were not included in this study.

In this study I used the concurrent planting and focal-only planting plots on the non-scraped side of each block. Within these plots I selected 50 sample sites in a randomised-block design to account for the three prevailing moisture regimes (wet, medium, dry), the different treatments across blocks, and the two podocarp species – kahikatea and totara.

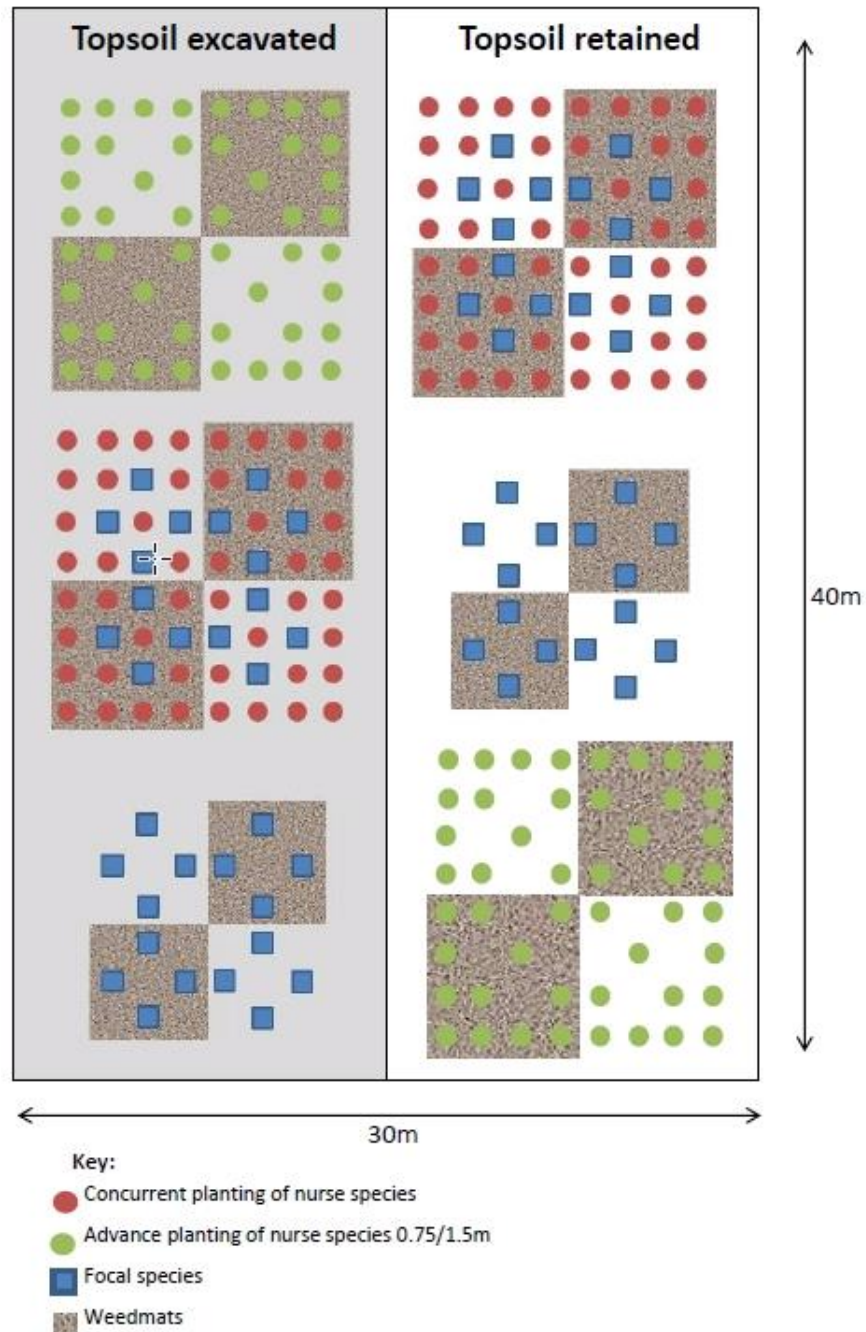


Figure 9. Planting treatments in each block of stage 3. This study focuses on totara and kahikatea trees (two focal species shown in blue squares) in the top soil retained side of the block, in the concurrent and focal plots. (Source: Johnson (2012)).

Field measurements

At each sample site, the survival of each tree was recorded. If the tree was alive, its height was measured.

Relative growth rate (R_h : cm cm⁻¹ yr⁻¹) of kahikatea and totara was calculated following the convention of Alvarez-Aquino *et al.* (2004),

$$R_h = (\log_e H_f - \log_e H_i) / (t_f - t_i)$$

where H_1 and H_2 are the heights of the trees at the time of initial planting (t_i) and five years later (t_f). Time was measured in years ($t_f - t_i = 4$).

Three replicate measurements of volumetric soil moisture were taken at each sampling position using a 'ML3 ThetaProbe Soil Moisture and Temperature Sensor' (Delta-t Devices, England)

Soil Cores

Two 8cm soil cores were taken within 50cm of the target tree. These soil cores were placed into airtight resealable bags and kept on ice for transport to the laboratory. They were then stored at 4°C until analysis. The soil samples were analysed for gravimetric moisture, carbon contents, bulk density, redox potential, pH and arbuscular mycorrhizal (AM) fungal spore numbers.

To determine gravimetric soil moisture a subsection of the length of one core was taken and the mass recorded (fresh weight). The subsample was then oven dried at 90°C for 6-8 hours or until dry. The mass of the dry subsample was then recorded (dry weight). Gravimetric soil moisture content of each soil core was then calculated as

$$\frac{(\text{fresh weight} - \text{dry weight})}{\text{dry weight}} \times 100$$

Carbon content was then measured on the same subsample of soil, using the mass loss on ignition method. Briefly, plant roots and stones are removed from the sample and

the mass of the remaining soil was recorded. The subsample was then placed in a muffle furnace (Manufacturer, City) set to 400°C for 16 hours, cooled to room temperature and reweighed.

To calculate bulk density, Soil Quality's method was used (Brown & Wherrett, 2016). Bulk density was calculated by weighing a soil core of known volume (fresh weight) and placing in a 90°C oven until dry. The soil core was then reweighed (dry weight). Bulk density was expressed as g/cm³ and calculated as:

$$\text{Dry soil weight (g)} - \text{Soil volume (cm}^2\text{)}$$

For measurements of pH and redox potential soil from the innermost portion of the second soil core was mixed with equal parts distilled water. The soil slurry was constantly mixed as the probe of the Thermo Scientific Orion Star A325 Portable pH/Conductivity Meter was placed in the soil slurry and pH and redox measurements (millivolts) were taken once the numbers had stabilised. Three replicate redox measurements were taken per sample and the mean value was used.

Arbuscular mycorrhizal fungal spores were counted using the method described by Brundrett *et al.* (1996) with some minor modifications. Fifty grams of soil was extracted vertically from a soil core to get a representation of the 0-11cm depth. The soil sample was suspended in distilled water and washed through a series of three sieves (660µm, 420µm, 210µm) until the water ran clear. The filtrate was collected and poured into large glass beakers until the large soil particles had settled to the bottom. The supernatant was then filtered to 47µm using a vacuum pump. The remaining slurry was placed directly into 15ml Eppendorf tubes and diluted with an equal volume of distilled water. The 47µm filter and 210µm sieve were scraped with a scalpel to removed retained particles. These were added to the Eppendorf tubes containing slurry of the correct size fraction. The soil slurries were then centrifuged for 5 minutes at 2,000 rpm and the supernatant, which contained organic debris, was decanted. Equal parts 50% sucrose solution to soil was then added to the pellet and mixed thoroughly. The samples were centrifuged for 1 minute at 2,000 rpm, causing the fungal spores to be suspended in the supernatant while the soil particles formed a

pellet. The supernatant containing the spores was transferred to clean labelled Eppendorf tubes. The supernatant from the $>210\mu\text{m}$ filtrate was filtered onto the $250\mu\text{m}$ paper, and then filtered through the $47\mu\text{m}$ filter to get any particles that were missed. The $250\mu\text{m}$ and $47\mu\text{m}$ filter papers were placed into separate petri dishes marked with 1 cm^2 squares. Spores were counted using dissecting and compound microscopes, respectively. Following Brundrett *et al.* (1996), four spore categories were counted: viable spores; hyphae shown; dead or burst spores; and sporocarps. However, sporocarps were too few to be included in the analysis.

Nutrient analysis

To determine the plant available nutrients in the soil, plant root simulator probes (PRS) (Western Ag Inc., Saskatoon, Canada) were inserted into the soil in July 2016 (mid-winter). PRS probes come in pairs, one cation probe has a negatively-charged membrane that attracts and absorbs all positively-charged cations such as NH_4 , K^+ , Ca^{2+} , and Mg^{2+} . The anion probe has a positively-charged membrane that attracts and absorbs all negatively-charged anions such as NO_3^- , H_2PO_4^- , SO_4^{2-} , and micronutrients.

Due to the budget of this study, 30 of the 50 sample sites were used to test nutrient availability in the wetland. Samples were categorised into treatment + species, from these categories, a random number generator was used to pick which sample site would have nutrient probes buried.

Two pairs (anion + cation) of nutrient probes were buried at 30 sample sites. Wairio wetland experiences strong westerly winds, and the trees primarily lean to the east, so a compass was used to place one pair of probes due north, and the other due south of each sapling. Probes were inserted at a distance of 0.5 metres from each tree, to avoid tree roots. In 3 of the sample sites however, this was increased to 1 metre because the trees were larger and root biomass had visually spread further.

Statistical analysis

A chi-squared test of independence was used to determine if there was a significant effect of kahikatea, totara, concurrent planting or focal-only planting on the survival of trees. Linear regression was used to determine the effect of moisture level on the growth of trees. GLMs were used to determine the effect of planting regime and species on the growth of saplings. GLMs were also used to determine the effect of planting regime on nutrient content on growth of totara trees. Pearson-Kendall correlations were used to determine the effect of spore counts on totara growth. All analyses were done using STATISTICA version 22 software (IBM SPSS Statistics 22, Armonk NY).

Results

Survival

Overall, survival of planted kahikatea and totara saplings in this study was 38.5% and was slightly higher for kahikatea than for totara (Table 3, 4). Survival of totara saplings was sensitive to planting treatment, with saplings planted near nurse trees surviving significantly better than those planted alone (Chi-square = 3.84, $p = 0.04$, Table 4). The survival of kahikatea saplings was unaffected by planting treatment.

Table 3. Survival for all planted sapling trees by species and treatment type.

Category	# Alive	# Dead	# Total	% Survival
All trees	62	99	161	38.5
Kahikatea	37	44	81	45.7
Totara	25	55	80	31.25
Focal	25	55	80	31.25
Concurrent	37	44	81	45.7

Table 4: Survival of kahikatea and totara saplings by treatment.

Grouping Variable	Total Survival	Treatment	Surviving Trees	Chi-squared test statistic	p-value	Significance
All trees	62	Kahikatea	37	2.32	0.12	N.S.
		Totara	25			
Kahikatea	37	Concurrent	19	0.11	0.73	N.S.
		Focal	17			
Totara	25	Concurrent	18	3.84	0.04	*
		Focal	8			

Initial heights of saplings

At the time of initial planting, kahikatea saplings were on average, 15cm taller than totara saplings. This difference was statistically significant ($t=5.82$, $p<0.0001$, Figure 10).

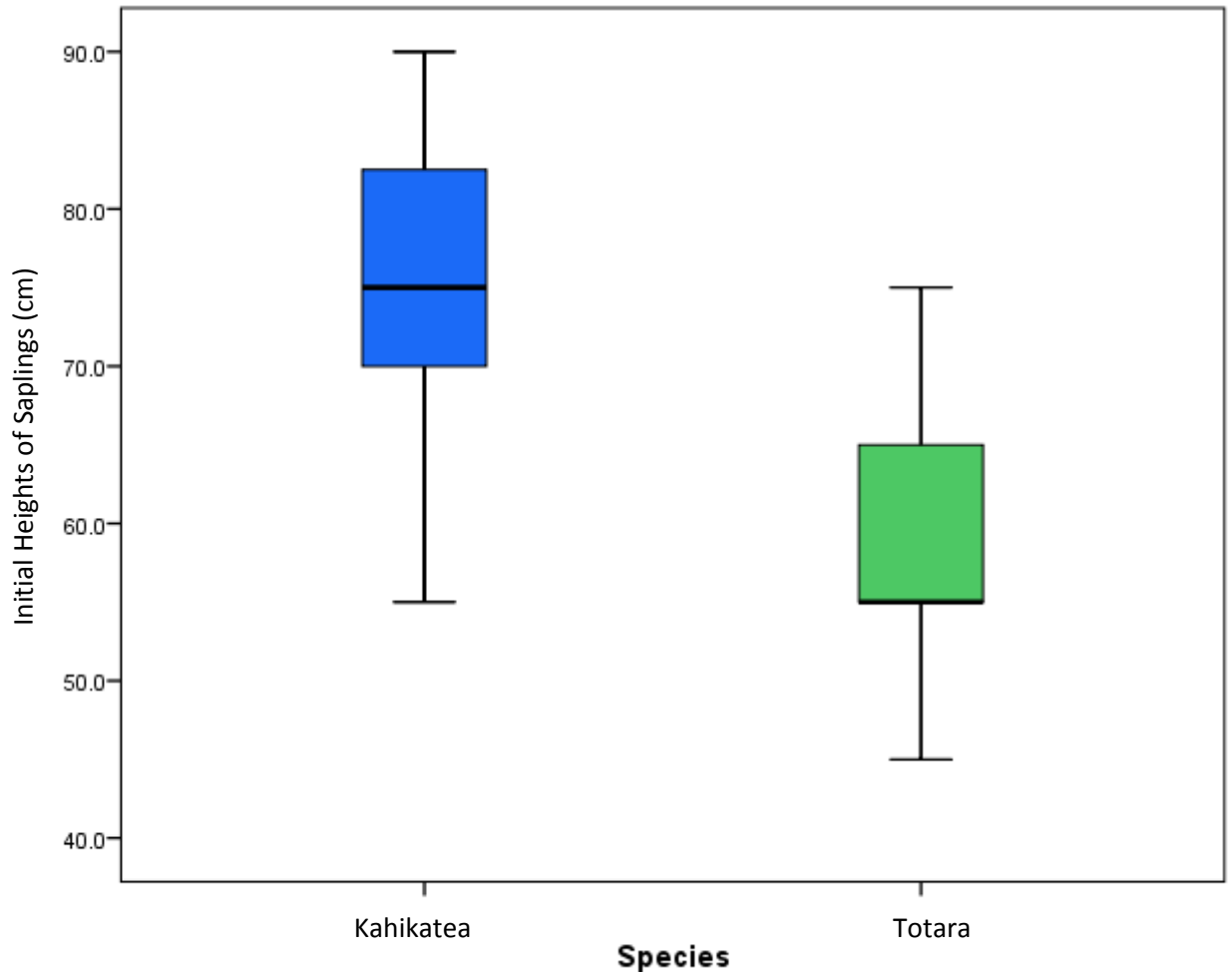


Figure 10. At the time of planting, kahikatea was on average, 15cm taller than totara.

Despite the initial difference in height of the two species, the relationship between relative growth rate and the absolute growth of saplings was significantly linear ($F = 650.5$, $p = 0.001$, Figure 11), suggesting that the growth of the two species was similar throughout this study.

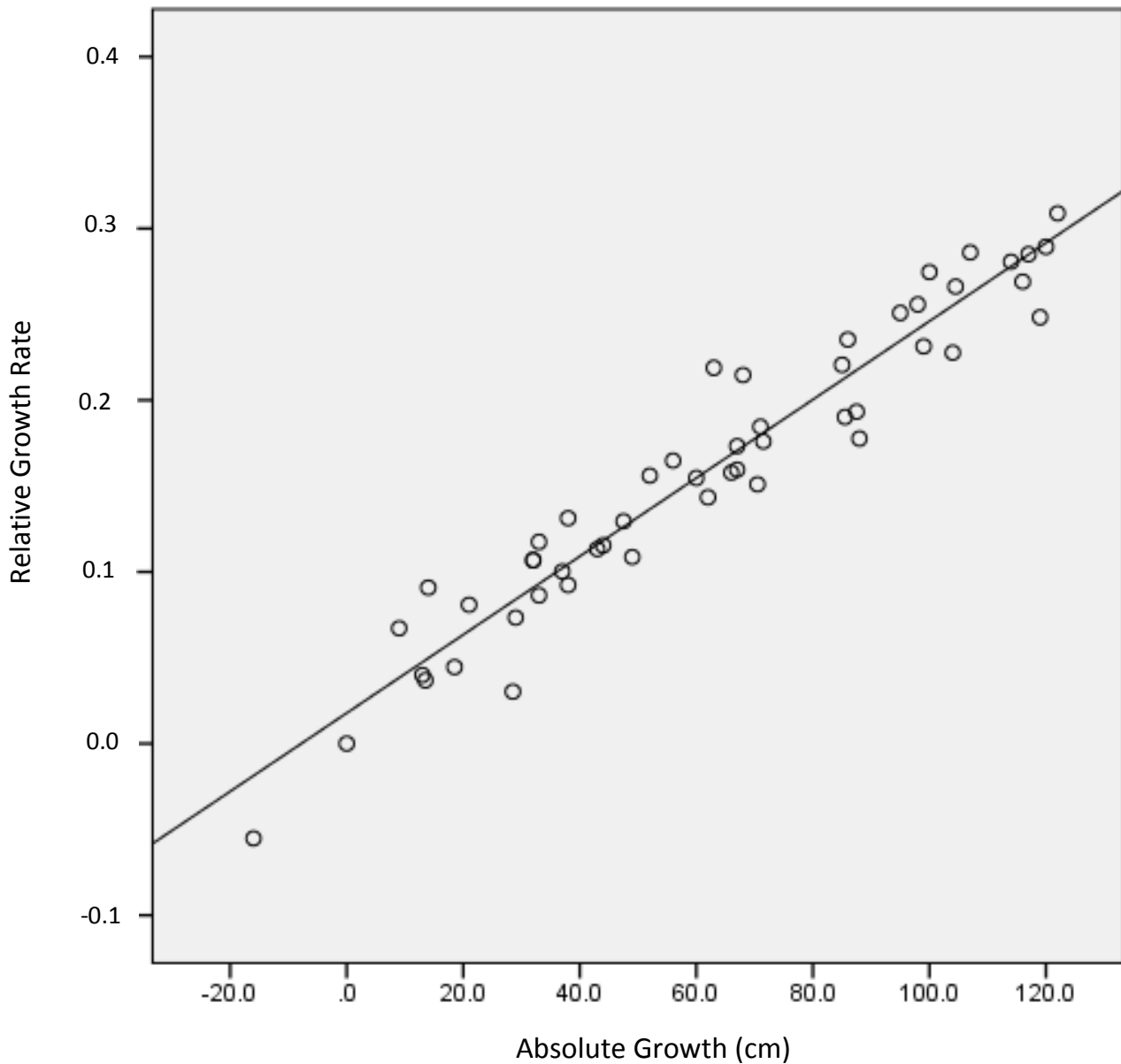


Figure 11. The linear relationship between the relative growth rate and absolute growth measurements of kahikatea and totara in this study.

Growth in response to moisture

Overall, soil moisture had a weak but significantly negative effect on the relative growth rate of kahikatea and totara saplings ($r^2 = 0.114$, $f = 6.328$, $p = 0.015$, Figure 12). In areas of very high soil moisture, kahikatea saplings were the only species to survive, where they grew little or died back (i.e. negative growth). In areas of Wairio wetland with low to moderate soil moisture, totara saplings survived and grew better than kahikatea saplings (Figure 10).

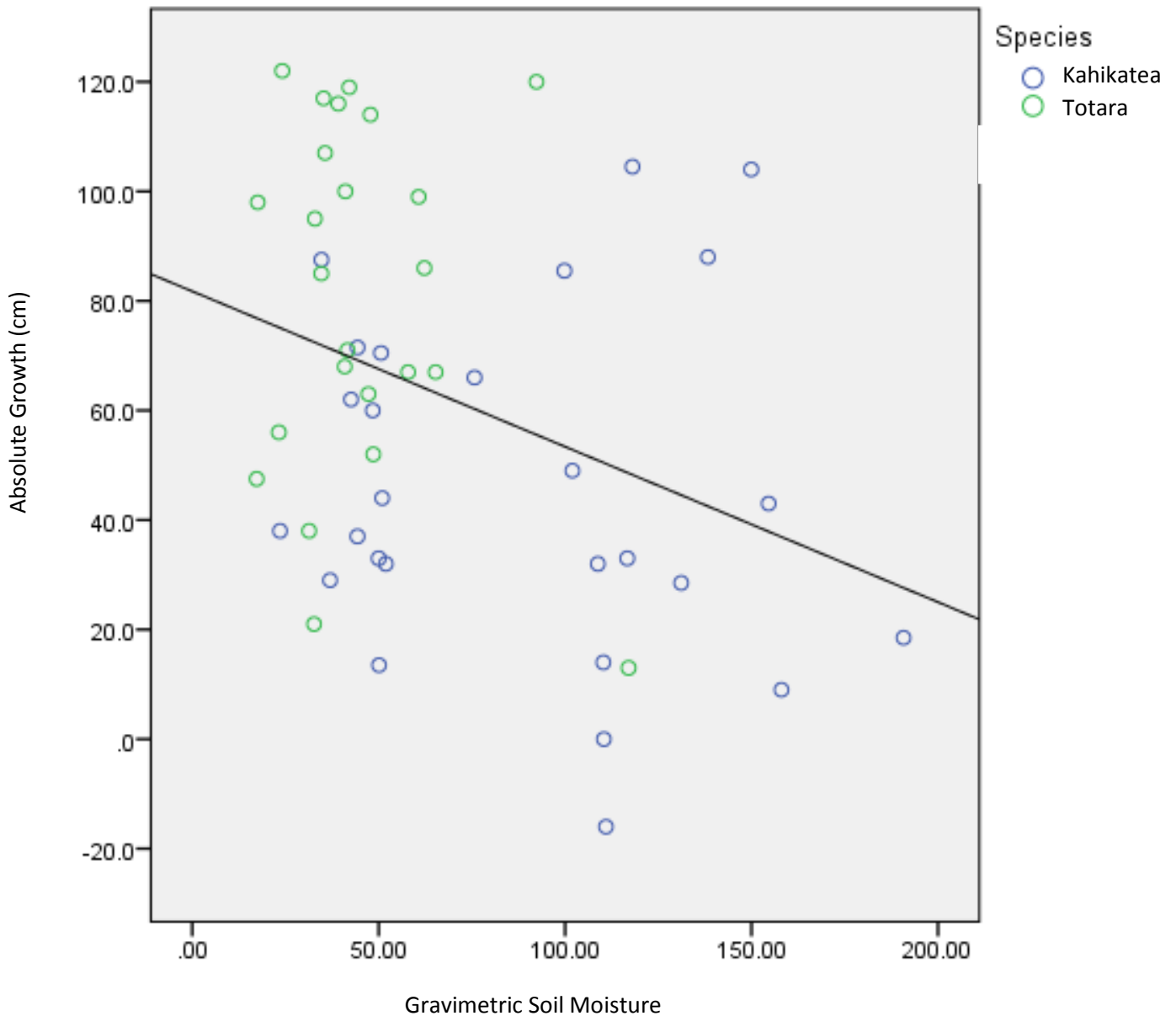


Figure 12. Growth of kahikatea and totara saplings as a function of gravimetric soil moisture.

Planting treatments

Overall treatments, absolute growth of totara was significantly greater than for kahikatea ($f = 10.5$, $p = 0.002$, Figure 4). Growth of all saplings did not show a significant interaction between planting treatments and species ($f = 3.4$, $p = 0.079$, Figure 13), however totara grew better in the presence of a nurse tree. I believe this effect was not significant due to the high mortality of totara, which lead to a small and uneven sample for growth measurements (concurrent = 16, focal = 8), and even sample sizes may have shown a significant interaction. Nonetheless, mean absolute

growth of totara with a nurse tree present was 24cm greater than for saplings planted without a nurse tree.

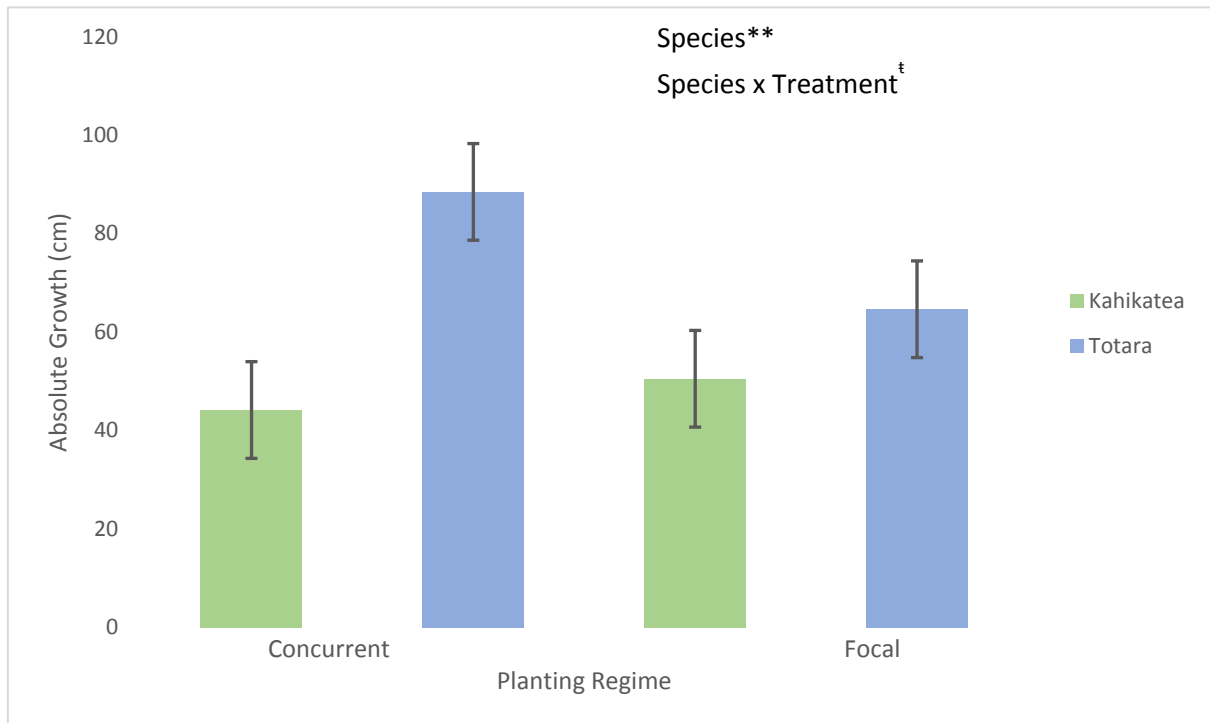


Figure 13. Absolute growth of kahikatea and totara saplings at Wairio wetland between 2011-2016 by planting treatment; symbols denote levels of significance ** = $p < 0.01$, † = $p < 0.1$.

AMF Abundance

Overall, large spores had a negative impact on the growth of kahikatea and totara ($\tau = -0.282$, $p = 0.045$), and small spores had a positive impact on the growth of the same trees ($\tau = 0.219$, $p = 0.122$). In addition, total spore count (large and small), had a weakly significant positive impact on the growth of all trees ($\tau = 0.217$, $p = 0.127$), however it's important to note that the number of small spores counted in this study were much higher than large spores, therefore the total spore effect is skewed towards the small spore effect.

Planting treatment (concurrent or focal) had no effect on the number of spores in any category or on the sum of all spores (Figure 14). Likewise, tree species had no effect on the number of spores in any category or on the sum of all spores (Figure 15).

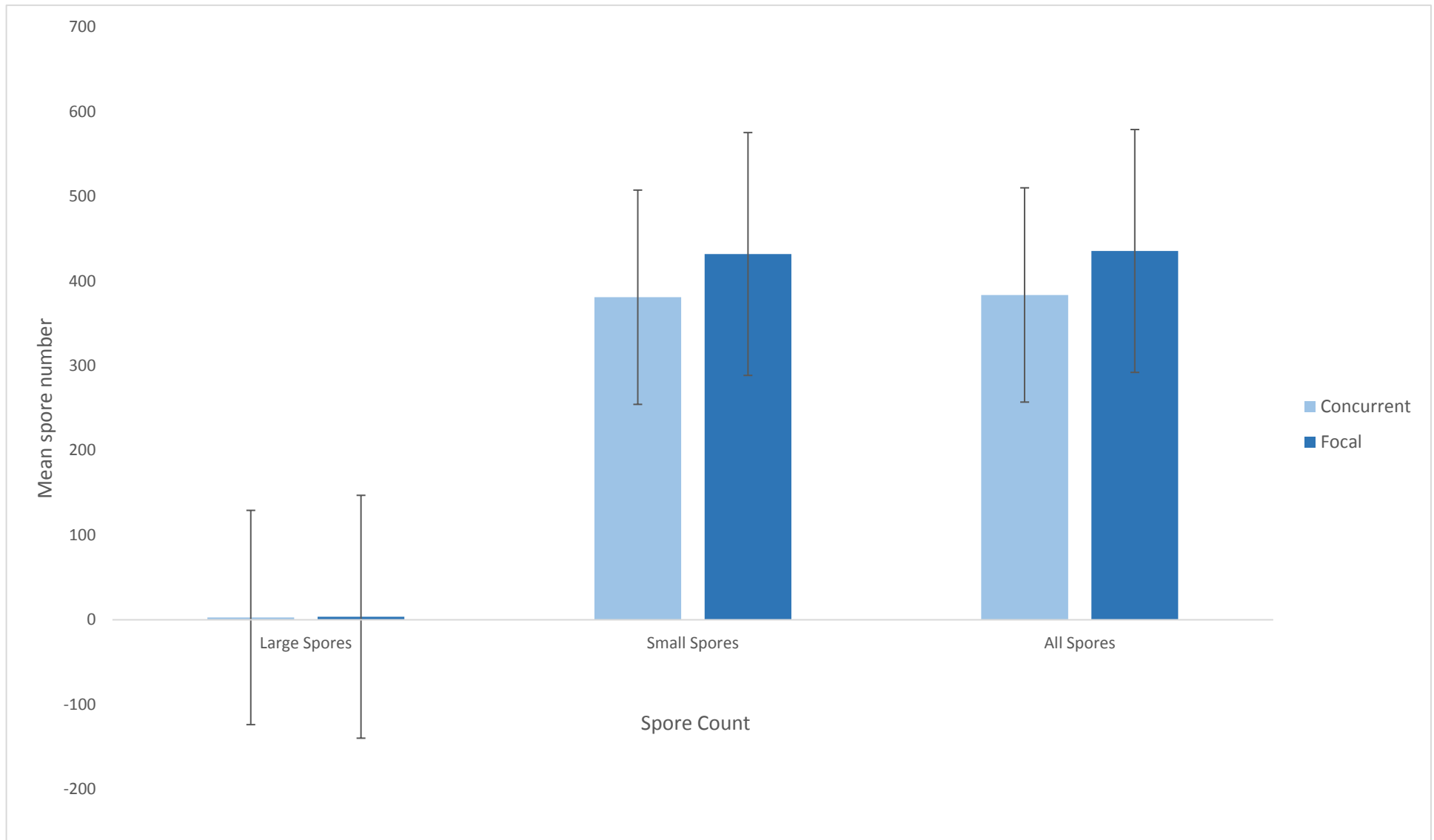


Figure 14. Mean number of spores depending on treatment. Spore number did not differ between treatments.

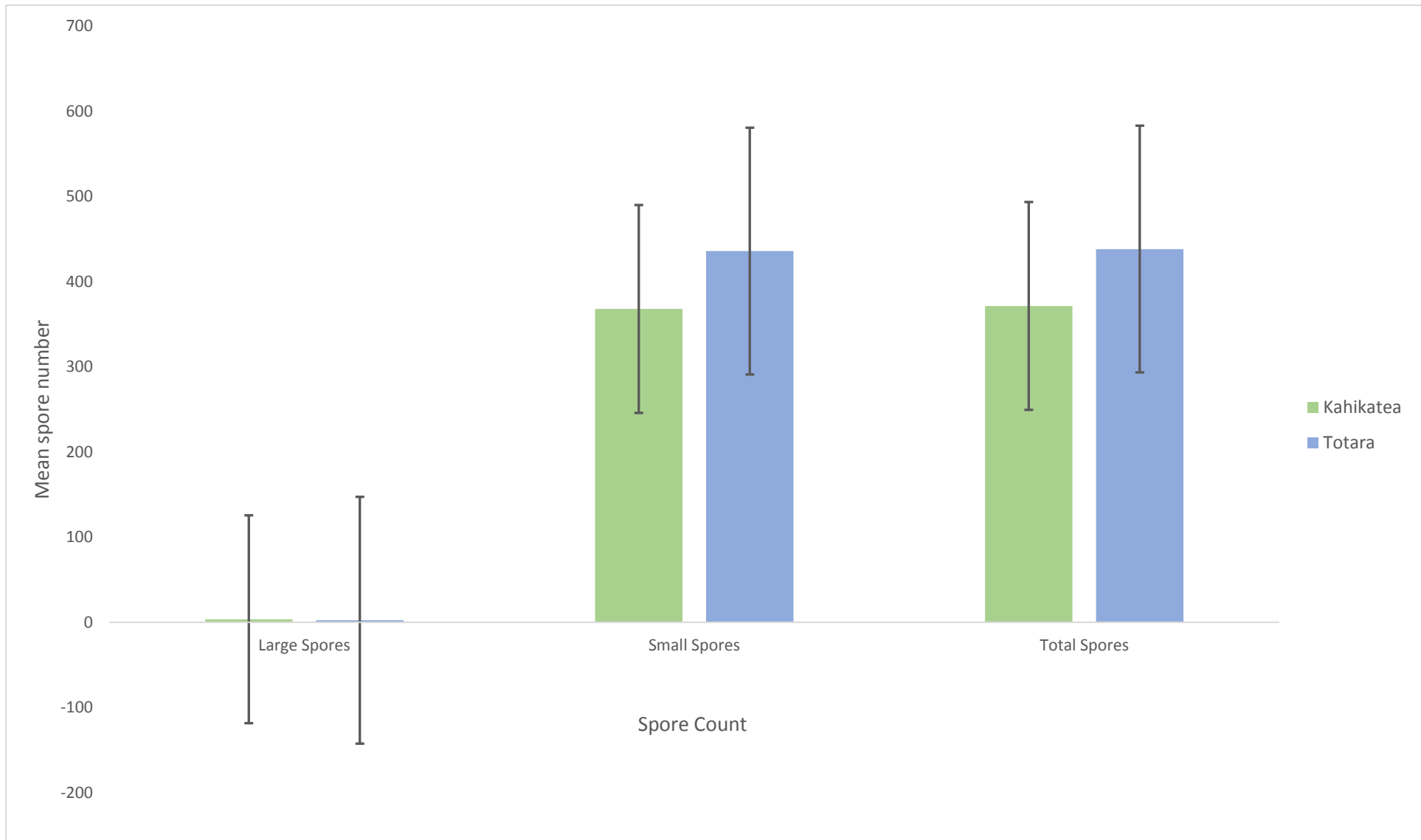


Figure 15. Spore number depending on species. Spore number did not differ depending on species.

Moisture effects on spore abundance

Gravimetric soil moisture was significantly negatively related to the sum of all spores in soil samples ($f = 10.43$, $p = 0.002$, Figure 16), indicating that there were a greater number of spores found at the drier sites, compared to the wet sites.

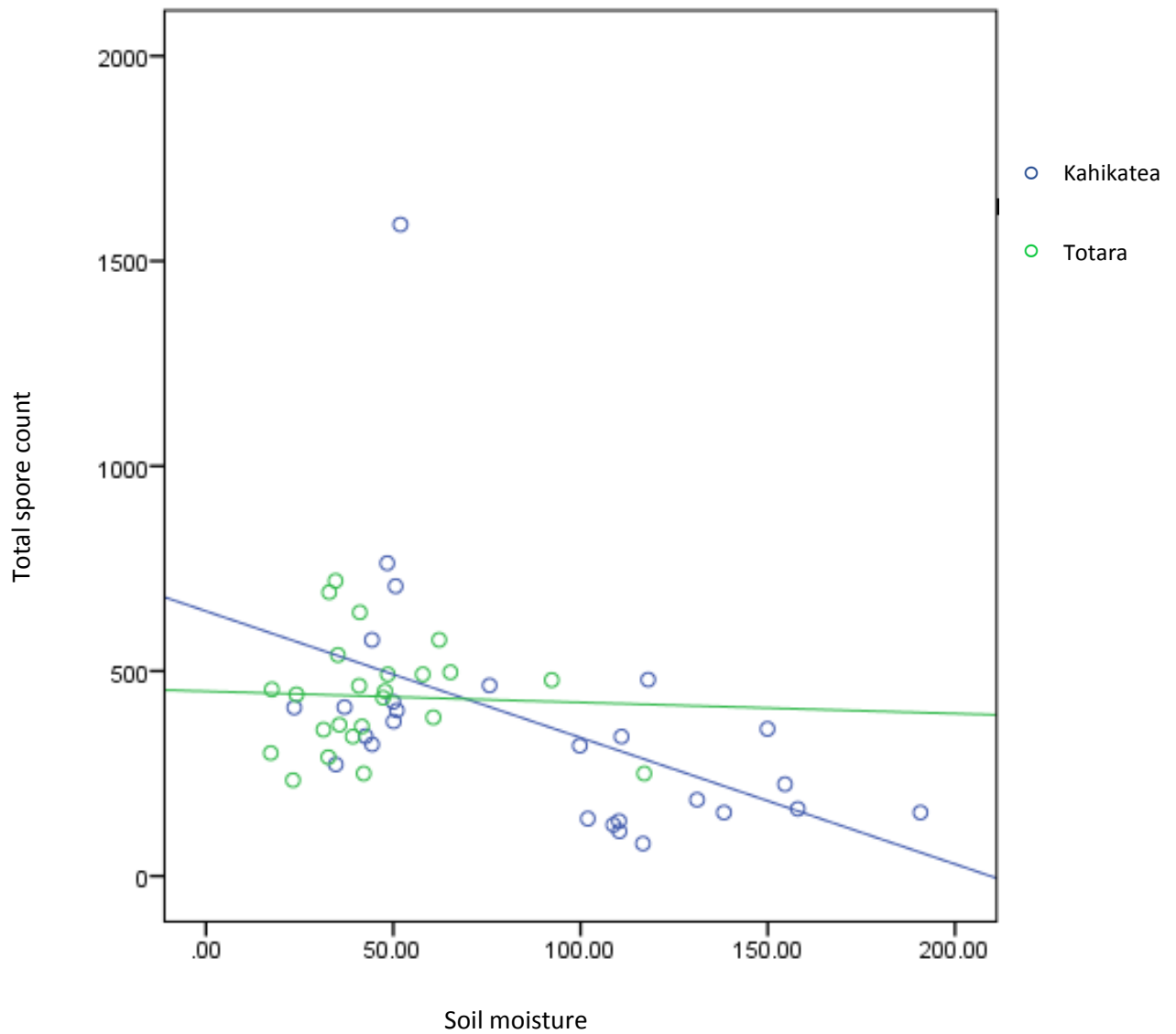


Figure 16. Total spore count as a function of gravimetric soil moisture. The dots and lines are coloured depending on species and show the correlation between species, moisture and spore counts.

Plant available nutrients

The presence of a nurse plant significantly increased the available soil nitrate ($\text{NO}_3\text{-N}$) to totara saplings ($f = 5.453$, $p = 0.036$). When a nurse tree was present, mean available $\text{NO}_3\text{-N}$ was near twice (609.9 mg) as much as that available when a nurse tree was absent (369.0 mg, Figure 17). Likewise, the presence of a nurse plant significantly increased the available soil potassium (K) to saplings ($f = 7.420$, $p = 0.017$). Mean K was 623.2 mg in the presence of a nurse tree, compared to 478.0 mg without a nurse tree (Figure 17).

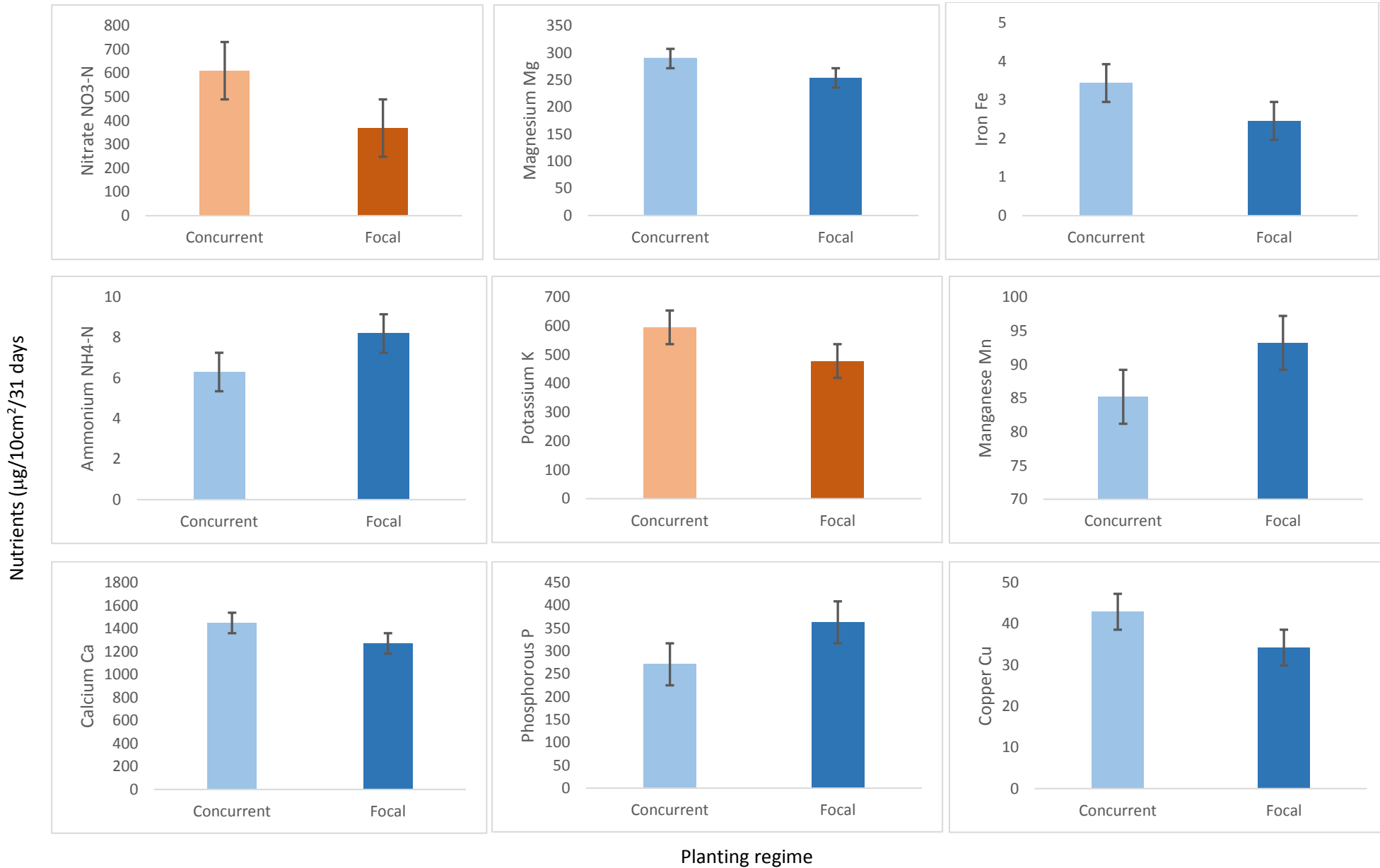


Figure 17. Mean plant-available nutrients by treatment. The plots shown with orange bars represent statistically significant relationships and the rest are shown in blue. The lighter coloured bars represent concurrent planting treatment and the darker coloured bars represent the focal planting treatment.

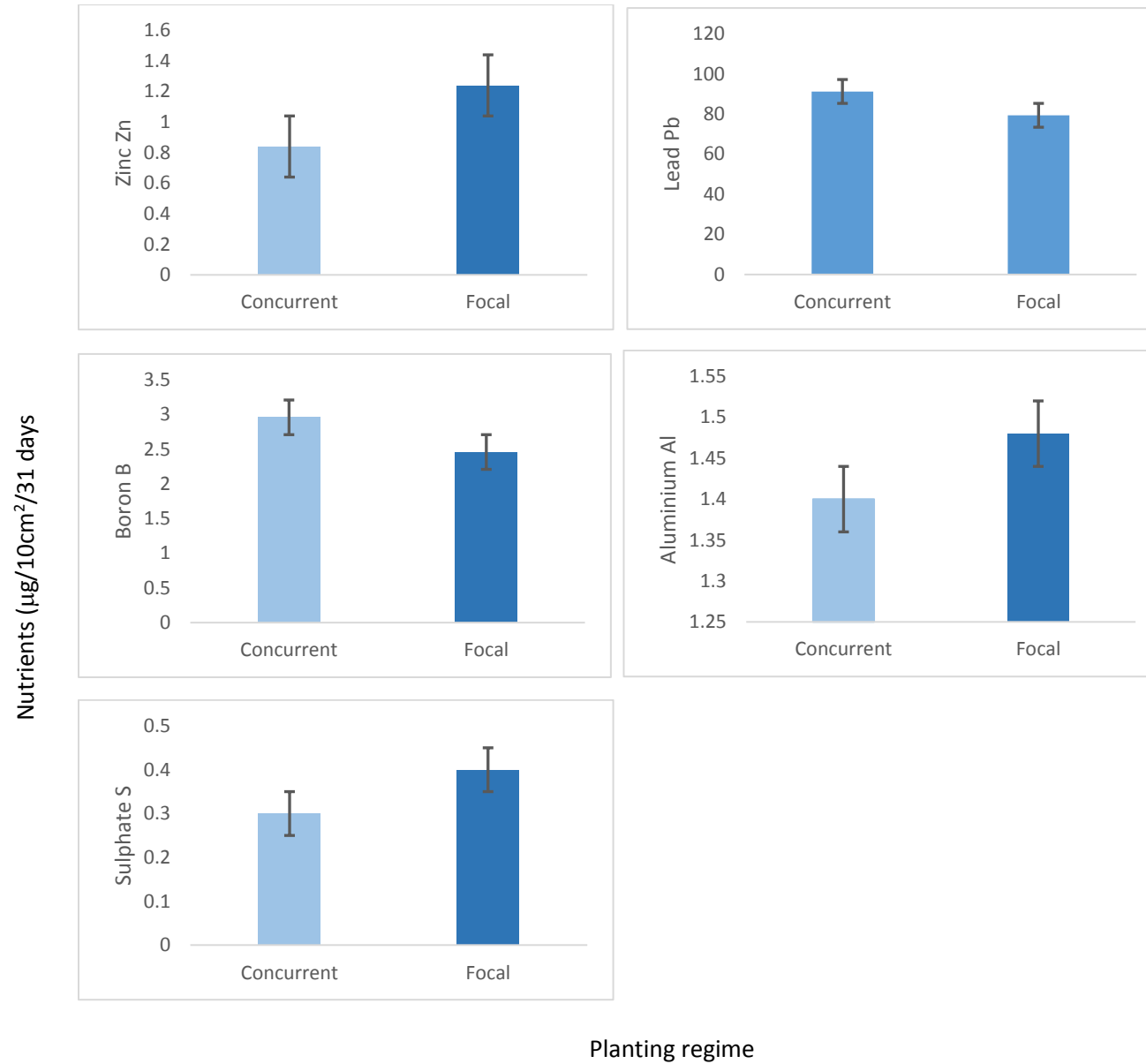


Figure 18. Mean plant-available nutrients by on treatment. The lighter coloured bars represent concurrent planting treatment and the darker coloured bars represent the focal planting treatment.

Discussion

Survival

Overall, the trees in this study had a survival rate of 38.5% since planting in 2011, with kahikatea surviving better than totara, and trees with a neighbour in the concurrent treatment, surviving better than those without a neighbour. Pennington and Walters (2006) studied the survival of woody tree species in wetland sites of 2 and 5 years old. They found that survival of their tree species ranged from 98-48% in the 2-year-old sites and 92-12% in the 5-year-old sites. However, this range represents upland, transition, and low wetland sites, and the wetland 5-year-old sites survival rates were very low (<15%). Compared to the wetland areas in this study, kahikatea and totara overall and individually had better survival.

Totara survival was significantly better in the presence of a neighbour, indicating that the dominant interspecific plant-plant interactions between totara and the established native tree species were positive. This suggest that the established vegetation had a 'nurse effect' on totara, facilitating its establishment at this wetland site. This effect was driven by totara trees being in the upland areas of the wetland, where nurse trees were larger and more abundant. Only two totara trees survived in soil that was more than moderately wet, meaning they are vulnerable to high moisture levels, regardless of the presence of a neighbouring tree.

Conversely, the survival of kahikatea was unchanged by the presence of a neighbour, suggesting that kahikatea was not significantly affected by positive or negative interactions with other plant species. However, this pattern emerged as a result of very poor survivorship of all species in very wet blocks. Of all tree species planted in this ecological restoration project, kahikatea was most tolerant to inundation, and was the only plant species present in the wettest blocks. Therefore, kahikatea trees exhibit a stress-tolerant strategy of survival (Grime, 1977) where they are less susceptible to particular forms of stresses, in this case, inundation, compared to all other tree species planted in the same area.

Consequently, I was unable to assess the effect of interspecific plant-interactions for kahikatea in this study, however, my results suggest the conspecific plant interactions

may be more important for kahikatea establishment in wetland areas that experience long periods of inundation. Moreover, this prediction aligns with the structure of mature kahikatea swamp forests, which tend to be relatively species poor and strongly dominated by kahikatea.

Absolute and relative growth

At the time of planting, kahikatea was significantly taller than totara. This may have led to potential problems in our study when comparing growth rates of both, since taller trees may grow slower than smaller trees. Relative growth rate is a growth measurement that standardises growth of different plant species that may grow differently depending on factors such as size, or genetically-determined differences in growth (Grime & Hunt, 1975). However, the saplings were all of an equal age at planting and since kahikatea and totara are both podocarp species, we assumed their growth would be similar. We found a strong correlation between absolute growth and relative growth rate of these trees, showing that despite the height difference, they grew very similarly during the study, and therefore absolute growth was a valid measurement to represent growth of both species.

Growth in response to moisture

The growth of totara and kahikatea were significantly affected by soil moisture level in the wetland. In areas of very high moisture, kahikatea was the only species to survive, and even these individuals grew slowly or died back. Where they survived in upland areas of the wetland with low to moderate soil moisture, totara dominated blocks and grew better than kahikatea. In the upland dry areas of the wetland nurse species were large and abundant, therefore their root biomass would be larger, allowing them to explore larger volumes of soil for water and nutrients that could facilitate the growth of totara saplings.

This result demonstrates that although kahikatea and totara are both podocarp species, they occupy different ecological niches, which supports the body of literature

identifying the hydrology of wetlands as being the most important factor that determines plant zonation and community development (Casanova & Brock, 2000; Clarkson *et al.*, 2013).

Additionally, although wetland plants experience inundation and drought stresses, these periods of water scarcity are often brief, only lasting a season. In New Zealand, the driest season is summer and the wettest season is spring, where wetland systems often see the greatest species richness and germination (Britton & Brock, 1994). Often this decrease in both productivity and survival are short-lived (Touchette *et al.*, 2007), unless there has been draining and water diversion in the area which increases drought periods and decreases flooding. As in Wairio wetland, following the recent hydrological changes to the site, the duration of floods generally lasts less than 10 days per year (Hicks, 1993).

Species-specific growth in response to planting treatments

Totara grew significantly better than kahikatea over the course of this study, but only where it survived in relatively dry areas of the wetland. Species-specific differences in survival and growth have been documented in other restoration ecology planting trials. For example, Walters and Reich (2000) found that trees varied greatly in their ability to survive and grow due to distinct mechanisms such as seed size or shade tolerance, however they found that trees that had higher mortality, grew better and vice versa, in some cases. The species-specific variation in survival and growth results in the plant zonation seen in wetlands, and this distinct difference between totara and kahikatea survival and growth is why there is such distinct species distribution among blocks. Moreover, some studies show that tree species of similar wood qualities can vary significantly when it comes to survival rates, pest problems and growth (Haggard *et al.*, 1998).

Totara grew better in the presence of an established interspecific nurse tree, but surviving totara that occurred only in relatively dry upland areas of the wetland drove this pattern. This may be due to totara relying on the facilitative effects of a nurse for not only their survival, but also, their growth. Moreover, the dry areas of the wetland,

where totara trees are found, may be sufficiently stressful for trees to grow because close proximity to a neighbour doesn't result in competition, rather positive facilitative effects. Wairio wetland has areas of very high moisture levels that don't dry out during the summer and only a few kahikatea trees have survived. It also has areas that dry out completely in the summer and stay like this for much of the year. These are among the areas in which totara have survived, and although literature has shown that inundation can negatively impact seedlings and trees (Kozłowski & Pallardy, 1997), much of the literature shows that desiccation and tree mortality are more likely in the dry seasons and the dry areas of a wetland (Britton & Brock, 1994). It is unsurprising then that the competitive effects between trees are outweighed by facilitative effects and totara relies so heavily on these.

AMF abundance

Based on the size of the small spores (<210 μ m), and the number of spores counted in this study, it's likely that they belong to the genus *Glomus*. Furthermore, the unique nodulated roots of podocarp species have been shown to have strong, but not exclusive associations with *Glomus* species (Russell *et al.*, 2002). Although this is likely, it cannot be confirmed without species identification which is notoriously challenging for AMF species, and therefore size, shape and colour was used to best understand the diversity of AMF in this study. Additionally, it's important to note that although there were far more small spores in this study, that is not a reflection on spore species richness (Sene *et al.*, 2012), and the response of growth to both small and large spores is likely a much more complex story of species interactions in the soil.

It's well documented that AMF strongly influence plant growth and fitness (van der Heijden *et al.*, 2015), and studies have shown that spore density is seasonal and is impacted by moisture levels, AMF species and temperature (Escudero & Mendoza, 2005), but little research has investigated the relationship between spore number and diversity and their effects on tree growth in wetlands. Furthermore, AMF are known to associate with a wide range of host species, but a growing body of literature suggests that the effect of AMF and the sporulation rates will change depending on the host

species involved (Bever *et al.*, 1996). Therefore, it's vital to understand these effects on kahikatea and totara to understand if AMF symbiosis significantly impacts their growth, and consequently restoration efforts.

The abundance of large spores was negatively associated with the growth of trees. This may be a result of trees sacrificing too many resources such as carbon to this symbiosis, that it damages their growth (Egerton-Warburton & Allen, 2000).

Large spores were far fewer than small spores, which may be a result of high nitrogen levels in the soil from the surrounding agricultural land. High soil N is associated with reduced AMF species richness and can lead to a loss of large-spored species (Egerton-Warburton & Allen, 2000).

More attention has been given to the relationship between AMF and plants, and whether the interaction is exclusively mutualistic (Klironomos, 2003). Evidence suggests that the costs and benefits of maintaining this symbiosis can differ significantly for plants and the resulting plant responses varies greatly (Klironomos, 2000; Raju *et al.*, 1990). The smaller spores in this study had a strongly positive effect on the growth of kahikatea and totara, and this contrast to our large spore results is likely a reflection of the symbiotic continuum ranging from parasitism to commensalism to mutualism between the various AMF species in the soil and kahikatea and totara.

Total spore number was also positively related to tree growth, however due to the number of spores counted for both large and small, total number was heavily weighted towards the small spores and therefore a similar effect would be expected. Our results also showed no effect of species or treatment, indicating that if there are any positive or negative responses of growth for kahikatea and totara aren't due to the species of tree, but rather the AMF species.

Unsurprisingly, we found a strong relationship between spore count, and moisture, with lowered spore number in areas of high moisture. This is consistent with previous literature that show AMF are aerobic (Truu *et al.*, 2009), and while they have mechanisms for surviving in anoxic conditions (Ernst & Brooks, 2003; Wang *et al.*, 2011), they are more abundant in aerobic soils (Bohrer *et al.*, 2004).

Interestingly, when we look at spore number in relation to species, it becomes clear that the areas of low moisture, where spores are abundant, totara individuals are dominant in these areas and grow better than kahikatea. This shows that totara and spores are highly correlated, which may be consistent with our previous results that show that totara are more reliant on facilitative effects than kahikatea. As mentioned above, these drier areas can be very limiting for plant growth and seedling germination, and the association between totara and AMF spores is likely strong in the drier areas because AMF may increase drought tolerance in plant species (Ma *et al.*, 2006). The reliance of totara on any positive effects from a neighbouring tree, and the possible lack of these same effects by kahikatea, could also explain why growth is significantly better overall for totara than kahikatea.

The impact of AMF spores and effects of a nurse tree on kahikatea growth is unknown. However, we do know that in areas of high soil saturation, external forms of AMF such as hyphae and spores are particularly sensitive (Wolfe *et al.*, 2007), and therefore it's possible that kahikatea are less reliant on the effects of increased AMF abundance in their vicinity. These results highlight the fact that although kahikatea and totara species are both podocarps, they occupy very different ecological niches.

Plant available nutrients

To explore the factors influencing totara survival and growth depending on treatment, we analysed whether the amount of soil nutrients available to totara is also effected by the presence of a neighbour. The results show that in the presence of a neighbour, soil nitrate ($\text{NO}^3\text{-N}$) was almost double, compared to totara trees without a neighbour.

Nitrate and potassium are among the most mobile water-soluble nutrients in soils, and totara trees experienced significantly higher root available $\text{NO}^3\text{-N}$ and K in the presence of a neighbour. Their increases availability suggests that neighbouring trees increased water availability in the root zone of totara. Neighbouring trees had much greater root lengths and surface areas than the planted saplings and it's probably that hydraulic redistribution of water to totara saplings in summer was likely the mechanism underpinning this nurse effect. Furthermore, our results showed that totara trees and

spore counts are highly correlated, and fungi are much better at mobilising NO³-N and K than trees alone, and this mobilisation would be promoted by the increased water availability in the root zone of totara trees.

Surprisingly, no other nutrients analysed in this study was significantly impacted by treatment for totara saplings. As we saw an effect of nitrogen, we would have expected to see a similar result of phosphates, as they are the other common nutrient that wetlands are often lacking in, and given our spore results that show such an influence on plant growth, phosphates are commonly foraged by AMF for plants (van der Heijden *et al.*, 2015). It's possible that phosphorous isn't a limiting nutrient in Wairio wetland, and therefore differences in tree proximity that may impact nurse effects aren't shown (Brown & Bledsoe, 1996). Additionally, the difference in nutrient acquisition in our treatments varies depending on AMF species, and this isn't represented by looking at spore counts overall.

Conclusion

It's important to note that in this study, soil samples weren't taken from dead trees, and therefore a significant amount of the variability in biophysical soil attributes experienced by the saplings may have been lost. Future research should sample from both dead and alive trees to understand the mechanisms that effect survival and growth. Considering that, the results of this study show that closely related species in the same environment can occupy very different ecological niches. Out of all the variables measured in this study, treatment type was vital for totara survival and growth, and moisture, spore counts and nutrient availability were vital overall for the survival and growth of these podocarp trees. Therefore, totara and kahikatea should be considered individually for each species in a wetland sites and then used increase successful restoration.

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IV GENERAL DISCUSSION AND CONCLUSIONS

Overview

Here I present the main conclusions from the two studies that comprise this thesis. The overall aim of my thesis was to determine the factors that had the biggest impact on the survival and growth of kahikatea and totara in Wairio wetland, in order to inform future restoration and management practices. My first study (chapter 2) assessed the importance of the distance from a mature tree and the ability to connect to an underground network of arbuscular mycorrhizal fungal hyphae on the survival and growth of kahikatea and totara saplings. My second study (chapter 3) assessed how the survival and growth of kahikatea and totara saplings responded to the presence of a nurse tree. I quantified the positive and negative effects of a nurse on the saplings by assessing a range of physical, chemical and biotic environmental variables for trees planted with and without a neighbour.

Summary of key findings

Kahikatea

Wairio wetland shows exhibits patterns of vegetation zonation that are typical for wetlands, with species with similar ecological tolerances assembling in space in response to hydrological gradients (Johnson, 2012; Pennington & Walters, 2006). Although kahikatea and totara are both podocarp species, kahikatea trees survived better than totara across Wairio wetland, and this was driven by kahikatea occupying the very wet blocks where no other species survived. Although their survival is very high in the first year of planting, this research found that survival continues to decline until at least year 5. Previous research on tree survival in Wairio wetland suggests that plant mortality is greatest in the first year of planting, but that of the species planted at Wairio, kahikatea is the slowest to die (Gillon, 2014). Other wetland plant studies have found that a plateau in plant mortality is achieved within the first 1-3 years of planting, depending on plant species and site differences in hydrological regime (Valades, 2013).

As kahikatea trees were the only species survive in the very wet blocks, there were no established nurse trees to facilitate their survival and growth, or to compete with them. In areas of inundation, decreased light and root oxygen results in reduced photosynthetic capabilities and oxygen for respiration can be detrimental to plant survival and growth (Maurenza *et al.*, 2012), these stressful conditions could cause trees to rely more heavily on the facilitative effects of a nurse tree or a symbiosis from AMF (Brown & Bedford, 1997; Butterfield, 2009). However, my results suggest that this is not the case for kahikatea. Kahikatea is a masting species, releasing large quantities of seed sporadically in favourable years. Pure stands of kahikatea forests were historically common, and still exist in some parts of New Zealand today (Robertson & Hackwell, 1995). They occur in stable river channels and established wetlands in areas of high rainfall (Robertson & Hackwell, 1995). After a major disturbance event, kahikatea seedlings germinate in large numbers, and form even-aged stands, reinforcing the stability of the riparian zone through their network of interconnected roots (Robertson & Hackwell, 1995; Wardle, 1974). In my chapter 1, I found that where kahikatea grew in close proximity to a nurse tree, their biomass was negatively affected. Biomass increased with increased distance from the nurse, and regular disturbance of the AMF mycelium. This result supports previous research that illustrates how conspecific competition is a stronger driver behind plant community composition than interspecific competition because individuals of the same species occupy the same ecological niche (Svanbäck & Bolnick, 2007). Furthermore, kahikatea trees are light demanding species and seedlings suffer underneath the canopy of their parent tree because they require high light levels for seedling establishment and growth (Ebbett & Ogden, 1998).

Totara

Plant survival and growth, species composition and zonation patterns are strongly influenced by flooding (Carvalho *et al.*, 2001), and totara trees were strongly associated with this pattern in Wairio wetland. The survival of totara was limited to the drier sites of Wairio wetland in both studies, across the ten blocks and near the remnant kahikatea trees, which are drier sites of the wetland. This research does not support some

literature that indicates that totara trees grow on wetter soils and are drought avoiders, rather than drought tolerators (Bergin, 2000; Innes & Kelly, 1992). Totara were found in areas of the wetland that are dry for most of the year, and may experience drought in the summer months. We did find a strong association between AMF, nurse trees and totara trees however which is likely a contributor to the distribution of totara.

Totara saplings survival was significantly better in the presence of a nurse tree, regardless of size or species of nurse tree. Survival declined if a nurse tree wasn't present, or if totara was two metres from the dripline of the nurse tree, showing that if totara trees experience any competitive effects of being in close proximity to a neighbour, this is outweighed by the facilitative effects provided. Furthermore, a connection to AMF hyphae was vital for the survival of totara in the first year of planting. This supports literature that shows AMF enhances the survival of trees through increased water and nutrient availability, lengthening root life, pathogen protection and aggregating soil particles (Brown & Bledsoe, 1996; Perry *et al.*, 1987).

In the upland soils of the wetland, totara trees grew better than kahikatea. Although totara has been labelled a 'drought avoider', within these upland areas, totara growth is strongly linked to AMF spore number. Furthermore, AMF may increase the drought tolerance in plant species (Ma *et al.*, 2006), therefore allowing totara to persist in these areas. The nurse trees present in these upland areas have greater root lengths and cover a larger area of the soil than totara saplings could alone. This leads to increased water availability in the dry soils and increased mobility of plant limiting nutrients (Cavagnaro *et al.*, 2015; Lambers *et al.*, 2008), which is what was seen in this research. When a nurse tree was present, N and K availability increased significantly in the root zone of totara trees. N and K are both water-soluble and therefore mobility in drier soils can be reduced (Liebersbach *et al.*, 2004), however this hydraulic distribution of water in the soil results in increased acquisition for totara. Furthermore, the positive impact of AMF spores on totara growth is likely linked in with this, as fungi are effective at promoting nutrient mobilisation in the soil.

Implications for the successful restoration of Wairio wetland

Based on the results gathered in this study, it's clear that kahikatea and totara occupy very different ecological niches, based on the hydrological and stress gradient across the wetland. Therefore, future restoration of Wairio wetland should consider these differences when attempting to revegetate the area to increase survival and growth rates and achieve a native cover.

Kahikatea trees should be the only species planted in the wetter areas of the wetland, at a reasonable distance from other kahikatea trees, such as 2 metres from the dripline of large and established trees. No other nurse tree species or focal species should be planted in these areas because they will not survive.

Furthermore, in moderately wet areas of the wetland, kahikatea trees should not be planted with a nurse tree as this interaction will not benefit the survival and growth of kahikatea trees and may result in competition.

Totara trees can be planted in the dry – moderate soil moisture sites, however survival and growth will be best at the driest sites. Totara should always be planted with a nurse tree, but this can be of any species and of any age. Totara trees are reliant on the facilitative effects of nurse trees and mycorrhizal networks for increased water availability in the root zone and N and K availability; therefore, care should be taken not to manually disturb the roots of totara trees once planted.

A connection to mycorrhizal fungi and fungal spores are beneficial to both species, so future research should investigate whether inoculating kahikatea and totara seedlings before planting, and inoculating the soil with fungi is more beneficial for their survival and growth. Additionally, in dry areas of soil, nurse trees and AMF should be used across the wetland to increase plant available N and K, where possible.

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