Functional trait variation along a hydrological gradient and trait-based predictions of the composition of a wetland plant community

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Abstract

Predicting the assembly of plant communities is considered the Holy Grail of functional ecology and has never been more important as we head into an era of environmental change. Studying plant functional traits provides the best opportunity for understanding the community assembly processes that determine the abundance and distribution of plant species. Plant functional traits provide information on the direct physiological adaptations of plants to various environmental conditions. The assembly of plant communities is driven by filtering processes that select for or against certain functional traits and a plant can only be present within a community if it contains the functional traits necessary to germinate, survive and compete in the environment of the community. An understanding of how functional traits are filtered by the environment and biotic interactions provides the foundations for predictive community assembly models. However, the understanding of how functional traits are filtered along hydrological gradients is poor for the majority of functional traits.

In this thesis I aimed to identify how plant functional traits respond to variation in soil hydrology in the presence and absence of grazing and determine whether a trait-based model of environmental filtering could predict the composition of an ephemeral wetland plant community. To achieve these aims, I performed a survey of the plant community in an ephemeral wetland in grazed and ungrazed transects. The survey was conducted along a hydrological gradient that was split into an elevation gradient above the flood line and a flooding gradient below the flood line. I measured nine root, leaf and shoot traits on 885 plant samples collected during the community survey and investigated the response of community-weighted and individual-level traits along the hydrological gradient using Generalized Additive Models (GAMs) and Generalized Linear Models (GLMs). To determine whether a trait-based model of environmental filtering could predict the composition of the plant community, I incorporated the individual-level trait relationships into a community assembly model known as Traitspace and predicted the relative abundance of the 23 most common species found within the Kettlehole.

Community-weighted root traits were more strongly related to the hydrological gradient than aboveground traits. Root aerenchyma increased as the number of days submerged increased while root dry matter content (RDMC), specific root length (SRL), root tissue density (RTD) and root branching intensity (RBI) decreased with increasing flooding. Community-weighted specific leaf area (SLA) and root tissue density were more closely related to the elevation gradient than any other traits. SLA decreased as elevation above the flood line increased while RTD increased with elevation. The relationships between individual-level traits and the elevation and flooding gradients were far weaker than community-weighted traits but showed similar trends in the directions of trait responses along the gradients. Grazing reduced the community-weighted trait values of all traits except SLA and aboveground dry matter content (AGDMC) along the flooding gradient and had little effect on the trait values observed along the elevation gradient.

Using environmental filtering of functional traits, the Traitspace model was able to predict the distribution and abundance of a number of key species within the wetland plant community but was unable to accurately predict the abundance and distribution of other species. The accuracy of the Traitspace model was best when all nine functional traits were used to produce predictions of species abundances but SLA and height were recognised as the two traits to provide the most predictive ability.

The strong filtering of root traits illustrate the important information that root traits provide in terms of the adaptations of plants in environments with varied soil hydrology. Easily measurable aboveground traits are often favoured in functional ecology but these results highlight the importance of measuring root traits in trait-based research. Traitspace has the ability to predict the abundance and distribution of some species within a wetland plant community using environmental filtering of functional traits. Community assembly at small spatial scales is mostly driven by biotic interactions rather than environmental filtering which may limit the power of current predictive models. The inclusion of biotic interactions into trait-based models will improve the predictions of community composition at small spatial scales in wetland ecosystems and help us to

progress towards achieving the goal of accurately predicting the composition of plant communities.

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Table of Contents

List of Figures

List of Tables

1 Chasing the Holy Grail: The current state of predictive community ecology in wetland ecosystems

1.1 Introduction

Predicting the assemblage of plant communities is a difficult task that has challenged ecologists for over 100 years and we are still far from being able to make accurate predictions of plant communities anywhere in the world (Schimper 1902, Clements 1916, Gleason 1926). The inability of ecologists to make hard predictions has led to criticisms of ecology as a 'soft' science, simply describing interactions rather than making predictions (Keddy 1992). Producing accurate predictions of the composition of plant communities has been referred to as the Holy Grail of community ecology and has never been more important as we head into an era of environmental change (Lavorel and Garnier 2002, McGill et al. 2006). Despite the difficulties, theoretical advances in the processes that govern the assembly of plant communities and new statistical models have made great strides towards the goal of predicting the assemblage and composition of plant communities (Diamond 1975, Keddy 1992, Shipley et al. 2006, Laughlin et al. 2012).

The most promising approach to predicting the composition of plant communities revolves around plant functional traits. Studying functional traits provides information on the direct physiological adaptations of plants to certain environments. The environment places direct selection pressure on functional traits and filters out species with traits that are not suitable for that environment (van der Valk 1981, Keddy 1992). As trade-offs exist between traits and plant strategies (i.e. a trait might increase tolerance of stressful conditions but reduce competitiveness in favourable conditions) there is no set of traits that provide the optimal fitness in every environment (Grime 1974). By understanding how different environments and biotic interactions select varying trait values, and collecting information on the functional trait values of different species, we can predict which species are most likely to occur and dominate in different environments (Keddy 1992, Laughlin et al. 2012).

To make accurate predictions in any environment it is necessary to determine the key environmental gradients and biotic interactions that drive changes in functional traits in different ecosystems. Temperature and precipitation are known to influence global vegetation patterns and can be used to accurately predict the vegetation types in a given landscape (Whittaker 1975). These climatic variables have also been shown to filter leaf traits on a global scale (Wright et al. 2005, Ordoñez et al. 2009, Maire et al. 2015). On smaller geographical scales, soil variables and disturbances have been shown to have a greater influence on leaf traits and the assembly of local plant communities than climatic variables (Weiher and Keddy 1995, Ordoñez et al. 2009, Roccaforte et al. 2012, Baastrup-Spohr et al. 2015, Maire et al. 2015). Biotic factors, such as competition and grazing, add additional selection pressures on functional traits within plant communities (Darwin 1859, Díaz et al. 2001).

Wetland ecosystems cover more than 10% of the Earth's land and are defined as any land saturated with water that leads to anaerobic soil conditions (Keddy 2010, Voesenek and Sasidharan 2013). The assembly of wetland plant communities are driven most strongly by the stresses associated with soil saturation and submergence (Weiher and Keddy 1995). Other factors such as soil fertility, salinity and grazing pressure can also apply selection pressures on various functional traits in wetland ecosystems (Weiher and Keddy 1995, Eallonardo et al. 2013). By understanding how functional traits respond along environmental gradients and to biotic interactions in wetland ecosystems, we can begin to make predictions on the assembly of wetland plant communities.

In this review I examine our current understanding of how environmental and biotic filtering of functional traits determine the assembly of plant communities and how that information can be used to predict the assembly of wetland ecosystems. I first discuss the current ecological theory necessary to consider when pursuing the goal of predictive community ecology. I then discuss important environmental variables and biotic interactions in wetland plant communities and provide the current evidence for how they filter various functional traits. Finally, I discuss two trait-based community assembly models

and how they are able to use trait-environment relationships and species-trait data to create predictions of the composition of wetland plant communities.

1.2 Community assembly rules

The concept of 'assembly rules' was first introduced by Diamond (1975) after finding that interactions between birds led to non-random assemblages of species. The theory was developed by Keddy (1992) who proposed that community assemblages were driven by ecological filters that remove species from the species pool. Hubbell (2001) elucidated the importance of dispersal limitation on community assembly as species that are unable to disperse into a community are not going to be included, regardless of whether they are suitably adapted to the environment. It is now commonly accepted that the ecological assembly of plant communities is driven by three filtering processes: dispersal limitation, environmental filtering and biotic filtering. Together these three filtering processes reduce the greater pool of available plant species down to the realised plant community.

Dispersal limitation reduces the global species pool down to the species that can disperse into the area of interest. It has been shown to be an important factor in community assembly and can potentially create initial zonation patterns of vegetation along environmental gradients through non-random seed dispersal (Fraaije et al. 2015). The influence of dispersal limitation reduces as the geographical area of interest decreases and in many studies, across small geographical areas, the influence of dispersal limitation on community assembly is assumed to be negligible.

Environmental filtering removes species from the plant community that are unable to germinate, survive or reproduce in the specific environmental conditions of a given area. Multiple climatic factors (e.g. temperature and precipitation), soil properties (e.g. hydrological regime, fertility, salinity, pH and porosity), disturbances (fire, flooding, and storms) and other environmental variables (light, topography, elevation, latitude) have been shown to influence the composition of plant communities through environmental filtering (Pearsall

1920, Whittaker 1975, van der Valk 1981, Weiher and Keddy 1995, Pearson and Dawson 2003, Shipley et al. 2011, Laughlin et al. 2012, Roccaforte et al. 2012). Species that pass through the dispersal and environmental filters must then deal with the pressures of competition and additional interactions with other organisms (Weiher et al. 2011).

Understanding the effects of biotic interactions is arguably the greatest challenge to overcome in the field of predictive ecology. Teasing out the different effects of biotic interactions such as competition, facilitation and grazing to make generalizable rules across ecosystems requires rigorous study. Competition and facilitation are direct opposites in terms of the influence they have on plants within a community and can both influence the composition of a plant community along a single environmental gradient (Santoro et al. 2012, Merlin et al. 2015). For the remainder of this review, I will focus on the processes of environmental and biotic filtering.

These filtering processes place selection pressures on organisms at a physiological level. From the regional species pool, only plants with the traits necessary to pass through the environmental and biotic filters will be found in the realised plant community. Environmental filters and predation select for species with converging trait values (Weiher et al. 1998). Competition forces different species into distinct niches and can remove species with a competitively inferior set of traits (Hardin 1960, Weiher et al. 1998). Competitive interactions can therefore force a divergence of trait values within the plant community. Keddy (1992) proposed that a list of the species pool, a trait matrix for each species and a series of 'deletion rules' would provide the information necessary to accurately predict the composition of plant communities. This trait-based approach to ecology provides the most promising method for producing accurate predictions. By focusing on traits rather than species, it is possible to build generalizable rules that work for all species in any environment. With a knowledge of the suite of trait values that are suitable across a gradient of environmental conditions we can begin to predict the presence and abundance of species, from the species pool, that will likely be a part of the realised plant community.

1.3 Environmental filtering on wetland plant communities

A huge array of environmental variables can apply filtering pressure on the species within a wetland plant community. In order to make generalizable predictions across ecosystems and landscapes, it is necessary to identify a common set of environmental gradients that provide predictive ability in any environment (Weiher et al. 1999). Key variables that drive community assembly can change significantly between environments creating a large number of environmental gradients that need to be included to make generalizable predictions. In contrast, ecologists need to optimise their investment of time, energy and money so it is important to identify which environmental variables place the strongest filtering pressure on key functional traits and provide the greatest predictive ability (McGill et al. 2006). Local scale environmental variables, such as soil properties and disturbances, are considered to have stronger filtering effects than climatic variables on the traits of plants in local communities, as climatic variables are typically homogenous within local sites (Matthews et al. 2009). Because environmental filtering generally increases with stress (Butterfield 2015), measuring environmental variables that apply the greatest amount of stress to a plant community are likely to provide the most predictive ability.

A study by Weiher & Keddy (1995) comparing the effects of six environmental variables on wetland community assembly, found that water level placed the strongest filtering on the wetland plant community followed by fertility and leaf litter. Many other environmental variables, such as salinity, drought and disturbance regimes, may also play important roles in the assembly of wetland ecosystems (Figure 1.1). Climatic variables, such as temperature and precipitation, may influence the composition of plant communities between wetland sites (Matthews et al. 2009).

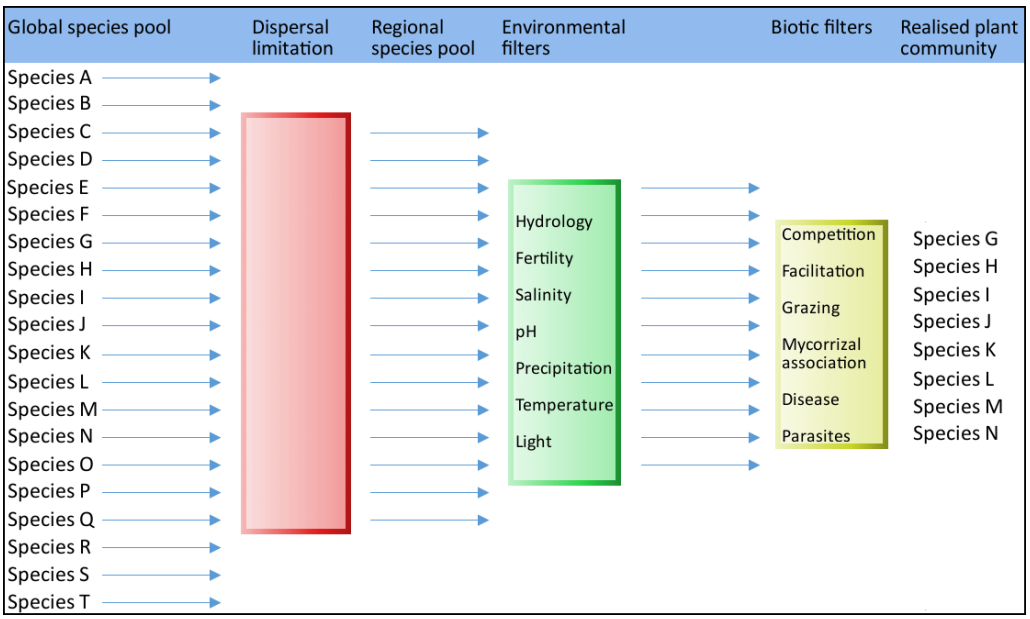

Figure 1.1. Conceptual diagram of dispersal limitation, environmental filters and biotic filters reducing the global species pool down to the pool of species found in a realised wetland plant community.

1.3.1 Flooding and soil moisture

Flooding and soil moisture place the greatest amount of stress on wetland plant communities and are the main drivers of community assembly in wetlands (Weiher and Keddy 1995, Blom and Voesenek 1996). Flooding imposes two main stresses on plants. Saturation of the soil causes a depletion of the oxygen available to roots in the soil leading to soil anoxia and hypoxia (Armstrong et al. 1994, Blom and Voesenek 1996). Submergence causes reductions in the availability of light, CO_2, oxygen and other atmospheric gases to the aboveground organs (Vervuren et al. 2003, Mommer et al. 2005a).

Plants have evolved successful strategies to either avoid or tolerate the stresses associated with flooding and saturated soils (Blom and Voesenek 1996, Garssen et al. 2015, Voesenek and Bailey-Serres 2015). Avoidance can be spatial or temporal. Spatial avoidance is achieved with traits that remove the plant from the associated stresses and temporal avoidance is achieved by only growing during times when flooding is unlikely. Functional trait responses known to help plants avoid the stresses of anoxic soil and submergence include the formation of aerenchyma, growth of adventitious roots, shoot elongation, increased angle and elongation of petioles and modified leaf anatomy (Tsukahara and Kozlowski

1985, Jackson and Armstrong 1999, Polko et al. 2011, Voesenek and Bailey-Serres 2015). Plants that use a stress-tolerant strategy enter a state of dormancy during periods of flooding during which time a plant will reduce its metabolism, grow no new tissue and maintain cellular homeostasis through a number of aerobic and anaerobic respiration pathways (Blom and Voesenek 1996, Gibbs and Greenway 2003, Voesenek and Bailey-Serres 2015).

Of the various traits that are known to be associated with flooding stresses very few have been measured at multiple points along a flooding gradient. Aerenchyma tissue is a network of gas-filled chambers through roots, shoots and leaves that many wetland plants produce as a mechanism to deliver oxygen from shoots to roots during periods of soil anoxia (Blom et al. 1990, Jackson and Armstrong 1999, Mommer et al. 2004) (Figure 1.2). Root aerenchyma, measured as root porosity, has been shown by a number of studies to increase with flooding (Przywara and Stepniewski 1999, Colmer 2003, Grimoldi et al. 2005, Luo and Xie 2009, Ryser et al. 2011, Baastrup-Spohr et al. 2015, Cheng et al. 2015, Zhang et al. 2015). Baastrup-Spohr et al. (2015) found that root porosity underwent the strongest environmental filtering of six functional traits along a flooding gradient. Adventitious roots increase access to aboveground oxygen and have been shown to increase with flooding and replace lateral roots in anoxic conditions (Kramer 1951, Blom et al. 1994, Colmer 2003). Shoot elongation following submergence improves a plants chance of emerging above the water table (Blom et al. 1990, Jackson 1990). The elongation of shoots has been shown to increase survival in adult plants in long floods (Garssen et al. 2015) but reduce survival post-flood and has been hypothesized to only be a beneficial response to long-term flooding (Setter and Laureles 1996). Increasing the angle and length of a leaf's petiole works in conjunction with shoot elongation and helps leaves to emerge above the water table (Groeneveld and Voesenek 2003). Observed changes to the anatomy of leaves in submerged plants include decreases in the thickness of leaves, cell walls and cuticles to reduce the resistance for gas diffusion from water into leaf tissue (Front-Christensen et al. 2003, Mommer et al. 2007); reorientation of chloroplasts towards the epidermis (Mommer et al. 2005b); and the development of a gas film covering newly grown submerged leaves (Pedersen et al. 2009). To the best of my knowledge, of all these

functional traits, only root aerenchyma has been measured at multiple points along a flooding gradient, while the remaining trait relationships have been observed in flooded and non-flooded treatments.

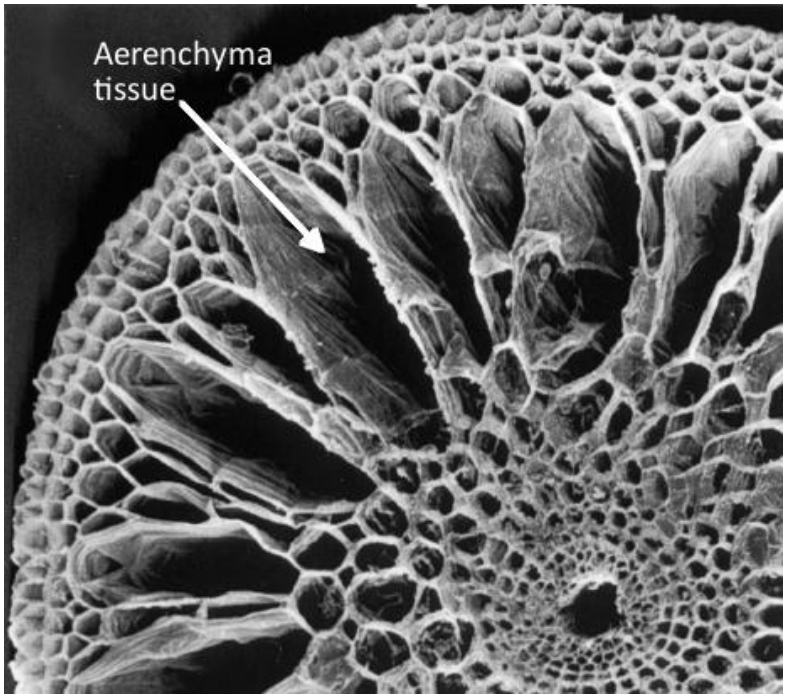

Figure 1.2. SEM micrograph showing the aerenchyma tissue in a transverse section of root tissue from a wetland plant. Image modified from Longstreth and Borkhsenious (2000).

Rather than focusing on the response of traits that are known to provide adaptive value to certain stresses, the selection of functional traits are often impacted by selection criteria. Weiher et al. (1999) and Westoby and Wright (2006) proposed criteria such as the ease of measurability alongside the functional or physiological importance of traits in key phases of a plant's life cycle. To date, much of the trait selection has revolved around the ease of measurability and has aligned with the L-H-S scheme focused on leaf, height and seed traits (Westoby 1998). Leaf traits, height and seed mass are argued to be indicators of a plant's strategy within Grime's CSR triangle of competitors, stress-tolerators and ruderals and to be important for dispersal, germination and establishment (Grime 1974, Westoby 1998, Pierce et al. 2014). Because root traits require more effort to sample, they are often avoided in trait-based studies. Additionally, trait-environment relationships are typically considered with single

traits but multiple trait combinations may provide adaptive value and form a 'plant strategy' for dealing with particular environments (Reich et al. 2003, Laughlin and Messier 2015).

Specific leaf area (SLA), height and seed mass, along with various other easily measurable traits, have been measured along flooding gradients and have shown evidence of environmental filtering. Specific leaf area is considered one of the most responsive traits to filtering (Götzenberger et al. 2012) and has been shown to increase in newly grown leaves following submergence of plants (Mommer et al. 2007), to increase along a flooding gradient (Jung et al. 2010, Violle et al. 2011) and to decrease along a flooding gradient (Baastrup-Spohr et al. 2015, Fu et al. 2015, Howison et al. 2015)(Table 1.1). Height has also had mixed responses to flooding and has been shown to reduce with flooding (Jung et al. 2010, Baastrup-Spohr et al. 2015, Fu et al. 2015), have no relationship with a flooding gradient (Howison et al. 2015) and height above flooded water has been shown to improve chances of survival in riparian vegetation (Garssen et al. 2015). Seed mass was found to be larger in wet, frequently flooded riparian zones (Stromberg and Boudell 2013), smaller in moist areas with no sedimentation (Stromberg et al. 2011), and Violle et al. (2011) found no relationship between seed mass and a flooding gradient. Multiple studies have looked at the response of leaf dry matter content (LDMC) along flooding gradients, one study found LDMC increased with flooding (Baastrup-Spohr et al. 2015) while two others found no relationship (Jung et al. 2010, Fu et al. 2015).

Besides root porosity, the response of root traits to flooding have been rarely studied mostly due to sampling difficulties. The majority of studies that have looked at the relationship between flooding and root traits have looked at intraspecific responses to flooding rather than the community response along a flooding gradient. Specific root length (SRL) and root dry matter content (RDMC) have both been observed to increase with flooding (Rubio et al. 1997, Ryser et al. 2011, Shi et al. 2015) and root tissue density (RTD) has been observed to decrease with flooding (Craine and Lee 2003, Ryser et al. 2011)

The response of various traits have been measured along soil moisture gradients. For aboveground traits, height, wood density and leaf toughness have been

shown to reduce while leaf area and SLA increases (Cingolani et al. 2007, Cornwell and Ackerly 2009). Specific root length has been observed to both increase and decrease with increasing soil moisture availability (Arreola et al. 2008, Thorne and Frank 2009, Olmo et al. 2014).

Table 1.1 Observed response of functional traits to flooding and soil moisture gradients. ↑ = trait has been observed to increase along gradient, ↓ = trait has been observed to decrease along gradient, — = trait has exhibited no relationship to environmental gradient.

Environmental gradient	Functional trait	Response	
Flooding	Root porosity	↑	(Przywara and Stepniewski 1999, Colmer 2003, Grimoldi et al. 2005, Luo and Xie 2009, Baastrup-Spohr et al. 2015, Cheng et al. 2015, Zhang et al. 2015)
	Adventitious roots	↑	(Kramer 1951, Blom et al. 1994, Colmer 2003)
	Root DMC	↑	(Ryser et al. 2011)
	Specific root length	↑	(Rubio et al. 1997, Ryser et al. 2011, Shi et al. 2015)
	Root tissue density	↓	(Ryser et al. 2011)
	Height	↓—	(Jung et al. 2010, Baastrup-Spohr et al. 2015, Fu et al. 2015, Howison et al. 2015)
	Specific leaf area	↑↓	(Jung et al. 2010, Violle et al. 2011, Baastrup-Spohr et al. 2015, Fu et al. 2015, Howison et al. 2015).
	Leaf DMC	↑—	(Jung et al. 2010, Baastrup-Spohr et al. 2015, Fu et al. 2015)
	Seed mass	↑—	(Violle et al. 2011, Stromberg and Boudell 2013)
Soil moisture	Specific root length	↑↓	(Arreola et al. 2008, Thorne and Frank 2009, Olmo et al. 2014)
	Specific leaf area	↑	(Cingolani et al. 2007, Cornwell and Ackerly 2009, Thorne and Frank 2009, Gotsch et al. 2010)
	Leaf area	↑	(Cornwell and Ackerly 2009)
	Leaf toughness	↓	(Cingolani et al. 2007)
	Height	↓	(Cingolani et al. 2007)
	Wood density	↓	(Cornwell and Ackerly 2009)

1.3.2 Soil properties

The influence of the water table on the assembly of wetland plant communities reduces as the water table recedes therefore infrequent flooding may be less of a driver of community assembly than other environmental filters such as soil fertility (Shi et al. 2013). Soil properties include a wide range of measurements such as nutrient concentrations, pH, salinity and soil porosity. Of the variables

they studied, Weiher and Keddy (1995) found soil fertility to be the second strongest environmental filter on the assembly of wetland plant communities.

As the root system is located within soil, soil properties are likely to filter root traits. To date however, very little research has studied the response of root traits along gradients of soil properties in wetland ecosystems but have instead focused on easily measurable aboveground traits. Along a soil fertility gradient in a warm temperate rainforest, root dry matter content, root tissue density, root porosity and root branching intensity were observed to decrease with soil fertility while root nitrogen was found to increase with fertility (Kramer-Walter 2015). Across general fertility gradients, SLA, growth rate, leaf phosphorus content and leaf nitrogen content have been shown to increase with fertility while leaf and stem dry matter content, leaf thickness, stem tissue density and bark thickness decrease with fertility (Ordoñez et al. 2009, Eallonardo et al. 2013, Jager et al. 2015, Kramer-Walter 2015, Laughlin et al. 2015). Height has been shown to increase and decrease with soil fertility (Naaf and Wulf 2012, Eallonardo et al. 2013, Jager et al. 2015) and seed mass has been shown to increase, decrease and show no relationship to fertility gradients (Leishman et al. 2000, Jager et al. 2015).

Soil fertility is the combination of numerous soil variables and the influence of different variables is likely to change between sites. In order to produce comparable results across studies, the filtering effects of these variables are better studied individually. At a global scale, pH and phosphorus availability were found to be the two soil properties that have the strongest relationship with leaf traits (Maire et al. 2015). Leaf nitrogen, leaf phosphorus and maximum photosynthetic rate all increased with pH while SLA decreased (Maire et al. 2015). Increases in leaf phosphorus, leaf nitrogen and stomatal conductance of water have been observed along gradients of soil phosphorus availability (Ordoñez et al. 2009, Maire et al. 2015).

Salinity places severe stress on plants in many wetland ecosystems. Salinity reduces a plant's ability to photosynthesise, take up water and grow due to osmotic and ion toxicity stress and energy expenditure of ion transport (Jefferies 1981, Munns 2002). Salt tolerant plants commonly have high ionic

concentrations in vacuoles of their cells, high leaf nitrogen, succulent tissue, perennial life history, C4 photosynthesis and low SLA (Munns 2002, Eallonardo et al. 2013, Munns and Gilliham 2015).

1.4 Biotic filtering on wetland plant communities

Biotic interactions can filter out certain species from wetland plant communities that are not aptly adapted to stresses such as competition and herbivory. Alternatively, positive biotic interactions can help plants to perform better when they are found near facilitative species (Callaway and Walker 1997). Quantifying the strength of interactions between species, and the influence of biotic interactions on community assembly, remains a significant challenge for community ecologists (Chesson 2008). The influences of competition, facilitation and grazing on functional traits have been studied far more than other biotic interactions such as disease, mycorrhizal associations and parasites.

1.4.1 Competition

Competition is the most commonly studied biotic filter. It is a main factor driving the evolution of traits (Darwin 1859) and a strong factor in wetland ecosystems, especially during dry periods when plants are relieved from the stresses associated with flooding and saturated soils (Merlin et al. 2015). Competition between species is strongest in habitats of low stress and infrequent disturbance where environmental conditions are suitable for many species (Grime 1977). In low stress environments, competition is greatest for the limiting resources (Tilman 1977, 1982). Finding generalizable effects of competition on community assembly is difficult as competition has been argued and experimentally shown to cause both the divergence and convergence of plant traits within plant communities (Weiher and Keddy 1995, Weiher et al. 1998, Grime 2006, Mayfield and Levine 2010, Naaf and Wulf 2012).

Traits associated with competition can be split into effect traits and response traits (Navas and Violle 2009). Effect traits are traits that change the environment, such as increased canopy cover which reduces light availability, and response traits are traits that respond to changes in the availability of

resources, for example, increasing leaf area to increase the interception of light in a shaded area. Response and effect traits vary depending on which resource is being competed for. Two further mechanisms that have been proposed to improve performance in competitive environments are increased maximum growth and increasing trait dissimilarity with competitor species to reduce competition (Kunstler et al. 2015).

Plants compete for light, water, soil nutrients and space (Grime 1974). Acquisition of light is performed by aboveground organs therefore competition for light is likely to have a greater influence on aboveground traits. Likewise, the acquisition of water and nutrients is performed by the root system and root traits are likely to be more responsive to competition for these resources. When competing for light, Gaudet and Keddy (1988) found above and belowground biomass, followed by height and canopy cover, explained the most variation in competitive ability of the 12 traits they studied. Greater leaf area and light transmission through leaves provided a competitive advantage for light in grass species studied by Teughels et al. (1995) and increased SLA has been shown to increase competitive ability as light decreases (Violle et al. 2009b). Low wood density and high SLA have been shown to provide a competitive advantage by increasing the growth rate of tree species (Kunstler et al. 2015). High wood density is correlated with shade-tolerance and helps plants to survive in environments of low light (Kunstler et al. 2015).

Traits that have been shown to be associated with greater competitive ability when competing for nutrients and water include height, seed nitrogen concentration, pre-dawn leaf water potential and increased rooting depth. Naaf and Wulf (2012) found a convergence of taller species in productive, fertile areas where competition is assumed to be high. Seed nitrogen concentration reduces when plants are exposed to competition but only when nitrogen is not highly available (Violle et al. 2009a). Violle et al. (2009b) found that rooting depth was the strongest effect trait on water availability with deeper rooting species reducing the availability of water. Pre-dawn leaf water potential was the strongest response trait to reductions in water availability and decreased as water availability decreased. Seed mass was not shown to decrease with

competition for nitrogen (Violle et al. 2009a) but seedlings from larger seeds have a better chance of surviving through early stages of development when in competition with seedlings from small seeds (Leishman 2001).

In certain conditions the interactions between plants can become positive and help to improve the growth and survival of other plants (McAuliffe 1984, Callaway and Walker 1997). Common examples of this facilitation include legumes providing their neighbours with nitrates and plants providing shade for neighbours in hot, arid environments. Santoro et al. (2012) found facilitation was a stronger driver of community assembly than competition in harsh environments.

1.4.2 Grazing

Grazing is a common disturbance in most ecosystems, including wetlands, and can significantly alter the composition and dynamics of a plant community (Kempel et al. 2015). The response of plant communities to grazing may be complex and depend on grazing history, environmental conditions and interactions between plants (Coley et al. 1985, Milchunas et al. 1988, Graff et al. 2007, Howison et al. 2015). Plants in areas that have had a long history of grazing have had the chance to evolve strategies to cope with grazing pressure whereas the plants in areas with little history of grazing are likely to be poorly adapted (Milchunas et al. 1988, Rosenthal and Kotanen 1994). In productive areas, grazing has been shown to increase diversity, whereas reductions in diversity have been observed in unproductive sites (Milchunas et al. 1988, Rosenthal and Kotanen 1994, Proulx and Mazumder 1998). Grazing has also been shown to increase facilitative interactions between plants where palatable plants can increase in biomass when found in close proximity to unpalatable plants (Briske 1996, Graff et al. 2007, Howison et al. 2015).

Plants have evolved two opposing strategies to deal with grazing stress (Belsky et al. 1993). Grazing drives shifts in the composition of plant communities towards species with strategies to avoid or tolerate grazing (Cingolani et al. 2005). Avoidance can be achieved with either defensive strategies or escape strategies (Belsky et al. 1993). Defensive strategies include the growth of chemically or physically unpalatable tissue (Coughenour 1985). Lignin, toxic compounds and

physical armour, such as thorns and spines, are known to reduce palatability and act as defensive mechanisms against herbivory (Coley et al. 1985, van Soest 1994). Escape from herbivory is achieved either spatially or temporally (Briske 1996). Spatial escape strategies include short, prostrate or decumbent growth forms and growing in close proximity to unpalatable species. Temporal escape strategies include inducible defences, such as the production of toxic compounds, that are produced at particular times due to increases in grazing pressure (Briske 1996). Strategies to improve grazing tolerance include fast growth rates, flexible photosynthetic rates, increased nutrient uptake, nutrient storage and altered nutrient allocation following grazing (Coughenour 1985, Belsky et al. 1993, Rosenthal and Kotanen 1994). This includes functional traits such as high SLA, low LDMC, low leaf toughness and high leaf nitrogen concentration (Cingolani et al. 2005, Strahan et al. 2015). Increased protection, number and basal positioning of meristems and deciduous shoots are also considered to be advantageous functional traits for grazing tolerance (Coughenour 1985, Grubb 1992).

In productive areas, a grazing-tolerant strategy is likely to prevail as traits associated with tolerating grazing, such as fast growth rate and increased nutrient uptake, also provide competitive advantages in those areas (Briske 1996) (Table 1.2). In unproductive areas, defensive strategies are likely to provide the most benefit against grazing because they are typically more conservative in their nutrient use and competition is likely to be reduced (Herms and Mattson 1992, Rosenthal and Kotanen 1994, Briske 1996, Díaz et al. 2007).

Table 1.2. Predominant relationships between environmental stress, competition, grazing and trait filtering.

Environmental stress	Competition	Grazing survival strategy	Dominant trait filter
High	Low	Avoid	Environment
Low	High	Tolerate	Competition

The response of a large number of traits to grazing have been studied and the majority of work has focused on height, seed mass and leaf traits. The majority of studies looking at the response of height to grazing have found height is reduced

with grazing (Díaz et al. 2001, Cingolani et al. 2005, Cingolani et al. 2007, Díaz et al. 2007). Seed mass varies over 10 orders of magnitude and optimal foraging theory predicts that granivores will select seeds that provide the most energy for the least amount of foraging effort (Radtke 2011). In forests, where seeds are relatively large compared with herbaceous ecosystems, smaller seeds are most commonly preferred by granivores (Radtke 2011). In herbaceous plant communities, granivores select large seeds (Hulme 1998, Radtke 2011, Wu et al. 2015). However, the preference of seed size is relatively similar between ecosystems with seed size preference typically ranging between 0.001-1 g. There is also evidence that seed longevity helps to pass through grazing filters because persistent seed banks are more commonly buried and buried seeds are less likely to be eaten (Hulme 1998).

The relationship between grazing and a number of leaf traits have been studied. SLA has been shown to increase under grazing pressure (Díaz et al. 2001, Cingolani et al. 2007, Zhao et al. 2009) but was a poor predictor of grazing response compared to height and leaf mass (Díaz et al. 2001). Leaf area, leaf toughness, leaf thickness and maximum photosynthetic rate have all been observed to decrease with grazing (Cingolani et al. 2007, Zhao et al. 2009). Bär Lamas et al. (2013) found no relationship between leaf nitrogen concentration and grazing. Although it has not been directly measured, it is believed to be necessary for long living leaves to be grazing resistant and leaf lifespan has been shown to be correlated with low palatability (Wright et al. 2004).

The response of various other traits to grazing have also been observed. Internode length, water use efficiency, aboveground biomass and belowground biomass all decreased with grazing (Zhao et al. 2009) while leaf : shoot mass ratio increased with grazing (May et al. 2009). Grazing has also been shown to select annuals over perennials, prostrate growth over erect growth and stoloniferous or rosette growth forms over tussock growth form (Díaz et al. 2007, May et al. 2009).

1.5 Trait-based community assembly models

Two mathematical models have recently been developed to predict the composition of plant communities using trait-environment relationships. The CATS model (Shipley et al. 2006) and Traitspace model (Laughlin et al. 2012) focus on environmental filtering and trait convergence and both assume the environment is filtering traits. The effects of competition and dispersal limitation are ignored when producing predictions of community assemblage, although Traitspace has the potential to identify the effects of competition by including the full variation of inter- and intraspecific trait variability within the plant community. Because the filtering effects of dispersal limitation are ignored, both models work with the regional species pool but have the ability to include additional species in their predictions and identify the fitness of exotic species to the local environment. Trait-based models have also been produced to predict global vegetation distribution to replace models that have traditionally focused on plant functional groups (van Bodegom et al. 2014, Lu et al. 2015, Sakschewski et al. 2015).

The data requirements differ between the two models. The CATS model uses mean species trait values and predicted community-weighted trait means based on environmental conditions. The community-weighted trait means are produced using observed species abundances along environmental gradients. Trait databases can provide the species trait data but data is ideally sourced from databases with known environmental conditions to account for environmental trait plasticity (Laughlin and Laughlin 2013). The Traitspace model uses individual level trait values to incorporate intraspecific trait variation when predicting species relative abundances. The data requirements for Traitspace are harder to meet than the CATS model as it requires a dataset of trait measurements from individual plants from known environmental conditions rather than mean species trait values.

Various studies have tested the effectiveness of these two models. Shipley et al. (2006) proposed the CATS model and tested the model using data on eight functional traits for 30 species at 12 sites along a 42 year chronosequence. Using observed community-aggregated trait values from each site the study predicted

94% of the observed relative abundances of species along the chronosequence. Shipley et al. (2011) produced the first comprehensive test of the CATS model by testing its ability to predict the relative abundance of 79 species across 96 quadrats using 12 environmental variables. The 12 environmental variables explained 53% of the variation in observed relative abundances and the model predicted 72% of that variation. The dominant species was correctly predicted in over half of the quadrats and 83% of rare species were correctly predicted to have a relative abundance of less than 0.05.

Laughlin et al. (2012) produced the first test of the Traitspace model and compared the predictions to those of the CATS model. The study used three functional traits (SLA, wood density and bark thickness) to predict the relative abundances of nine tree species across an elevation and temperature gradient. Both models produced predictions that were positively correlated with observed relative abundances (Traitspace r = 0.66, CATS r = 0.74) and Traitspace was able to predict the dominant species in over half of the 196 plots.

Two recent studies have tested the CATS model across flooding and water depth gradients. Baastrup-Spohr et al. (2015) performed the first test of the CATS model in a wetland plant community. They measured six traits including leaf area, LDMC, SLA, root porosity, height and resistance to water loss on drying and identified the response of each trait along a flooding gradient that was produced by a principle component analysis (PCA) from 14 environmental variables. Root porosity increased with flooding and was the strongest filtered trait along the flooding gradient. The flooding gradient explained 59% of the variation in root porosity. SLA (r^2 = 0.44) and resistance to water loss on drying (r^2 = 0.41) were the next most filtered traits along the flooding gradient and both decreased with flooding. Using the single PCA gradient that accounted for 59% of the environmental variation, the CATS model was able to explain 66% of the variation in observed relative abundances for 32 species and, using a fitted model, predicted 37% of the variation in relative abundances. Fu et al. (2015) tested the CATS model on a macrophyte community of a freshwater lake across a water depth gradient from 0.5 m to 3.0 m at depth intervals of 0.5 m. The study measured 16 functional traits that included flowering, leaf, shoot and root traits

of 17 plant species. The CATS model predicted 99.8% of the variation in relative abundance for the 17 species using the observed community-weighted mean trait values. When applying a fitted model of community-weighted means, the CATS model predicted 32% of the variation in relative abundances using all traits and 22.1% of the variation using only leaf carbon content and leaf nitrogen content. Further studies testing the predictions of these models using additional traits will help to advance our current ability to predict the assembly of wetland plant communities.

1.6 Conclusions

We are still a long way from making confident, accurate and generalizable predictions of the composition of any wetland plant community anywhere in the world. However, advances in predictive ecology in the last decade have led to significant progress towards this goal. This has been enabled by new modelling techniques and advances in our understanding of the processes that determine the assembly of wetland plant communities. Progress will continue if we focus on elucidating the response of key functional traits to important environmental variables; the filtering effects of different biotic interactions on key functional traits; and the particular conditions when filtering is driven by abiotic or biotic interactions.

It is commonly accepted that the hydrological regime is the main driver of community assembly in wetland plant communities and the physiological adaptations to deal with the stresses associated with flooding and anoxic soils are well understood. However, little research has incorporated this physiological understanding into the design of studies interested in the processes of environmental filtering along flooding and soil moisture gradients. Soil anoxia caused by flooding applies direct stress to the root system rather than the aboveground system and root traits likely undergo more filtering and provide more predictive power than aboveground traits in flooded environments. To date, roots traits have been heavily neglected due to sampling difficulty and identifying the response of root traits along flooding gradients is a key task on the path towards accurately predicting the assembly of wetland plant

communities. The research focus for this thesis was to identify the response of functional traits to flooding and grazing in a wetland ecosystem, paying particular attention to root traits such as root porosity, root tissue density and SRL.

Once a set of functional traits and environmental gradients has been identified that show strong, consistent patterns of trait filtering then models such as the CATS model and Traitspace model may begin to provide a mechanism for producing confident and accurate predictions of community composition across a range of environments regardless of location. This thesis also provides the first test of the Traitspace model in a wetland ecosystem and helps to identify which traits are important predictors of wetland community assembly. Advances in modelling may be seen with developments that include the filtering effects of biotic interactions and dispersal limitation, including combinations of multiple traits into modelling algorithms or the integration of local community assembly models with global vegetation models to improve the generality of local models and specificity of global models.

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2 Root functional traits respond more than aboveground traits along a hydrological gradient in an ephemeral wetland

2.1 Abstract

1. Trait-based ecology provides a promising approach for predicting the composition of plant communities. Recently developed mathematical models use variation in plant functional traits to predict species abundances along environmental gradients. However, it is still unclear how traits respond along environmental gradients and whether environmental filtering of functional traits can be used to predict the assembly of plant communities. Here I determine the response of plant functional traits along a hydrological gradient, inside and outside of grazing exclosures, and test the ability of a trait-based community assembly model.
2. Plant community composition was surveyed in grazed and ungrazed transects along a hydrological gradient that was split into a flooding gradient and an elevation gradient for areas above the flood line. Nine root, leaf and shoot functional traits were measured on 885 plant samples collected from the survey. I investigated the individual-level and community-weighted response of the nine traits along the hydrological gradient in both grazing treatments. Predictions of relative abundance for 23 species across the gradient were generated using the Traitspace model.
3. Of the nine traits examined, the five root traits exhibited the strongest relationships to the flooding gradient. Root aerenchyma increased with frequency of flooding while root dry matter content, specific root length, root tissue density and root branching intensity decreased with frequency of flooding. Specific leaf area and root tissue density were the two traits with the strongest relationships with the elevation gradient and decreased and increased with elevation, respectively. Grazing reduced the strength of the trait-flooding relationships for most traits and reduced the community-weighted trait values for all traits except SLA and SRL. Grazing had little influence on the trait relationships above the flood line.
4. Generalized Linear Models of individual-level traits identified specific leaf area and height as the two traits that varied most predictably along the hydrological gradient. The Traitspace model produced accurate predictions of species relative abundances and distributions for a number of key species within the plant community.

Synthesis. The strong filtering of root traits along the hydrological gradient provided important information about the functional adaptation of plant to variations in soil hydrology. Functional trait-based research should focus more on measuring root traits as they provide unique information about a plant's fitness in their environment. It is possible to predict the relative abundance of species within a wetland plant community using trait-based predictive models but the inclusion of biotic interactions in models will improve predictions of plant community composition at small spatial scales in wetland ecosystems.

2.2 Introduction

Accurately predicting the composition of plant communities is a key goal in ecology and has never been more important as we head into an era of environmental change (Lavorel and Garnier 2002, McGill et al. 2006). With an ability to predict the composition of plant communities in varied environments, we can predict how communities will shift with changing climates (Araujo and Rahbek 2006), build resilient communities through ecological restoration (Laughlin 2014a) and guide political action on environmental issues (Keddy 1992). Studying functional traits provides the best opportunity to understand how the environment, competition and other ecological interactions drive the composition of plant communities (Westoby and Wright 2006, HilleRisLambers et al. 2012). Observed shifts in the trait values of plants in different environments are the direct outcome of selective pressures from the environment and ecological interactions (van der Valk 1981, Keddy 1992). As environmental conditions and ecological interactions change, the suite of traits that provide optimum fitness change accordingly. With an understanding of how plant functional traits vary across a range of environmental conditions and a knowledge of the range of trait values for any given species, it is possible to predict the outcome of plant community assembly (Shipley et al. 2006). Our ability to predict the assembly of plant communities is limited by our understanding of how plant functional traits respond to environmental changes and which traits provide the most predictive power across a range of environmental conditions.

The hydrological regime is considered the main driving factor in the assembly of wetland plant communities (Weiher and Keddy 1995). Predicted changes in precipitation and evaporation dynamics around the world will alter the hydrological regime of wetland ecosystems (IPCC 2014). Increases in precipitation or flooding will alter the composition of plant communities to species that are better adapted to wet, flooded environments. Decreases in precipitation or flooding will select for species with functional traits that perform well in dry environments. Aside from the environmental conditions, the assembly of wetland plant communities are also influenced by biotic interactions such as

competition and grazing that select for competitive and grazing resistant species (Kempel et al. 2015, Merlin et al. 2015).

The flooding of soil imposes stress on plants by limiting oxygen availability in the soil and by reducing the availability of light, CO_2 and other atmospheric gases to submerged plants (Armstrong et al. 1994, Blom and Voesenek 1996, Vervuren et al. 2003, Mommer et al. 2005). Anoxic soil is the greatest stress that wetland plants are subjected to and plants have evolved adaptations to combat the lack of available oxygen in saturated soil (Blom and Voesenek 1996). Many wetland plants have a network of gas-filled chambers, called aerenchyma, through their roots and into their stems which allows the transportation of oxygen from shoots into roots (Jackson and Armstrong 1999, Colmer 2003b). Root aerenchyma has been shown by multiple studies to increase with flooding (Przywara and Stepniewski 1999, Colmer 2003a, Grimoldi et al. 2005, Luo and Xie 2009, Baastrup-Spohr et al. 2015, Cheng et al. 2015, Zhang et al. 2015). Other than root aerenchyma, root functional traits have rarely been studied along hydrological gradients. A study by Craine and Lee (2003) found root tissue density (RTD) decreased in wetter environments and hypothesised that the relationship was due to increased root aerenchyma causing a reduction in the root mass to volume ratio. Ryser et al. (2011) found that within a single species root dry matter content (RDMC) increased in a flooded environment, RTD decreased with flooding and SRL increased with flooding in mature plants but decreased in seedlings. Several studies have examined the plasticity of SRL with soil moisture availability and flooding. SRL has been observed to increase with flooding (Rubio et al. 1997, Shi et al. 2015), increase with soil moisture (Thorne and Frank 2009), and increase and decrease with drought (Olmo et al. 2014). To the best of my knowledge, this is the first study that has examined relationships of community-weighted mean SRL, RTD, RDMC and root branching intensity (RBI) along a hydrological gradient.

Varied responses have been observed in leaf, shoot and seed traits along flooding gradients. Specific leaf area (SLA) has been observed to both increase with flooding (Mommer et al. 2007, Jung et al. 2010, Violle et al. 2011) and decrease with flooding (Baastrup-Spohr et al. 2015, Fu et al. 2015, Howison et al.

2015). Leaf dry matter content (LDMC) has shown both no response to flooding (Jung et al. 2010, Fu et al. 2015) and to increase with flooding (Baastrup-Spohr et al. 2015). The majority of studies examining the response of height along flooding gradients have found height to decrease with flooding (Cingolani et al. 2007, Jung et al. 2010, Baastrup-Spohr et al. 2015, Fu et al. 2015) but one study also found no response (Howison et al. 2015).

In dry environments, a lack of water imposes severe physiological stress on plants. Dry soil can make a plant vulnerable to cavitation of xylem tissue which prevents photosynthesis due to halted water transport through the plant (Pockman and Sperry 2000). Multiple root traits are known to be responsive to variation in soil moisture (Eviner and Chapin 2003). Rooting depth is a trait commonly associated with improving performance in dry soil but studies have shown that plants with both shallow and deep roots can perform well if soil moisture availability reduces (Reader et al. 1993, Groom 2004, Yang et al. 2011). Plants with deeper roots are able to access lower water supplies but deeper roots may require a greater investment of energy to maintain and may be at a competitive disadvantage against shallow roots (Ackerly 2004, Yang et al. 2011). In order to tolerate dry environments, plants typically have traits associated with a conservative growth strategy such as slow growth rates, low SLA, smaller leaf area, high dry matter content and long leaf lifespan (Westoby et al. 2002, Reich et al. 2003). Shorter height may help to tolerate low soil moisture availability because less energy is required to transport water through the plant (Westoby et al. 2002).

Grazing is a common disturbance in most ecosystems and has been shown to drive shifts in functional traits and the composition of plant communities (Cingolani et al. 2005, Kempel et al. 2015, Strahan et al. 2015). The response of plant communities to grazing is dependent on grazing history, productivity and the interactions between plants (Coley et al. 1985, Milchunas et al. 1988, Graff et al. 2007, Howison et al. 2015). Plants in areas with a long history of grazing are likely to be well-adapted to the associated pressures (Milchunas et al. 1988, Rosenthal and Kotanen 1994). The strategy to deal with grazing is likely to switch between tolerance strategies in productive sites to avoidance strategies in

unproductive sites as the traits associated with each strategy are better suited to the different environments (Herms and Mattson 1992, Rosenthal and Kotanen 1994, Briske 1996, Díaz et al. 2007). Avoidance traits include short growth forms, physically or chemically unpalatable tissue, and thorns and spikes (Coley et al. 1985, van Soest 1994, Briske 1996). Traits associated with grazing tolerance strategies include high SLA, high leaf nitrogen concentration, low LDMC, low leaf toughness and basal meristems (Coughenour 1985, Grubb 1992, Cingolani et al. 2005, Strahan et al. 2015). These traits help plants tolerate grazing by improving growth rates, photosynthetic rates and nutrient uptake to allow a quick recovery following grazing (Coughenour 1985, Grubb 1992, Belsky et al. 1993, Rosenthal and Kotanen 1994, Cingolani et al. 2005, Strahan et al. 2015).

This study asked two questions to improve our understanding of trait-based environmental filtering and the assembly of plant communities. 1) How do community-weighted functional traits vary along a hydrological gradient in the presence and absence of grazing? 2) Can a trait-based statistical model of environmental filtering be used to predict the composition of the plant community within a wetland ecosystem? To answer the first question I conducted a study along a hydrological gradient in an ephemeral wetland, above and below the flood line, inside and outside gazing of exclosures. I measured nine functional traits and analysed how they varied along the gradient. To answer the second question, I used a trait-based, community assembly model known as Traitspace (Laughlin et al. 2012) to determine our ability to predict the assembly of an ephemeral wetland plant community through the process of environmental filtering.

2.3 Methods

2.3.1 Study site

The study was conducted in an ephemeral wetland, known as the Wairepo Kettlehole, found in North Otago, New Zealand (latitude 44.374143°S, longitude 169.890052°E) (Figure 2.1). The Kettlehole was formed by glacial scouring and is nested in the middle of a valley of gently rolling hills that is surrounded by

mountain ranges. The Kettlehole is fed by rainwater and is frequently inundated with water, especially over winter when potential evapotranspiration is low, and can also fill following a heavy rain event in summer (Figure 2.2). The site is regularly invaded by rabbits and hares and over 1600 have been caught within the Kettlehole since 2009 (unpublished data from the Department of Conservation). The Kettlehole is also an important feeding ground for a range of bird species. Within a distance of less than 50 metres from the edge of the Kettlehole, there is a strong hydrological gradient from above the maximum flood line (hereafter, the 'flood line') down to land submerged for an average of more than 250 days per year. Because the study site was small (less than 300 metres between the furthest points), the influence of dispersal limitation was assumed to be negligible, and community assembly was assumed to be driven by environmental and biotic filters.

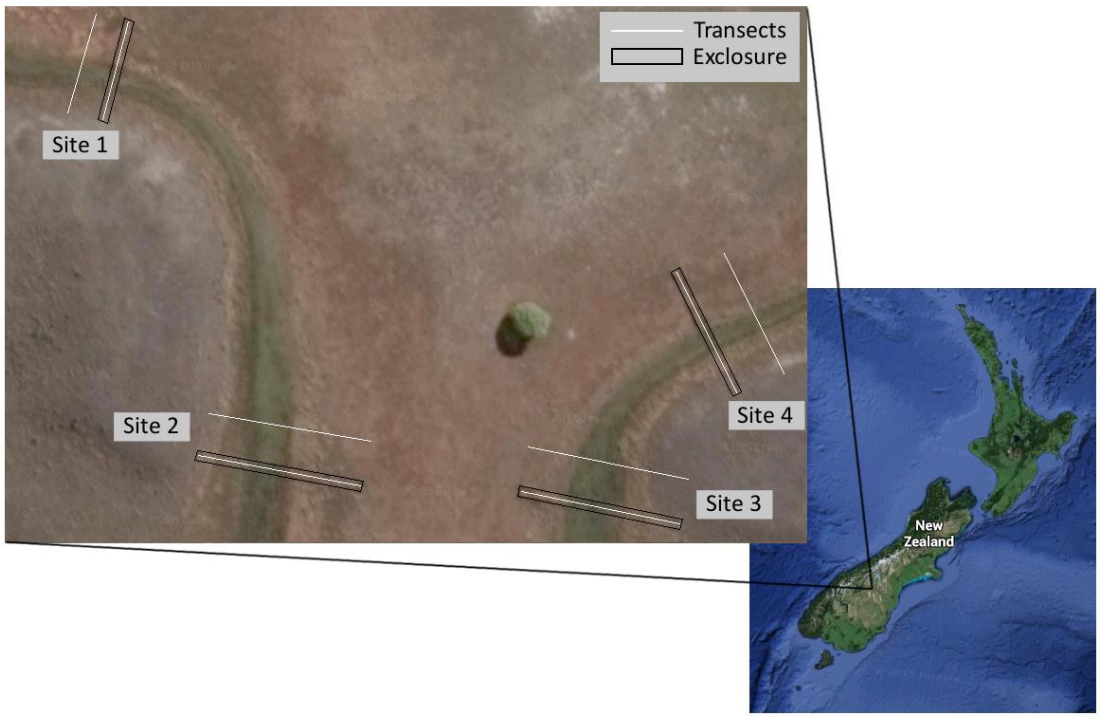

Figure 2.1. Image of the study site and its location within New Zealand. Four replicated sites were surveyed within the Kettlehole, each with a grazed transect and ungrazed transect. Fenced exclosures prevented grazing along the ungrazed transects.

The Kettehole consists of four replicated sites, where each site contains two parallel transects that run from low-down within the wetland to above the flood

line. One transect in each site is located within a permanent exclosure built in 2008 to prevent grazing by birds and invasive mammals (Figure 2.1). The second transect in each site is located 12 m from the grazing exclosure. Each transect line is permanently marked by wooden pegs located every 10 m along the transect lines.

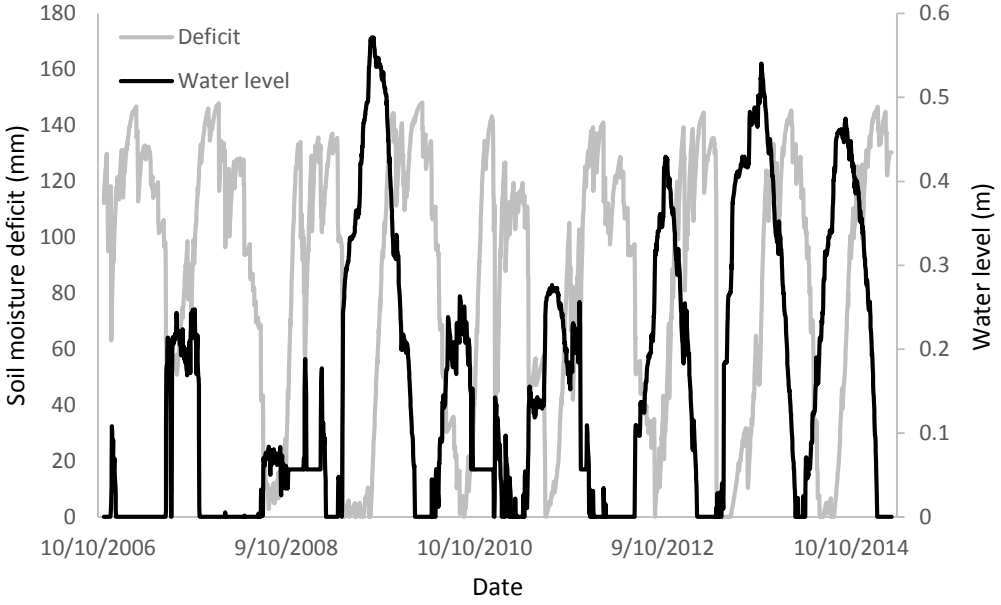

Figure 2.2. Mean daily water level (cm) measured from the lowest point in the Wairepo Kettlehole and soil moisture deficit measured at a climate station 17 km from the Wairepo Kettlehole from 2006 to 2015. Soil moisture deficit is driven by differences in incoming

Flooding data was collected using a capacitance probe that continuously recorded the water height above the lowest elevation in the Kettlehole between November 1, 2006 and March 17, 2015 (Figure 2.2). Known elevations at every 50 cm along each transect and water level data were used to calculate the mean annual number of days submerged at every 2 m along the transects. The mean annual number of days submerged was used as our flooding gradient and ranged between 0-253.25 days. For all the quadrats that were found above the flood line, I used the elevation above the flood line as the environmental gradient.

2.3.2 Community composition

To answer the first question, I measured and analysed how nine functional traits varied along the hydrological gradient. The responses of traits were observed inside and outside of the grazing exclosures to examine how grazing influenced the response of traits along the hydrological gradient. I measured community composition within 184 quadrats along the eight transects. Beginning at 0 m and repeating at 2 m intervals along each transect line, a 25x25 cm (0.0625 m^2) quadrat was placed directly alongside the transect line. All individuals within the quadrat were identified to species. Canopy cover was estimated for each species using the following cover estimates: 0.5%, 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 30%, 40%, …, 100%. Estimations were performed by the same individual through the entire process to reduce variability. Maximum vegetative height (cm) was measured on the highest photosynthetic part of the tallest individual for each species in each quadrat.

2.3.3 Functional traits

To quantify how traits varied along the two gradients, I measured nine plant functional traits on each species that occurred in each quadrat. To avoid disturbing the vegetation, I collected one individual of each species perpendicular to the transect, mostly within 1 m of the quadrat. The vegetative height (cm) of the collected sample was measured before the whole plant was carefully dug out with a knife or trowel. Each individual sample was stored in a sealed ziplock bag with a moist paper towel and was stored in a chilled container. Samples were refrigerated in their ziplock bags and separated into above and belowground material within 28 hours.

After weighing the aboveground fresh mass (mg), leaves were removed for additional sampling and the remaining aboveground tissue was dried in the ovens at 60 °C until a consistent dry mass was obtained. Aboveground dry matter content (AGDMC) was calculated as the dry mass divided by the fresh mass (mg mg^{-1}) (Table 2.1).

Depending on the size of the leaves for each species, three, five, ten or 30 leaves were removed from each individual specimen and were weighed on analytical

balances to obtain fresh mass (mg). Leaf area measurements were obtained from photographs of the leaves using ImageJ software. A known distance was entered into the program using the scale bar in the image and the area of the leaves was selected by shade and colour saturation. Dry mass of the leaf samples were obtained after drying the samples in an oven at 60 °C. The dry weights of the leaf samples were added to the aboveground tissue sample to give a total dry mass of the aboveground material. The dry weights and areas of leaf samples were used to calculate specific leaf area (SLA), the one-sided projected leaf area divided by dry mass ($mm^2\ mg^{-1}$). Leaf dry matter content (LDMC) was calculated as dry mass divided by the fresh mass ($mg\ mg^{-1}$).

Table 2.1. The traits measured on samples collected from the Wairepo Kettlehole. The abbreviations, units and range of values observed in this study for each trait are provided.

Plant section	Traits	Abbreviation	Units	Range
Roots	Specific root length	SRL	$m\ g^{-1}$	1.1 - 559.5
	Root dry matter content	RDMC	$mg\ mg^{-1}$	0.03 - 0.64
	Root aerenchyma index	-	%	1.5 - 68.9
	Root branching intensity	RBI	$tips\ cm^{-1}$	0.04 – 16.32
	Root tissue density	RTD	$mg\ mm^{-3}$	0.02 – 0.84
Shoots	Aboveground dry matter content	AGDMC	$mg\ mg^{-1}$	0.06 – 0.92
	Height	-	cm	0.1 – 83.0
Leaves	Specific leaf area	SLA	$mm^2\ mg$	0.94 – 53.47
	Leaf dry matter content	LDMC	$mg\ mg^{-1}$	0.10 – 0.88

Root samples were wrapped in a moist paper towel and stored inside ziplock bags in a refrigerator. The root samples were transported to the University of Waikato where they were cleaned, tamped dry and weighed to obtain a fresh mass (mg) and then stored in a refrigerator. Once all the samples had been weighed, a sub-section of fine roots from each sample was weighed and scanned on an Epson Expression 10000 XL scanner. The scanned images were analysed using WinRhizo Pro to quantify root diameter, length, volume and number of tips for the scanned sample. This information was used to calculate specific root length (SRL), root tissue density (RTD) and root branching intensity (RBI). SRL was calculated as the fresh length of the root sample divided by its dry mass ($m\ g^{-1}$); RTD was measured as the dry mass divided by the fresh root volume ($mg\ mm^{-3}$);

and RBI was measured as the number of root tips per centimetre of root length (tips cm^{-1}).

The 'microbalance method' was used to measure an index of root aerenchyma (Visser and Bögemann 2003). Ten short sections of fresh unsuberized roots, approximately 10 mm in length, were cut at least 10 mm back from the root tip. Whenever possible, sections were taken from first order terminal roots, otherwise second order roots with no secondary thickening were used. Roots were cleaned using fine brushes and surface moisture was removed using tissue paper prior to obtaining the initial mass (w_1). The root sections were transferred into a 20 ml glass vial that was then filled with water. The vial was placed into a vacuum dessicator three times for five minutes each, with pressure rapidly returned to atmospheric at the end of each five minute period. The root sections were then removed from the vial, placed on dry tissue paper, and briefly rolled to remove surface moisture prior to obtaining the final mass (w_2). Root aerenchyma was calculated as $100 \times (w_2 - w_1)/w_2$.

2.3.4 Statistical analyses

To identify the correlation structure of functional traits within the plant community along the hydrological gradient and identify the location of species within trait space, I performed a principal components analysis (PCA). Axes within the PCA with eigenvalues greater than 1 were considered significant. A biplot was produced plotting individual plants of the 23 most common species (Table 2.2) within trait space along the first two axes of the PCA. The biplot was overlaid with the correlation structure of the nine functional traits along the first two principle component axes and species were identified within the biplot by colours indicative of their location along the hydrological gradient. Species ellipses were produced to indicate 95% of the trait space occupied by each species.

To identify the community-weighted response of each functional trait along the hydrological gradient, I plotted the community-weighted mean (CWM) trait values for each quadrat against days submerged per year and elevation above the flood line. To calculate the CWM trait values of a quadrat, the mean trait

value of each species was multiplied by its relative abundance within the quadrat and the values of all species were summed together. The response of each trait was modelled above and below the flood line using Generalized Additive Models (GAMs) (Hastie and Tibshirani 1986). The GAMs were produced with maximum k values of five and smooth splines were used to produce a model fit for the response of each trait above and below the flood line in both the grazed and ungrazed treatments. R^2 values for the GAMs of each traits were used to determine the strength of the relationships the trait and the flooding and elevation gradients. An analysis of covariance (ANCOVAs) was performed within the GAM framework to determine whether the categorical factor of grazing influenced the relationship between the community-weighted trait values and the flooding and elevation gradients. Individual-level trait responses along the flooding and elevation gradients were identified using Generalized Linear Models (GLMs) (Nelder and Baker 1972) in both the grazed and ungrazed treatment for all nine functional traits. R^2 values were used to determine the strength of the linear models for each trait and the two gradients, both inside and outside the grazing exclosures. Plots were produced for both community-weighted traits and individual traits, separating data from the two grazing treatments.

To answer my second question of whether environmental filtering could be used to predict the assembly of plant communities, I used a community assembly model called Traitspace (Laughlin et al. 2012) to predict the abundances of the 23 most common species (Table 2.2) above and below the flood line. The Traitspace model includes six steps: step 1) quantifies the environmental filtering of each trait using GLMs weighted by the average biomass of each species (Appendix Table 4.2); step 2) computes the probability of trait values for each species using Gaussian mixture models with the 'mclust' library of R; step 3) simulates the assembly of the plant community by performing 1000 stochastic selections of trait values along the flooding and elevation gradients; step 4) computes the likelihood of each species to have the trait values produced in step 3; step 5) uses *Bayes Theorem* to compute the posterior distribution; and step 6) uses *Monte Carlo integration* to acquire the desired posterior distribution. Hellinger distances were used to measure the dissimilarity between the observed and predicted abundances for each species in each quadrat. *P*-values were

derived by comparing the number of times out of 999 permutations that randomly permuted species abundances had lower Hellinger distances than the modelled abundances.

Table 2.2. The 23 most abundant species observed in the Wairepo Kettlehole and used in the Traitspace model. The number of samples collected and the status of each species within the New Zealand flora are provided.

Species	6 letter codes	No. of samples	Status
Agrostis capillaris	AGRcap	78	Exotic
Alopecurus geniculatus	ALOgen	21	Exotic
Amphibromus fluitans	AMPflu	43	Indigenous
Anthoxanthum odoratum	ANTodo	41	Exotic
Carex gaudichaudiana	CARgau	48	Indigenous
Carex ovalis	CARova	10	Unknown
Eleocharis acuta	ELAacu	87	Indigenous
Eleocharis pusilla	ELApus	34	Endemic
Epilobium angustum	EPIang	30	Endemic
Festuca novae-zelandiae	FESnov	21	Endemic
Galium perpusillum	GALper	31	Endemic
Glossostigma elatinoides	GLOela	10	Indigenous
Juncus articulatus	JUNart	30	Exotic
Lachnagrostis lyallii	LAClya	31	Endemic
Lachnagrostis striata	LACstr	30	Endemic
Leucopogon fraseri	LEUfra	32	Indigenous
Lilaeopsis ruthiana	LILrut	31	Indigenous
Lobelia perpusilla	LOBper	33	Endemic
Myriophyllum propinquum	MYRpro	22	Indigenous
Parahebe canescens	PARcan	30	Endemic
Pilosella officinarum	PILoff	40	Exotic
Pilosella piloselloides	PILpil	30	Exotic
Potamogeton cheesemanii	POTche	11	Indigenous

The accuracy of the model with varying numbers of traits was tested by running the model with one trait through to nine traits. The order in which traits were added to the model was determined by the R^2 value of each trait produced by ANCOVAs within the GLM framework of the Traitspace model. Because the model was run with two environmental gradients, the R^2 values were weighted across the two gradients by the proportion of individuals found along each gradient. Weighted R^2 of each trait was calculated by multiplying the R^2 of the trait-flooding relationship by the proportion of individuals found along the flooding gradient, multiplying the R^2 of trait-elevation relationship by the

proportion of individuals found along the elevation gradient and summing the two values together. Traits were added to the Traitspace model in descending order of their combined ranking from the flooding and elevation gradients. Hellinger distances were used to measure changes in the accuracy of the Traitspace model as traits were added and *P*-values indicated the statistical significance of the Hellinger distances compared to stochastic permutations.

Predicted species abundances and distributions were plotted along the flooding and elevation gradients and compared to the observed species distributions. Observed distributions of each species were modelled using GAMs with maximum k values of 50 using observed relative abundance data. Smooth splines were used to fit the GAM of each species along the flooding and elevation gradients for both the predicted and observed species distributions.

2.4 Results

2.4.1 Trait-trait relationships

The PCA of the nine functional traits identified two significant independent axes that explained 61.1% of the functional trait variation (Table 2.3). Axis 1 of the PCA separated individuals due to covariation in tissue dry matter content (RDMC, AGDMC and LDMC), RTD and SLA. Axis 2 separated individuals by covariation in root aerenchyma and SRL (Figure 2.3).

A large amount of overlap was seen between species within trait space. The species found in the driest areas were clearly separated from other species and were found in the top right quadrant of the biplot (Figure 2.3). The PCA identified two functional strategies in the dry environment. Species either had the combination of high tissue dry matter content and high root tissue density or they had low SRL and low root aerenchyma. Species found in the wetter environment (lowland species) typically had high root aerenchyma and SRL but could also be fit into two functional groups. One group of lowland species had high SLA, low RTD and low tissue dry matter content and a second group had high tissue dry matter content and low SLA (Figure 2.3).

Table 2.3. First three axes from the principal components analysis for nine functional traits measured on the wetland plant community. Eigenvalues and proportion of variance are provided. Eigenvectors are provided for each trait. For the first two axes, eigenvectors greater than 0.4 are in bold.

	PC1	PC2	PC3
Eigenvalues	3.47	2.03	0.95
Proportion of variance	38.55	22.54	10.61
Cumulative proportion	38.55	61.09	71.70
Specific root length	-0.10	**0.59**	0.33
Root dry matter content	**0.45**	-0.11	0.13
Root aerenchyma index	0.02	**0.48**	-0.52
Root branching intensity	0.26	0.39	0.20
Root tissue density	**0.40**	-0.39	0.03
Aboveground dry matter content	**0.44**	0.11	0.24
Height	0.31	0.32	-0.20
Specific leaf area	-0.31	0.02	0.63
Leaf dry matter content	**0.42**	0.03	0.24

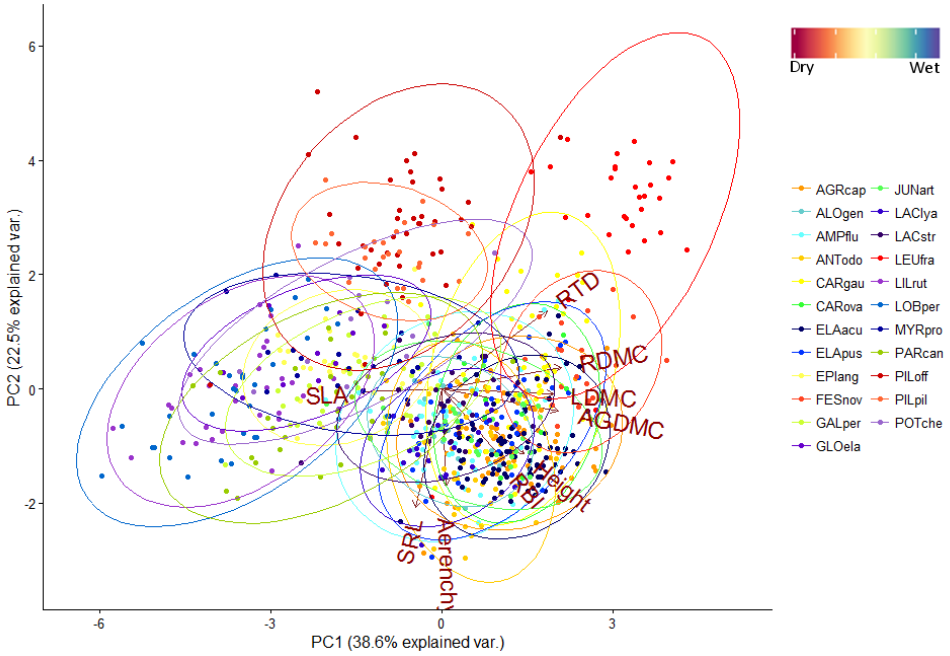

Figure 2.3. Principle component analysis of nine traits with species located along the first two principle component axes. Species are colour coded into their locations along the hydrological gradient ranging from red in the driest areas, yellow in middle of the gradient and blue in the wettest areas.

2.4.2 Trait-environment relationships

Strong relationships were observed for community-weighted mean trait values along the flooding gradient. Root aerenchyma and RDMC exhibited the strongest

community-weighted relationships with the flooding gradient (Table 2.4) where root aerenchyma increased with flooding (Figure 2.4a) and RDMC decreased with flooding (Figure 2.4b). A much broader range of community-weighted mean root aerenchyma measurements were observed in commonly flooded areas than in areas with little or no flooding. Community-weighted SRL and RBI decreased with flooding and the relationship between SRL and flooding was weaker in the grazed treatment (Figure 2.4c & Figure 2.4e). RTD increased along the flooding gradient up to approximately 80 days submerged per year and then began to decrease (Figure 2.4d). Models explained less than 20% of the observed variation in community-weighted AGDMC, SLA, LDMC and height. AGDMC and LDMC both showed weak trends of decreasing with flooding (Figure 2.4f & Figure 2.4h). SLA decreased with flooding in the ungrazed treatment but showed no significant response to flooding in the grazed treatment (Figure 2.4g). Height increased with flooding in the ungrazed treatment and decreased with flooding in the grazed treatment from 0 to approximately 120 days submerged per year and then began to increase but a wide variety of CWMs were observed (Figure 2.4i). The range of community-weighted mean traits for both height and SLA increased as flooding increased. Along the flooding gradient, grazing reduced the CWM trait values for all traits except SLA and SRL.

The strength of the relationships for community-weighted SLA and RTD along the water gradient were the strongest of all trait-environment relationships. SLA decreased with elevation above the flood line and 73% of the community-weighted variation was explained by the elevation gradient in the grazed treatment (Figure 2.4g). RTD increased with elevation and 70% of the community-weighted variation in RTD was explained by elevation in the ungrazed treatment (Figure 2.4d). SRL, height, root aerenchyma and RBI all increased with decreasing elevation (Figure 2.4). RDMC, AGDMC and LDMC all showed similar responses to water availability. All three dry matter content measurements only had significant relationships with the elevation gradient in the ungrazed treatment where they all increased either side of an elevation of approximately 1.0 m above the flood line. Grazing had little effect on the response of traits along the elevation gradient (Figure 2.4).

Table 2.4. Generalized Additive Models fit to two sets of data (grazed and ungrazed) to account for the interaction between the environmental gradients (days submerged per year and elevation) and the categorical factor of grazing. * = P-value < 0.05, ** = P-value < 0.01, *** = P-value < 0.001

Flooding gradient				
Trait	Days submerged : grazed	Days submerged : ungrazed	R^2	Deviance explained
Root aerenchyma	$F_{3,143}$=14.10***	$F_{3,143}$=35.02***	0.48	50.0%
Root DMC	$F_{3,143}$=22.77***	$F_{3,143}$=15.49***	0.44	45.9%
Specific root length	$F_{3,143}$=6.32***	$F_{3,143}$=11.25***	0.25	27.8%
Root tissue density	$F_{3,143}$=27.20***	$F_{3,143}$=10.88***	0.24	25.9%
Root branching intensity	$F_{3,143}$=15.12***	$F_{3,143}$=6.32*	0.20	22.1%
Aboveground DMC	$F_{3,143}$=8.79***	$F_{3,143}$=5.37*	0.19	21.7%
Specific leaf area	$F_{3,143}$=2.10	$F_{3,143}$=12.60***	0.18	20.1%
Leaf DMC	$F_{3,143}$=5.59**	$F_{3,143}$=8.51**	0.13	15.0%
Height	$F_{3,143}$=5.10**	$F_{3,143}$=10.56**	0.12	13.7%
Elevation gradient				
Trait	Elevation : grazed	Elevation : ungrazed	R^2	Deviance explained
Root aerenchyma	$F_{3,44}$=6.79**	$F_{3,44}$=9.41***	0.45	49.6%
Root DMC	$F_{3,44}$=2.65	$F_{3,44}$=8.36***	0.30	35.0%
Specific root length	$F_{3,44}$=8.74**	$F_{3,44}$=12.48***	0.46	50.2%
Root tissue density	$F_{3,44}$=10.86**	$F_{3,44}$=25.15***	0.59	61.9%
Root branching intensity	$F_{3,44}$=8.14**	$F_{3,44}$=11.95***	0.45	49.3%
Aboveground DMC	$F_{3,44}$=0.03	$F_{3,44}$=5.157*	0.16	21.9%
Specific leaf area	$F_{3,44}$=16.27***	$F_{3,44}$=18.65***	0.69	72.5%
Leaf DMC	$F_{3,44}$=0.45	$F_{3,44}$=7.11**	0.24	28.8%
Height	$F_{3,44}$=6.16**	$F_{3,44}$=9.67***	0.45	50.0%

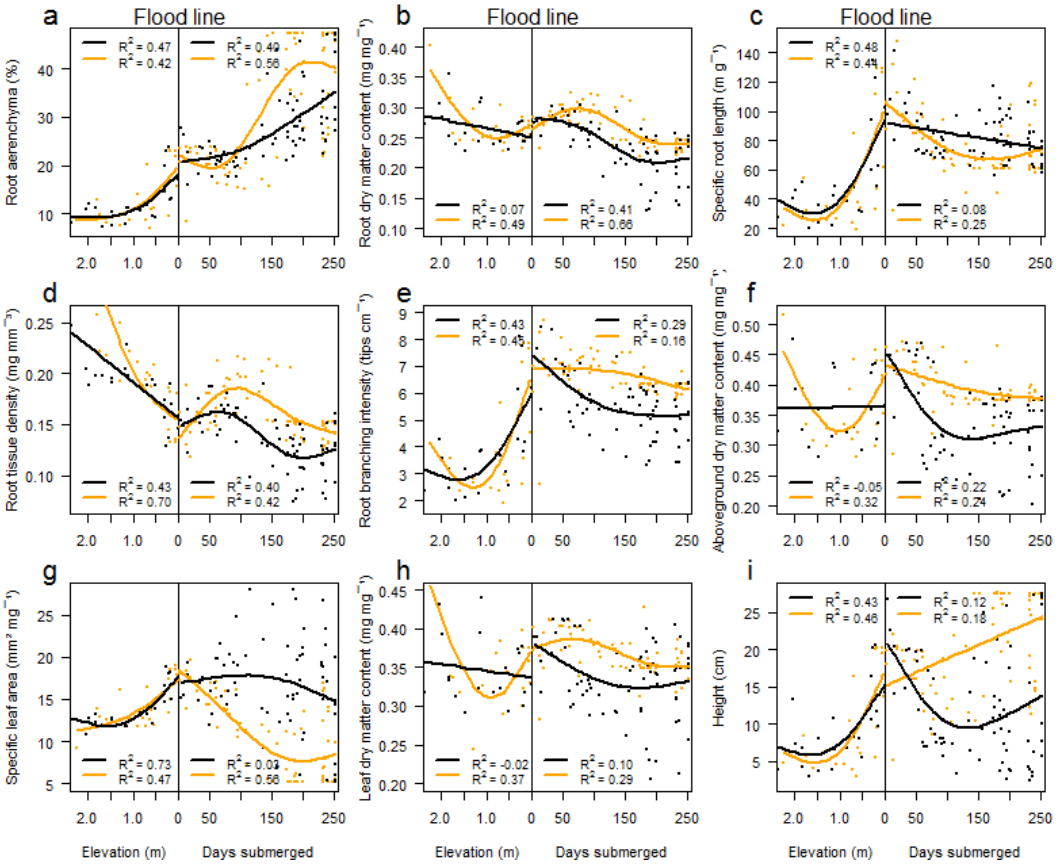

Figure 2.4. Community-weighted response of the nine traits above and below the flood line (0 on the x-axis) in grazed (black) and ungrazed (orange) treatments. Below the flood line, the responses of traits are observed along a flooding gradient measured as the number of days submerged per year. Above the flood line, the responses of traits are observed along an elevation gradient. R^2 values are provided for the GAMs of each trait along both gradients in the grazed and ungrazed treatment.

The individual-level trait responses across the hydrological gradient were much weaker than the community-weighted responses but typically showed the same general patterns. Root aerenchyma increased along the entire hydrological gradient (Figure 2.5a). Individual-level RDMC and RTD decreased along the entire hydrological gradient (Figure 2.5b). SRL only had statistically significant relationships with elevation and decreased with increasing elevation (Figure 2.5c). RBI only had a statistically significant relationship in the ungrazed treatment along the flooding gradient where it decreased with flooding (Figure 2.5e). Aboveground dry matter content had a general decrease along the hydrological gradient but only had significant relationships above the flood line in the ungrazed treatment and below the flood line the grazed treatment (Figure

2.5f). Specific leaf area increased as elevation decreased in both the grazed and ungrazed treatment and decreased with flooding in the ungrazed treatment (Figure 2.5g). Leaf dry matter content increased with elevation and had no significant relationship with flooding in either grazing treatments (Figure 2.5h). Height increased as elevation decreased for both grazing treatments but decreased with flooding in the grazed environment and increased with flooding in the ungrazed environment (Figure 2.5i). Root arenchyma, SRL, RTD, SLA and height all had restricted maximum values at one end of the hydrological gradient (Figure 2.5).

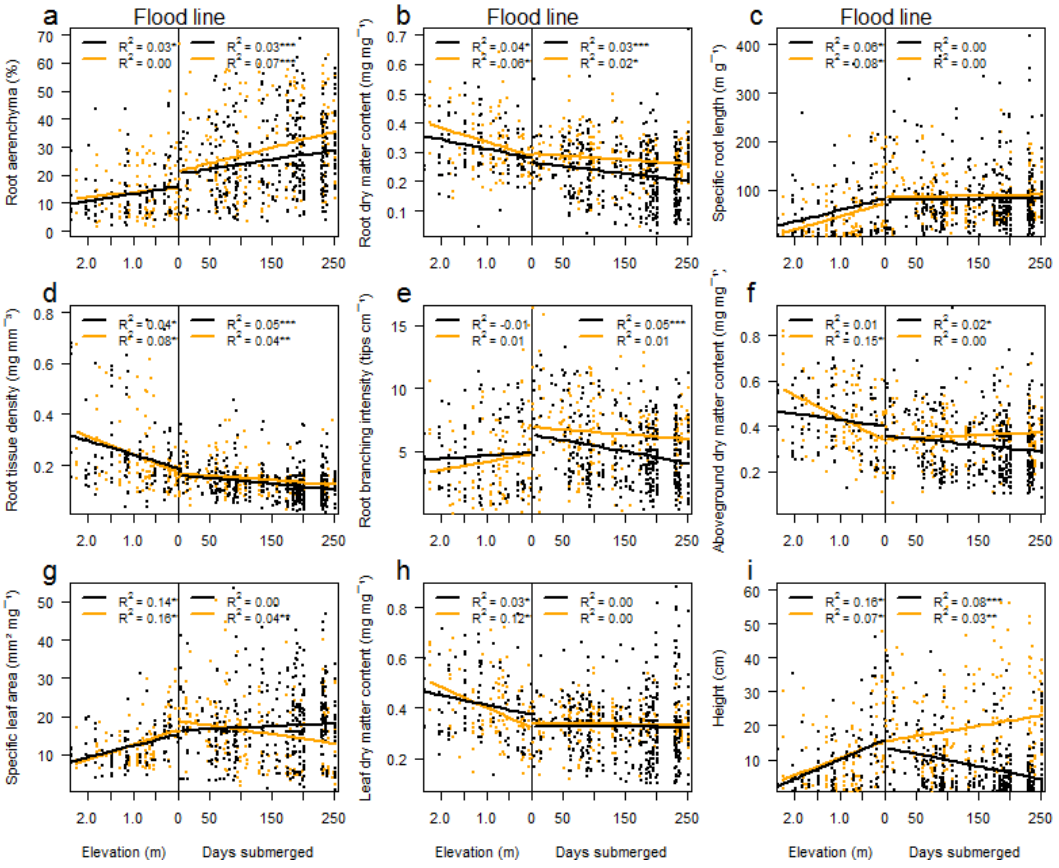

Figure 2.5. Individual-level trait values for nine functional traits observed along the hydrological gradient in the grazed (black) and ungrazed (orange) treatments. The gradient is split into an elevation gradient above the flood line and a flooding gradient measured by the number of days submerged per year in areas below the flood line. * = P-value<0.05, ** = P-value<0.01, *** = P-value<0.001.

2.4.3 *Species distributions and community composition*

Four different dominant species were identified with the species distribution models. *Elaeocharis acuta*, was the dominant species in areas flooded for more than 150 days and was the most abundant species in the entire study site. *Carex gaudichaudiana* was the most abundant species in areas flooded between 70-130 days per year but faced strong competition from *Galium perpusillum* for dominance in the grazed treatment (Figure 2.6a). *Agrostis capillaris* was the dominant species in the plant community in areas that were flooded up to approximately 70 days per year and was the second most abundant species in the study area. *Pilosella officinarum*, a small daisy, was the dominant species above the flooding zone (Figure 2.6a & Figure 2.6b). In the ungrazed community, *Leucopogon fraseri* competed for dominance in the highest elevations. Aside from *Pilosella officinarum*, grazing reduced the dominance of each dominant species and increased diversity in commonly flooded areas.

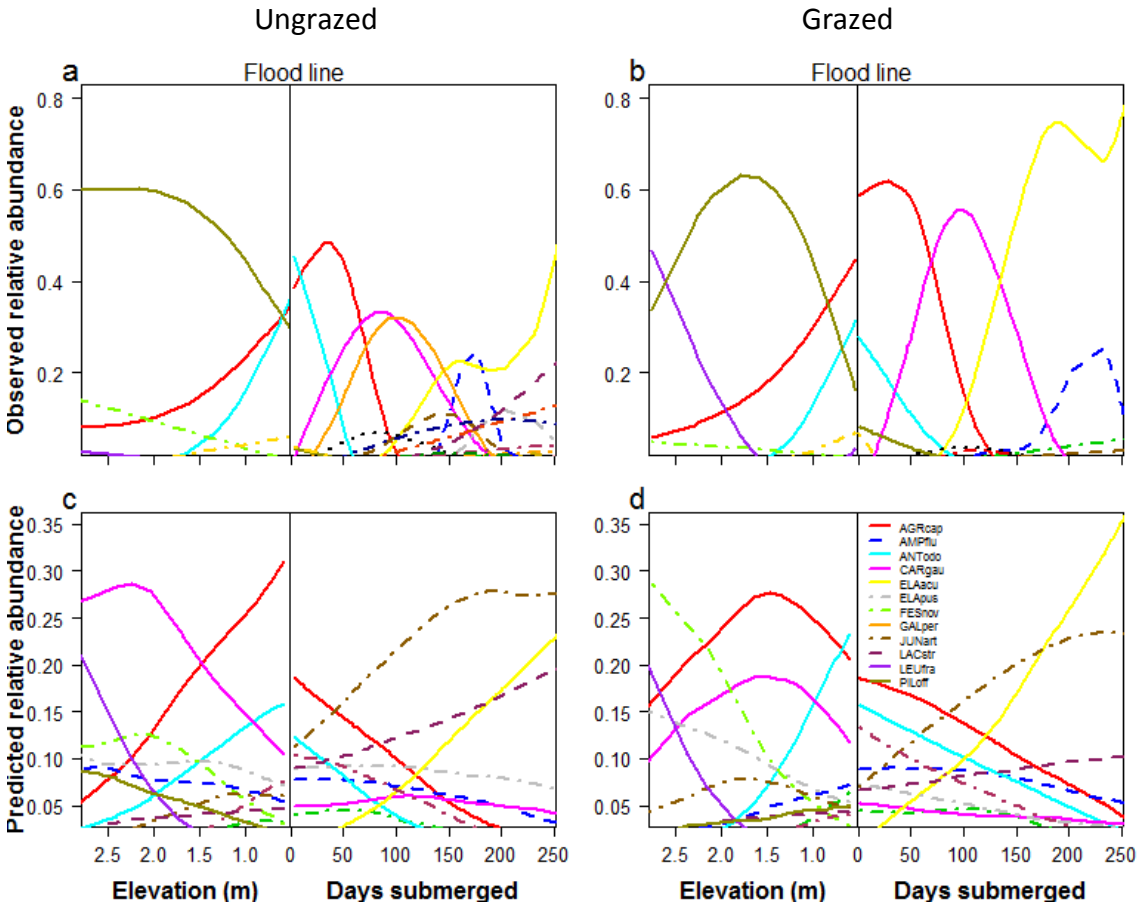

Figure 2.6. Observed and predicted relative abundances of the 23 most abundant species in grazed and ungrazed environments, above and below the flood line.

2.4.4 Traitspace predictions

Individual-level SLA exhibited the highest weighted ranking within the GLM framework and was selected as the first trait in the model (Table 2.5). The Traitspace predictions obtained using a single trait, SLA, was highly statistically significant (*P-value* = 0.001) and produced a Hellinger distance of 2.18 (Figure 2.7). Height produced the second highest ranking of all traits and the Hellinger distance of the modelled predictions reduced down to 2.03 with the addition of height in the model (Figure 2.7). The addition of RBI, SRL and root aerenchyma reduced the Hellinger distance down to 2.00, 1.95 and 1.91, respectively. The Hellinger distance continued to decrease with the addition of traits down to a value of 1.83 when the Traitspace model was run with all nine traits.

Table 2.5. R^2 values for the flooding gradient, elevation gradient and weighted R^2 of each trait within the GLM framework of the Traitspace model. * = P-value<0.05, ** = P-value<0.01, *** = P-value<0.001.

Trait	R^2 - Flooding	R^2 - Elevation	Weighted R^2
SLA	0.22***	0.10***	19.24
Height	0.12***	0.10***	11.54
RBI	0.10***	-0.01	7.47
SRL	0.07***	0.09***	7.46
Root aerenchyma	0.08***	0.05*	7.31
RDMC	0.03***	0.08***	4.15
AGDMC	0.01	0.11***	3.3
LDMC	0.01*	0.10***	3.07
RTD	0.00	0.10***	2.3

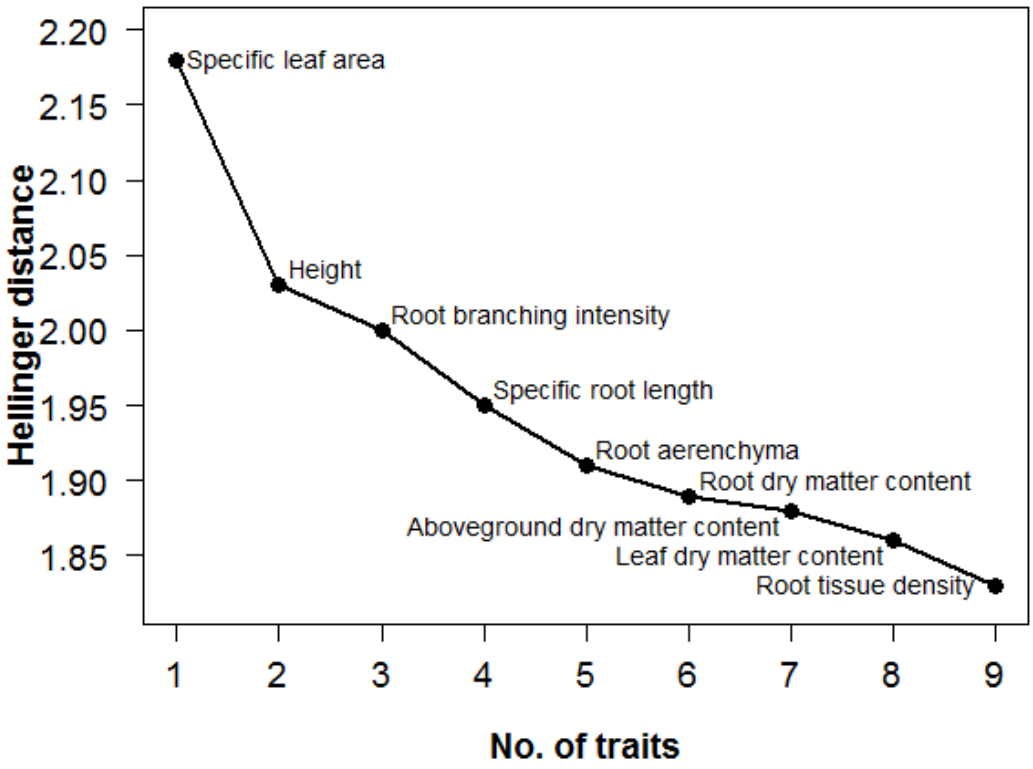

Figure 2.7. Hellinger distances indicating the level of dissimilarity between the predicted species distributions and the observed species distributions. The Traitspace model was first run with only specific leaf area. The model was then run with two traits - specific leaf area and height. Root branching intensity was added to SLA and height to produce the three trait model. Additional traits were added in the order identified in the figure for models run with 4-9 traits. All models were highly statistically significant (P-value = 0.001)

Using functional traits and environmental data, the Traitspace model predicted 12 of the 23 species to be common across the hydrological gradient (Figure 2.6c & Figure 2.6d). Predicted abundances of the dominant species were all lower than the observed abundances. Of the six common species found above the flood line, the Traitspace model accurately predicted the distribution for four of those species. *Festuca novae-zelandiae* and *Leucopogon fraserii* were accurately predicted to increase in abundance as elevation increased. *Agrostis capillaris* and *Anthoxanthum odoratum* were accurately predicted to increase in abundance with decreasing elevation in the grazed environment but the predicted abundance of *Agrostis capillaris* peaked in the middle of the elevation gradient in the ungrazed environment. *Pilosella officinarum*, the observed dominant species above the flood line, was only predicted to have a low abundance across the

elevation gradient. Above the flood line, the model predicted *Carex gaudichaudiana* to be abundant and predicted low abundances of *Amphibromus fluitans*, *Eleocharis acuta*, *Juncus articulatus* and *Lacnagrostis striata*, none of which were observed above the flood line.

Below the flood line the model predicted high abundances for three of the five most abundant species and accurately predicted their distribution along the gradient. *Agrostis capillaris* and *Anthoxanthum odoratum* were predicted to increase in abundance towards the flood line and *Eleocharis acuta* was predicted to increase in abundance as flooding increased (Figure 2.6c & Figure 2.6d). *Carex gaudichaudiana* and *Galium perpusillum* were not predicted to occur in high abundance along the flooding gradient but were observed in high abundance in areas flooded between approximately 50-150 days per year. *Juncus articulatus* was predicted to be a dominant species below the flood line but it was only ever observed in low abundances (Figure 2.6). The model accurately predicted an increase in the abundance of *Lachnagrostis striata* with flooding and correctly predicted a lower abundance in the ungrazed environment. The model was unable to predict the low abundances of a number of species found along the flooding gradient in the grazed environment (Figure 2.6c).

2.5 Discussion

This research had two goals: 1) to identify how functional traits respond along a hydrological gradient in the presence and absence of grazing; and 2) to determine whether a trait-based statistical model of environmental filtering can be used to predict the composition of plant communities along a hydrological gradient. My results highlight the close association between root traits and the hydrological variation within this ephemeral wetland. The majority of research in the field of functional ecology has focused on easily measureable, aboveground traits but this research suggests that we can obtain more information about a plant's fitness in varying hydrological environments by focusing on roots. The trait-based model was able to correctly predict the abundance and distribution of a number of species along the hydrological gradient but it is likely models will need to incorporate the role of competition on community assembly to

accurately predict the composition of plant communities at small spatial scales. From here I will discuss the observed responses of functional traits along the hydrological gradient, with and without grazing, before discussing the predictions of the trait-based model of environmental filtering and how they might be improved.

2.5.1 Functional trait responses

Many community-weighted traits exhibited strong relationships with the hydrological gradient. Community-weighted root traits had particularly strong relationships with the hydrological gradient. Root traits also responded independently from aboveground traits. The vast majority of functional trait research has focused on easily measurable aboveground traits and neglected root traits due to sampling difficulty. Evidence from this research suggests that important information about the functional adaptations of plants may be missed if root traits are neglected in functional trait-based research, at least when studying adaptation to variation in soil hydrology and other soil properties (Holdaway et al. 2011).

Root aerenchyma was more responsive to flooding than any other trait. The production of aerenchyma is a well-known response to flooded and saturated soil (Blom and Voesenek 1996, Drew 1997, Jackson and Armstrong 1999, Przywara and Stepniewski 1999, Colmer 2003b, Voesenek and Bailey-Serres 2015) and the increase in root aerenchyma I observed matched the observations of various other studies (Przywara and Stepniewski 1999, Colmer 2003a, Grimoldi et al. 2005, Luo and Xie 2009, Baastrup-Spohr et al. 2015). By producing aerenchyma tissue, plants are able to deliver oxygen to roots from other organs and leach oxygen into the rhizosphere to maintain an aerobic environment in flooded soil (Blom et al. 1994, Revsbech et al. 1999). Interestingly, root aerenchyma continued to respond above the flood line. Increases in elevation above the flood line are likely associated with reductions in water availability as the ground surface gets higher above the water table and surface water drains to lower elevations (Price 1997, Winter 2001). The response of root aerenchyma above the flood line suggests potential advantages of root aerenchyma in moist soil when soil saturation is rare.

The other root traits I measured also appear to be important for dealing with flooding stress. Reduced community-weighted means were observed for root dry matter content, specific root length and root tissue density as flooding increased indicating that lower values of these traits are advantageous in flooded environments. Reduced RDMC may help to increase the turgor of root tissue and provide rigidity, compensating for the loss of structural integrity due to the increase in aerenchyma tissue (Caliaro et al. 2013). A reduction in RTD with flooding aligns with the previous findings of Craine and Lee (2003) and Ryser et al. (2011).

The community-weighted means of specific root length and root branching intensity both peaked at the flood line suggesting that low values of these traits are beneficial in both dry and flooded environments. An increase in SRL is known to improve the ability for roots to absorb water (Eissenstat 1992, Comas et al. 2012). Various studies have observed SRL increase with flooding (Rubio et al. 1997, Ryser et al. 2011, Shi et al. 2015) and both increase and decrease in dry soils (Arreola et al. 2008, Thorne and Frank 2009, Olmo et al. 2014). This appears to be the first study to have observed the response of SRL along a hydrological gradient at the community level. The mixed results on the response of SRL to variations in soil hydrology suggest further investigation is required to determine the adaptive value of SRL in environments of varied water availability. The decrease in RBI with increases in elevation suggests that RBI is important for tolerating and competing in dry soil and may be associated with a conservative growth strategy that has been shown to be beneficial in xeric environments (Westoby et al. 2002, Reich et al. 2003, Farooq et al. 2009, Fort et al. 2013).

Leaf and aboveground dry matter content had weak relationships with the hydrological gradient. Previous community level studies have found either no response (Jung et al. 2010) or an increase in LDMC along a flooding gradient (Baastrup-Spohr et al. 2015) and no previous studies have looked at the response of AGDMC along a hydrological gradient. The correlation between RDMC, LDMC and AGDMC suggests that measuring all three of these traits is redundant (Laughlin 2014b). RDMC was more strongly correlated to both the flooding and

elevation gradients and measuring RDMC is likely to provide more information about a plant's fitness in wetland ecosystems.

SLA and height showed similar responses along the elevation gradient but responded differently to flooding. Both traits decreased with elevation above the flood line. These findings align with previous studies and the concept that increases in water availability improve growing conditions and selects larger SLA values and taller height as they provide a competitive advantage in favourable growing conditions (Westoby et al. 2002, Cingolani et al. 2007, Cornwell and Ackerly 2009, Thorne and Frank 2009, Gotsch et al. 2010, Kunstler et al. 2015). The decrease in height along the flooding gradient in the grazed environment supports the findings of previous studies (Jung et al. 2010, Baastrup-Spohr et al. 2015, Fu et al. 2015). An increase in height with flooding in an ungrazed environment, as I observed, may be advantageous because it reduces the probability of submergence (Garssen et al. 2015).

Grazing had a significant impact on the functional traits and the composition of the plant community within the Kettlehole. The observed reduction in height in the in the presence of grazing is indicative of a grazing avoidance strategy (Briske 1996). Height within the grazed treatment was much lower than the ungrazed treatment and tall dominant species such as *Eleocharis* acuta, *Agrostis capillaris* and *Anthoxanthum odoratum* all reduced in abundance when exposed to grazing. Traits associated with grazing tolerance include high SLA and low LDMC as they are associated with faster growth rates and reduce the time required to recover from grazing (Díaz et al. 2001, Wright et al. 2004, Cingolani et al. 2005). I observed an increase in the CWM values of SLA and a decrease the CWM values of LDMC and AGDMC in the grazed treatment, responses indicative of a grazing tolerance strategies. My results suggest that both grazing avoidance and tolerance strategies are employed by plants within the Kettlehole. There is a possibility that the observed effects of grazing on functional traits were an artefact of an altered environment within the grazing exclosures rather than the removal of grazing pressure.

The relationships between functional traits and the hydrological gradient were far weaker for individual-level traits than they were for community-weighted

traits although the general trends were similar. The wide spread and weak responses of individual-level traits suggest that a broad range of trait values from a range of functional plant strategies are able to pass through the environmental filters at any location along the hydrological gradient (Westoby et al. 2002). Five individual-level traits had observable changes to their upper limits along the hydrological gradient suggesting that the maximum values of those traits were restricted by environmental filtering or excluded due to weak competitive ability (Keddy 1992, Mayfield and Levine 2010).

The observed responses of functional traits along the hydrological gradient illustrate how changes in soil hydrology selects plants with varying physiological adaptations and influence the assembly of wetland plant communities (Keddy 1992). If the global climate continues down predicted trajectories (IPCC 2014), variation in precipitation and potential evapotranspiration will alter the composition of ephemeral wetlands like the Wairepo Kettlehole (Weiher and Keddy 1995, IPCC 2014). The task for ecologists is to determine how predicted climate changes will alter the distribution of plant species and the composition of plant communities like that of the Wairepo Kettlehole. A trait-based approach provides the most promising mechanism to complete this task.

2.5.2 Traitspace predictions

The Traitspace model provided varied evidence to answer whether or not environmental filtering can be used to predict the assembly of plant communities within wetland ecosystems. Using environmental filtering of functional traits, the model was able to produce accurate predictions on the relative abundance and distributions of a number of key species within the wetland plant community, including four of the six most abundant species found in the study site. However, the model also produced inaccurate predictions for a number of other species and predicted their presence in environments where they were not observed. Incorporating additional information into the modelling framework appears to be necessary in order to produce consistently accurate predictions of specie's abundances.

The limited number of variables measured for this research is a restraint on the predictive ability of the model. Environmental filtering of unmeasured functional traits along the hydrological gradient would provide further information to help improve the predictions of the Traitspace model. Unmeasured traits that are known to be important adaptations to flooding, such as adventitious roots (Blom et al. 1994), would likely provide important predictive information. Additionally, this study only used one environmental gradient at each location within the study site (elevation above the flood line and days submerged below the flood line) to identify environmental filtering of functional traits. Although the hydrological regime is considered the strongest driver of wetland community assembly (Weiher and Keddy 1995), other environmental variables are also important and including additional variables, such as soil fertility, in the model would likely improve predictions.

There is potential to improve the accuracy of trait-based models of environmental filtering by recognising suites of co-varying traits that form functional strategies (Laughlin and Messier 2015). The principle components analysis identified various trait combinations of different species found in similar environments along the hydrological gradient. For example, flood adapted species had either high SLA, low RTD and low tissue dry matter content or they had low SLA and high tissue dry matter content. Variation in functional plant strategies can allow plants to acquire resources using different combinations of traits (Westoby et al. 2002). Traits that reduce one aspect of a plant's ability to acquire resources or tolerate the environmental conditions can be offset by advantageous traits that improve a plant's ability to compete or tolerate the environment. This 'offsetting' can potentially allow detrimental traits to pass through environmental filters, thereby reducing the power of trait-based models of environmental filtering. Current trait-based models are built around environmental filtering of individual traits but recognising how various traits synergistically provide adaptive value and incorporating those relationships into trait-based models of environmental filtering will improve our ability to predict the assembly of plant communities (Laughlin and Messier 2015).

Environmental filtering is an important process that occurs along environmental gradients, but the composition of plant communities at small spatial scales within the environmental gradient are predominantly driven by interactions between species (Kraft and Ackerly 2010). Models built around environmental filtering can recognise the general trends in trait responses over the extent of the environmental gradient but fail to identify patterns at small spatial scales. Integrating biotic filtering processes, such as competition, may be needed to improve predictions at small spatial scales. The theories of limiting similarity and trait hierarchy can be used to guide the inclusion of biotic interactions into trait-based models (Macarthur and Levins 1967, Chesson 2000, Mayfield and Levine 2010, Kunstler et al. 2012).

A key goal in predictive ecology is to produce the most accurate predictions for the lowest amount of effort. A key question to answer for trait-based modelling is whether the greatest improvements in predictions will be produced by measuring more traits and environmental variables, incorporating multiple co-varying traits into the modelling framework, or adding competitive interactions to the modelling framework. As competition is a key driver of community composition at small spatial scales (Kraft and Ackerly 2010), I suggest that incorporating competitive interactions into the modelling framework is likely to produce the greatest improvements in the accuracy of trait-based models.

2.6 Conclusions

This study shows that studying root traits, rather than aboveground traits, is important for understanding how plants adapt to environments of varied soil hydrology. It is necessary for functional ecology to place more emphasis on the measurement of root traits as they provide unique information about a plant's fitness within its environment. As a statistical translation of environmental filtering of plant functional traits, the Traitspace model has the ability to identify which traits are likely to perform well across a range of environments and can begin to select species from the species pool that will likely exist in different environments. Improvements to the predictions of plant community composition

at small spatial scales in wetland ecosystems will be enabled by the inclusion of biotic interactions into the modelling framework.

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3 Synthesis

3.1 Conclusions

Producing accurate predictions of the composition of plant communities has challenged ecologists for over 100 years. In the literature review of my first chapter, I identified that recent developments in trait-based statistical models have led to significant advancements towards a predictive ecology. Developing our understanding of how functional traits respond to environmental variation and biotic interactions is a key task in continuing the progress towards the goal of accurately predicting the composition of plant communities. Previous research has identified the hydrological regime to be the strongest environmental filter driving the assembly of wetland plant communities. Understanding the functional responses of plants to flooding and water availability will provide information about adaptations that improve a plant's ability to survive and compete in wetland ecosystems. Soil anoxia associated with flooded soils applies direct stress to the root system of plants but the response of root traits to flooding is poorly understood as the majority of trait-based research has focused on easily measurable aboveground traits.

This research had two central objectives: 1) to identify how plant functional traits respond along a hydrological gradient in the presence and absence of grazing; and 2) to determine if the composition of a wetland plant community can be predicted by a trait-based model of community assembly.

In Chapter Two, I identified the response of root, leaf and shoot traits along a hydrological gradient that was split into a flooding gradient below the flood line and an elevation gradient above the flood line. The response of traits along the gradients were observed in the presence and absence of grazing. At the community level, root traits were more strongly related to the hydrological gradient than aboveground traits. Root aerenchyma increased as exposure to flooding increased while root dry matter content, root tissue density and specific root length all decreased as flooding increased. These results indicate that variation in root traits help plants survive and compete in environments of varied

water availability and highlight the importance of measuring root traits in trait-based research.

In my second chapter I also tested the ability of a trait-based model of environmental filtering to predict the composition of the wetland plant community. Using nine functional traits and the gradients of elevation above the flood line and number of days submerged per year below the flood line, the Traitspace model was able to predict the abundance and distribution of a number of species within the plant community but was unable to produce accurate predictions for a number of other species. Models built around environmental filtering can recognise the general trends in trait responses over the extent of the environmental gradient but fail to identify patterns at small spatial scales where biotic interactions likely drive the composition of plant communities. The inclusion of biotic interactions into the framework of trait-based community assembly models will improve predictions of community composition at small spatial scales.

3.2 Recommendations for future research

Before this study was performed, root aerenchyma was the only trait of the five root traits that I measured to have been studied along a hydrological gradient at the community level. All five root traits that I measured (root aerenchyma, root dry matter content, specific root length, root tissue density and root branching intensity) had strong relationships to the flooding and elevation gradients. Root aerenchyma has been observed to consistently increase in response to flooding but further research is required to determine the generality of the responses of other root traits in different wetland ecosystems.

Nine functional traits is only a small selection of the wide range of traits that can be measured on plants. To achieve the ability to make generalizable predictions of community assembly across ecosystems it is necessary to have a core list of functional traits to use in predictive models. However, the response of a large number of traits are yet to be comprehensively measured along environmental gradients. This research identified that root traits, which are often ignored,

provided important information about the fitness of plants along the hydrological gradient than above ground traits. Many other, potentially important, functional traits are yet to be measured within wetland ecosystems. For example, adventitious roots are a well-known adaptation for dealing with flooding but are yet to be measured at the community level. Measuring the response of novel traits along environmental gradients is an important first step in process of building a core list of traits. Once a comprehensive list of traits have been measured along environmental gradients we can begin to determine which traits provide the most predictive power across a range of environments for the least amount of effort.

Environmental filtering is driven by multiple variables in any ecosystem. The study of environmental filtering in wetland ecosystems has largely focused on filtering along hydrological gradients but other environmental variables are also important in the assembly of wetland plant communities. A more comprehensive understanding of environmental filtering within wetlands would be developed by measuring the influence of non-hydrological, environmental gradients such as soil fertility or salinity on wetland plant communities.

Trait-based models of environmental filtering will benefit if trait covariation is incorporated into models. In any given environment multiple functional strategies, determined by covariation of functional traits, enable the coexistence of multiple species. The incorporation of trait covariation into community assembly models will enable models to select species with a variety of strategies that can compete in the same environment but are composed of different functional traits combinations.

Current trait-based models of community assembly are limited by their inability to account for the influence of biotic interactions in the process of community assembly. By including biotic interactions into community assembly models, the accuracy of predictions at small spatial scales will increase because interactions between species have a significant influence on the composition of plant communities at small spatial scales. The addition of biotic interactions into predictive models can be guided by the theories of limiting similarity and trait hierarchy. Potential advances in trait-based assembly models and on-going

improvements in our understanding of how fitness advantages are associated with functional traits make it an exciting time in the field of predictive community ecology.

4 Appendix

Table 4.1. Number of samples collected for each species encountered in the community survey and the average trait values for specific root length, root dry matter content, root porosity (aerenchyma), root branching intensity and root tissue density from the data collected for each species.

Species	# of samples	SRL (m g^{-1})	RDMC (mg mg^{-1})	Root porosity (%)	RBI (tips cm^{-1})	RTD (mg mm^{-3})
AGRcap	78	99.21	0.29	23.77	6.64	0.15
ALOgen	21	91.65	0.23	22.72	5.69	0.12
AMPflu	43	121.04	0.22	21.23	5.81	0.13
ANTodo	41	159.03	0.27	22.41	9.08	0.11
CARgau	48	67.58	0.33	15.15	7.79	0.22
CARova	10	50.21	0.28	32.11	6.46	0.18
CELgra	9	9.04	0.25	9.67	2.90	0.25
COPper	1	24.29		10.96	3.37	0.27
COPpet	3	58.22	0.28	21.47	1.99	0.25
CRAsin	0					
DEYave	3	52.87	0.35	17.80	4.91	0.17
ELAacu	87	61.74	0.24	47.38	6.36	0.15
ELApus	34	138.34	0.32	30.04	7.19	0.15
EPIang	30	64.10	0.14	14.20	3.50	0.10
FESnov	21	48.90	0.35	12.82	5.47	0.20
GALper	31	81.83	0.24	23.87	3.69	0.12
GLOela	10	58.63	0.18	25.55	3.01	0.08
GONmic	1	27.18	0.22	9.88	4.90	0.17
HELfil	1	121.65	0.31	6.15	6.69	0.16
HERnov	2	15.69	0.25	8.98	2.44	0.20
HYDsul	6	110.35	0.13	8.97	3.20	0.13
JUNart	30	51.21	0.23	36.29	7.06	0.13
JUNcon	9	33.98	0.27	39.94	6.86	0.21
JUNeff	4	36.60	0.26	45.56	7.57	0.20
JUNten	0					
LAClya	31	100.82	0.24	27.85	5.84	0.11
LACstr	30	80.81	0.24	28.08	5.01	0.13
LEPman	8	93.67	0.10	20.11	2.46	0.06
LEUfra	32	19.70	0.45	8.82	4.49	0.52
LILrut	31	48.78	0.11	26.32	3.33	0.07
LOBper	33	102.32	0.10	20.39	2.76	0.06
MUEaxi	6	45.68	0.44	8.26	2.35	0.43
MYOlax	3	68.00	0.11	11.63	5.43	0.07
MYRpro	22	39.46	0.17	23.53	2.37	0.10
PARcan	30	172.47	0.15	22.58	3.04	0.08
PILnov	5	90.72	0.23	17.65	5.39	0.14
PILoff	40	17.50	0.22	6.63	1.73	0.19
PILpil	30	13.38	0.22	7.38	2.15	0.23

PIMore	4	5.23	0.23	11.03	2.65	0.24
PLAtri	5	57.54	0.14	18.02	2.83	0.07
POTche	11	15.83	0.14	34.53	2.02	0.10
PRUvul	1	23.26	0.15	10.34	2.49	0.15
RANlim	2	91.10	0.12	38.46	2.29	0.04
RUMace	3	19.23	0.32	6.85	3.34	0.24
RYTpum	7	49.77	0.40	11.28	4.22	0.21
STAmin	0					
TRIrep	0					
WAHalb	3	8.81	0.24	7.83	1.72	0.19

Table 4.2. Average trait values for aboveground dry matter content, aboveground biomass, height, specific leaf area and leaf dry matter content from the samples collected of species found in the community survey. Average aboveground biomass was used to weight the Generalized Linear models used in the Traitspace model.

Species	AGDMC (mg mg^{-1})	AG biomass (g)	Height (cm)	SLA (mm^2 mg)	LDMC (mg mg^{-1})
AGRcap	0.47	1.91	19.84	16.83	0.41
ALOgen	0.39	4.60	16.71	18.97	0.30
AMPflu	0.40	1.20	11.67	16.34	0.37
ANTodo	0.41	7.86	20.57	20.09	0.34
CARgau	0.39	0.32	10.24	10.80	0.39
CARova	0.39	7.32	25.45	13.09	0.39
CELgra	0.35	0.68	7.22	5.80	0.36
COPper	0.38	5.76	1.00	9.98	0.35
COPpet	0.37	4.28	2.33	10.01	0.40
DEYave	0.48	1.36	6.23	7.71	0.44
ELAacu	0.38	3.56	27.56	5.17	0.35
ELApus	0.46	0.03	3.22	19.37	0.51
EPIang	0.23	0.57	4.75	18.16	0.26
FESnov	0.59	5.97	18.84	9.32	0.51
GALper	0.20	0.10	4.65	28.26	0.27
GLOela	0.25	0.05	0.42	23.50	0.29
GONmic	0.29	0.07	3.50		0.37
HELfil	0.54	0.13	5.50	18.18	0.48
HERnov	0.33	0.35	2.60	7.85	0.34
HYDsul	0.16	0.07	1.30	21.18	0.17
JUNart	0.39	3.18	13.19	8.71	0.32
JUNcon	0.49	27.62	32.17	3.48	0.44
JUNeff	0.47	9.05	55.03	4.27	0.40
LAClya	0.38	1.35	11.66	20.07	0.36
LACstr	0.35	0.47	5.53	15.22	0.38
LEPman	0.17	0.08	0.79	27.38	0.17
LEUfra	0.56	0.55	3.83	8.51	0.60

LILrut	0.16	0.12	3.76	22.20	0.14
LOBper	0.18	0.14	1.87	33.57	0.22
MUEaxi	0.41	0.96	3.07	11.94	0.32
MYOlax	0.15	2.80	9.47	34.43	0.15
MYRpro	0.27	0.16	2.01	20.84	0.37
PARcan	0.21	0.11	2.51	22.02	0.24
PILnov	0.48	0.01	1.94	16.64	0.47
PILoff	0.27	0.47	2.50	12.66	0.28
PILpil	0.21	1.42	9.19	21.57	0.23
PIMore	0.41	25.61	2.88	9.40	0.42
PLAtri	0.14	1.42	2.60	11.01	0.17
POTche	0.32	0.04	1.42	20.00	0.35
PRUvul	0.24	0.89	3.80	14.28	0.28
RANlim	0.21	0.03	1.80	16.69	0.24
RUMace	0.27	0.60	10.30	21.01	0.16
RYTpum	0.57	0.42	4.19	8.54	0.60
WAHalb	0.30	0.15	4.97	8.36	0.34

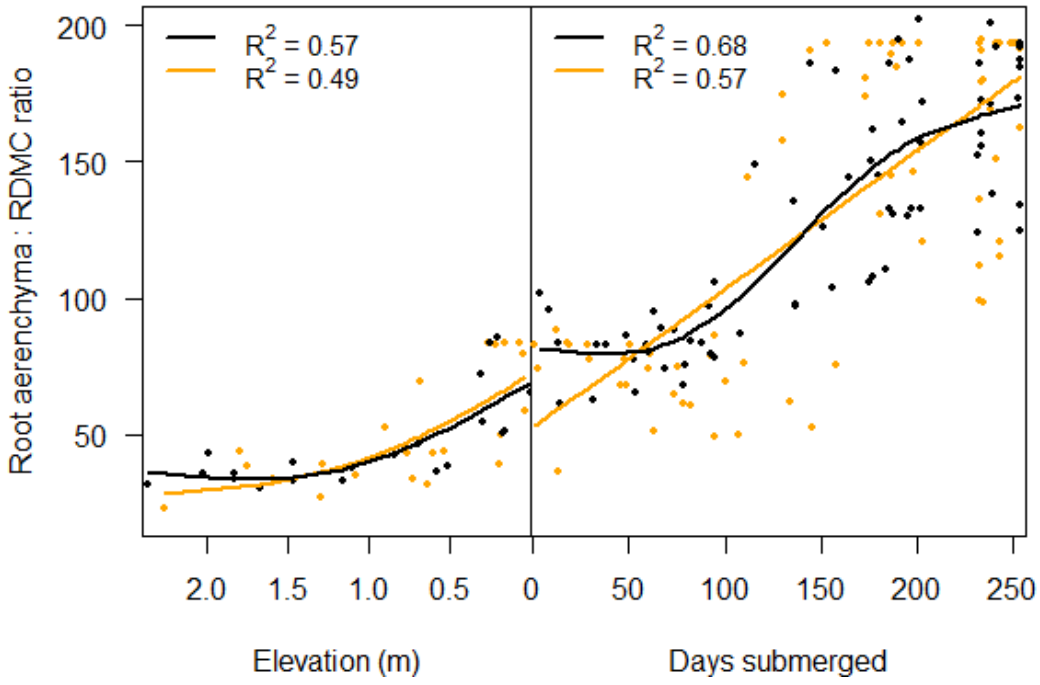

Figure 4.1. Plot illustrating how the ratio between CWM root aerenchyma and CWM root dry matter content changes along the hydrological gradient. In dry areas, plants have low root aerenchyma and high root dry matter content creating a low ratio. At the wet end of the gradient, plants have high root aerenchyma and low root dry matter content resulting in a high root aerenchyma to RDMC ratio.

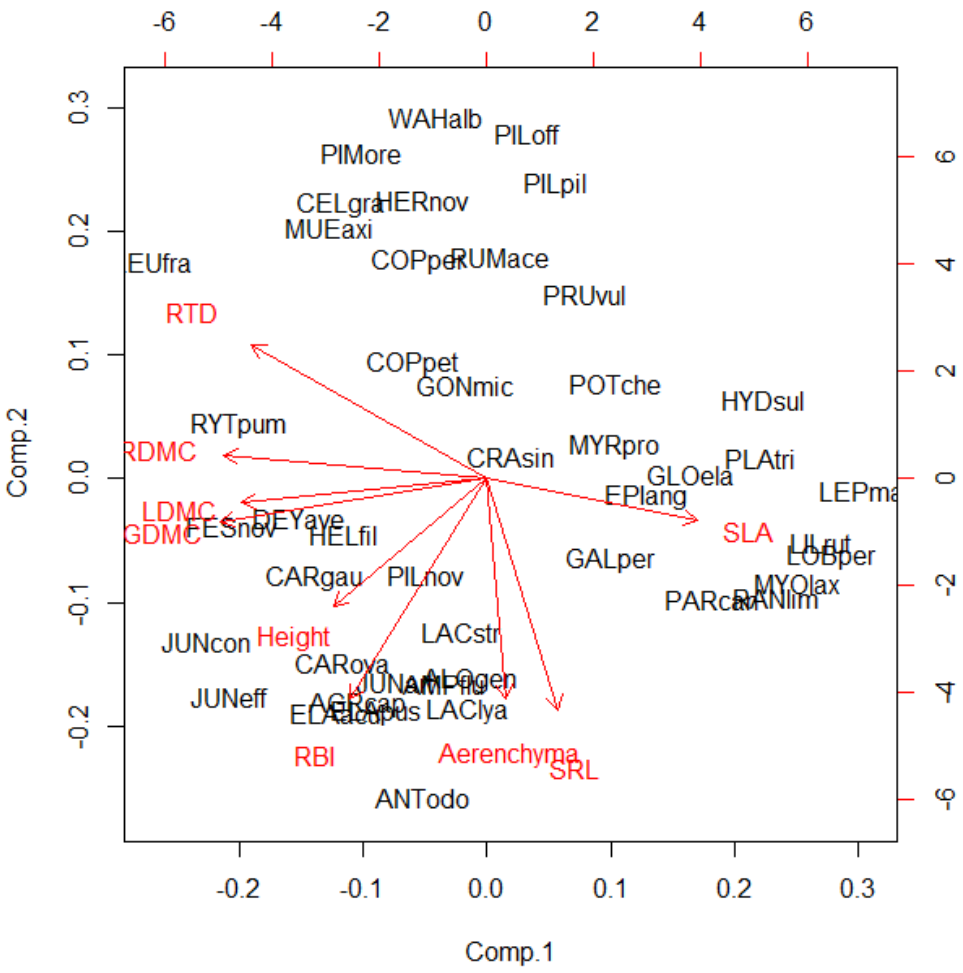

Figure 4.2. Principle component analysis plotting species within trait space produced with average species trait values.