Communities of Arbuscular Mycorrhizal Fungi in Salt Marsh Habitats: Diversity, Structure, and Ecosystem Function

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Summary

The relationship between ecosystem functioning and the biodiversity of microorganisms has been a central focus of recent ecological studies, and fungi are considered to play a key role in this relationship. For example, the Mycorrhizae's association with two-thirds of terrestrial plants makes them one of the most common forms of symbiosis on Earth. Although Arbuscular Mycorrhizal Fungi (AMF) have been shown to be important in nutrient cycling, soil stability and enhancing plant growth by increasing root mycelia, much of the information we have on them is restricted to a small sample of woodland and grassland habitats. Consequently, the mechanisms regulating the diversity and community structure of fungi remain poorly studied across many habitats. This is particularly true for salt marshes, which are key conservation priority habitats in many countries, including the UK. Using the latest sequencing technologies, this work examined the community ecology of fungi across six different salt marsh habitats over two locations in Essex and Lancashire, UK and related this to local environmental variables and sediment nutrient statuses. In addition, by linking with other datasets from the same sites, this study also examined the role of biotic factors that are likely to influence the relationship of rhizosphere fungal communities with each other. Although a range of local abiotic factors significantly influenced the composition of fungal communities within each salt marsh, at larger scales, the compositions of these fungal communities were significantly affected by the pattern of the biotic factors that also differed over seasons. Indeed, the ability of these variables to predict fungal richness and abundances differed greatly between sites, suggesting that drivers of fungal

community structure may be site-specific to some extent. Indeed, fungal richness in relation to abiotic or biotic factors explained considerably different amounts of variation in each site, and generalised poorly to other sites. Therefore, it is possible now to argue against deriving general environment-diversity relationships without empirical validation in multiple sites. Finally, functionally similar fungi ponded similarly to the environmental gradients, suggesting a possible disconnect between functional redundancy and resilience in microbial communities

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List of Abbreviations and Units

°C	Celsius
µl	Microliter
18S rRNA	A component of the small eukaryotic ribosomal subunit
AA3	Auto Analyser, third generation from Seal®
AH	Abbotthal Essex, UK
AIC	Akaike information criterion
AMF	Arbuscular Mycorrhizal fungi
CBESS	A hierarchical approach to the examination of the relationship between biodiversity and ecosystem functioning
CS	Cartmel Sand, Morecambe Bay, Lancashire, UK
FW	Finringhoe, Essex, UK
ITS	Internal transcribed spacer
mg	Milligram
$MgCl_2$	Magnesium Chloride
MQ	MilliQ water
NH_4^+	Ammonium
NO_3^-	Nitrate
OC	Organic Carbon
OUT	Operational taxonomic unit
PCR	Polymerase chain reaction
PO_4^{---}	Posphate
rpm	rotate per minute
S	Summer
TC/IC	Total Carbon/ In-organic Carbon
TM	Tillingham, Essex, UK
TOC	Total organic carbon
VTX	Virtual taxa, may also referred to as VT
W	Winter
WP	West Plain, Morecambe Bay, Lancashire, UK
Archetype	Groups with relating to behavior
Abundance	The number of individuals found per sample
WS	Warton Sand, Morecambe Bay, Lancashire, UK

Author’s Declaration

I Ahmad K Alzahrani declare that, except where explicit reference is made to the contribution of others, that this thesis is the result of my own work, and has not been submitted for any other degree at the University of Essex or any other institution.

Chapter One

1. Literature Review

1.1. Introduction

The term “biodiversity” includes differences at various levels of organisation, starting from variations at the genetic level, to variations between ecosystems and biomes (Tilman, 1997). Although this fact was recognised by Darwin, it has not been researched by ecologists and environmentalists until the last two decade (Loreau *et al.*, 2001). Species diversity is directly related to the provision of goods and services, and the regulation and modulation of ecosystem functions that underpin the delivery of ecosystem services (Balvanera *et al.*, 2006). Thus, an understanding of the relative importance of changes in different abiotic and biotic controls over species diversity has become a central theme in ecological research (Preston, 1948; Peet, 1974; Hamilton, 2005; Ieno *et al.*, 2006; Cardinale *et al.*, 2011).

Species distribution is arguably not random, thus, studies use indices that treat it as equivalent to species richness, to measure biodiversity (Magurran, 2004), based on three assumptions (Peet, 1974). First, species that make disproportionate contributions to communities are weightless, which means that all species are equal. This assumption treats the relative abundance of a species in an assemblage as a factor that determines its importance in a diversity measure; species richness measures therefore do not distinguish between species that are exceptionally abundant, and those that are extremely rare (Magurran, 2004). Second, all individuals are equal, which means that the intensity of sampling has no effect on the number of species. Finally, biodiversity measures assume that species abundance has been recorded using appropriate units, even if their estimates were based on different units (Magurran, 2004).

Under normal circumstances, only a few patterns of species richness have been documented to be temporally and spatially universal (Rahbek, 1997), given the complex correlation between ecological, historical, and evolutionary processes. However, it still not clear which entities should be compared and over what scales they can be investigated (Magurran, 2004). Determination of total species diversity in a landscape (γ), is a result of the mean number of species diversity (or richness) of the local community or habitat (α), and differences in diversity associated with differences in habitat or sampling scale (β) (Whittaker, 1960). Thus, ecologists aim to know if one domain is more diverse than another, or whether diversity is subjected to changes across different spatial scales over time. Use of the Whittaker concept for community ecology provides a testable framework to predict where species will shift in response to changing environmental conditions (Mckenney *et al.*, 2011). This used to be done by estimating species' niches across geographical space, that links observations of species presence (and sometimes absence) in abiotic or biotic conditions (Algar *et al.*, 2009). However, a major paradigm shift in the scientific perception of diversity occurred during the last two decades, towards biodiversity effects on ecosystem functions (BDEF), where diversity is analysed as a driver of ecosystem processes, and the main objective is to understand consequences of altered diversity in ecosystems (Hillebrand & Matthiessen, 2009). This shift in emphasis has revealed that local species extinctions, or even large changes in abundance have as much potential to affect ecosystem properties (Zimov *et al.*, 2012).

1.1.1. Ecology of microbial communities

Despite the fact that ecosystems are heavily dependent upon microorganisms, and microbial community structure, predicting how structure responds to environmental heterogeneity, or linkages between structure and ecosystem processes are yet to be fully determined (Bossio *et al.*, 1998). Soil harbours a huge variety of organisms including those inhabiting the narrow zone of soil that is influenced by plant root secretions (rhizosphere). Bacteria, fungi (including arbuscular mycorrhizal fungi (AMF)), oomycetes, viruses and archaea that live in this narrow zone are attracted by and feed on nutrients, exudates, border cells and mucilage released by the plant root, and known to have profound effects on growth, nutrition and plant health (Berendsen *et al.*, 2012; Philippot *et al.*, 2013).

As any microbial community, the compositions of the rhizosphere microbes differ in both qualitative and quantitative aspects. The relative abundance of individuals within these microbial communities is subject to physiological and metabolic changes caused by the organisms, and physico-chemical changes of the surrounding environment. Species of high abundance that are culturable under certain conditions may develop into dormant and possibly uncultured forms (Wintzingerode & Göbel, 1997; Philippot *et al.*, 2013). Many studies have shown that biotic variables can affect the composition and relative abundance of microbial populations in the rhizosphere (Costa *et al.*, 2006; Teixeira *et al.*, 2013). For example, the relative abundance of bacterial taxa detected in the rhizosphere of the graminoid *Avena fatua* were significantly different from those in the bulk soil (DeAngelis *et al.*, 2009). Although, bacteria do not monopolize the nutrient-rich rhizosphere niche, fungi such as those in the phyla Ascomycota and

Glomeromycota (for example, *Glomus* spp.) can respond rapidly to added nutrients.

Numerous studies have shown proteobacteria as dominant members of the rhizosphere microbiota (DeAngelis *et al.*, 2009; Uroz *et al.*, 2010; Mendes *et al.*, 2011), based on their ability as fast-growing strategists, utilising of a broad range of root-derived carbon substrates and provided $^{13}CO_2$ (Vandenkoornhuyse *et al.*, 2007). This fits the Baas-Becking's (1934) hypothesis, following the classical view that assumes all microbes are distributed globally due to their high dispersal potential (Kellogg & Griffin, 2006). According to this hypothesis, their small size and ability to enter dormancy enables prokaryotes and some microscopic eukaryotes such as protists and small invertebrates to acquire global distribution (Cáceres & Soluk, 2002; Bohonak & Jenkins, 2003; Fenchel & Finlay, 2006; Fontaneto *et al.*, 2008). However, this hypothesis has been challenged recently, as new methods have been developed in the field of microbial studies. The use of the this new technique was evidence of the distance–decay relationship that revealed a high degree of cryptic diversity and restricted dispersal in a variety of microorganisms (Whitaker, 2003; Taylor *et al.*, 2006). The latest developed molecular technique known as Next-generation Sequencing (NGS) is now frequently used to identify microbial taxa in the rhizosphere, and has the power to resolve several methodological issues (Philippot *et al.*, 2013). NGS has resolved methodological constraints related to data acquisition of the natural diversity of microbial soil, primarily issues related to high labour demand, and cost of molecular analyses (Öpik *et al.*, 2009). Such second-generation sequencing technologies offer greatly improved throughput at lower cost per

nucleotide sequence, and may be used for assessing the complexity of natural populations by targeting specific genes (Dowd *et al.*, 2008).

1.1.2. Applying advanced molecular methods in the studies of microbial diversity

Until the 1970s, microorganisms including those living in the soil could only be studied by morphological observation or culture-dependent methods. However, within less than a decade, a new technique was developed by Kelppe *et al*, (1971), which began to be used by researchers. This new technique used 5S rRNA molecules, directly extracted from mixed samples, belonging to various community members, and separated them using an electrophoreses gel, yielding a comparative sequence analysis of phylogenetic placements (Stahl *et al.*, 1984, 1985; Lane *et al.*, 1985). This technique included three microbial assemblages: The bacterial symbionts around deep-sea hydrothermal vents (Stahl *et al.*, 1984), bacteria inhabiting the 91°C source pool of a hot spring (Stahl *et al.*, 1985), and the microorganisms inhabiting a copper leaching pond (Lane *et al.*, 1985). Although, this study resulted in interesting insights, the information content in the approximately 120-nucleotide 5S rRNA molecule was relatively small. The requirement for electrophoretic separation of the different samples limited this approach to less complex ecosystems. Thus, to solve this issue, a larger rRNA molecules was introduced to the studies of microbial ecology (Olsen *et al.*, 1986). The bacterial16S rRNA molecule has a length of 1,500 nucleotides, and 23S rRNA molecules are around 3,000 nucleotides.

The late introducing of the polymerase chain reaction (PCR) allows the 16S rRNA gene fragments to be selectively amplified from mixed DNA (Saiki *et al.*, 1988; Amann *et al.*, 1995). The resulting gene libraries from the mixed amplification products should contain only defined fragments that can be rapidly

sequenced from known priming sites (Amann *et al.*, 1995). This approach was first applied in a study of Sargasso Sea picoplankton, where results indicated the presence of defined clusters of proteobacterial and cyanobacterial origin (Giovannoni *et al.*, 1990). This technique greatly reduced and even avoided lengthy screening procedures, which were previously necessary to identify the rRNA containing clones in shotgun libraries (Amann *et al.*, 1995). However, this technique was rather laborious and costly, with the result that most applications have assayed on the order of only 100 sequences per sample, that may not be sufficient to fully characterize all but the simplest communities (Degnan & Ochman, 2012). Thus, considerable progress has led to the development of alternative techniques for assessing rRNA variation (for example, ARISA, DGGE, tRFLPs) (Muyzer G Uitterlinden AG, de Waal EC, Uitterlinden AG, 1993; Liu *et al.*, 1997; Fisher & Triplett, 1999); however, with the advent of 454 pyrosequencing, hundreds of thousands of 16S rRNA gene amplicons may be surveyed in a single sequencing run (Sogin *et al.*, 2006).

Continuous developing of sequence technology has been carried out ever since, to develop more cost effective high-throughput sequencing technology. Thus, recent interest has focused on the applicability of other sequencing methodologies, most notably Solexa/Illumina (Lazarevic *et al.*, 2009; Claesson *et al.*, 2010; Gloor *et al.*, 2010; Caporaso *et al.*, 2011; Zhou *et al.*, 2011). The current cost is less than 1/100 the cost per read, compared with the 454 pyrosequencing (Degnan & Ochman, 2012). Although Illumina reads are much shorter, this new technique can be customised to yield sequences of increased lengths by merging the paired-end reads generated from the same amplicon (Gloor *et al.*, 2010; Rodrigue *et al.*, 2010; Zhou *et al.*, 2011). By integrating

sample-identifying index into the amplification primers, the Illumina platform, like 454 pyrosequencing, allows high level of multiplexing, which further increases its utility for examining large and complex sets of samples (Gloor *et al.*, 2010). Thus, this technique is now frequently used to identify microbial taxa in the rhizosphere, with considerable resolving power, providing descriptive analyses of the rhizosphere microbiome, where elucidation of the mechanisms underlying the selection of specific populations of microorganisms is required (Philippot *et al.*, 2013).

1.1.3. Arbuscular Mycorrhizal fungi

Among the rhizosphere microbes are the widespread plant symbionts - arbuscular mycorrhizal fungi (AMF). Arbuscular belong to the phylum Glomeromycota (Schüβler *et al.*, 2001), and form an important symbiosis with almost 80% of all terrestrial plant species (Smith & Read, 2008). By developing a specialised area called the symbiotic mycorrhizal interface that allows interaction with the host plant, AMF facilitate the horizontal transfer of a range of beneficial impacts including increased P and enhancing N uptake (Jin *et al.*, 2005; Leigh *et al.*, 2009; Hodge & Fitter, 2010). AMF are obligate root symbionts, exchanging their beneficial association for carbon form their host plants, via the intra radical mycelium (Helgason & Fitter, 2009). In saline habitats, mycorrhizal increase K^+/Na^+ ratio in the root area (Rabie & Almadini 2005; Sannazzaro ***et al***. 2006) and decrease shoot Na^+ concentration (Al-Karaki & Hammad 2001). Both strategies are known to prevent disruption of various enzymatic processes and inhibition of protein synthesis. Mycorrhizal fungi may act as a filter that helps in ion selection during the uptake of nutrients from the soil or during transfer to the plant host. Studies indicated that AMF can select elements such as K^+ and Ca^{2+}, which act

as osmotic equivalents while they avoid uptake of toxic Na^+ (Hammer et al. 2011; Evelin, Giri & Kapoor 2012). This suggests that AMF induce a buffering effect on the uptake of Na^+ when the content of Na^+ is within an average limit (Evelin, Kapoor & Giri 2009; Hammer et al. 2011). Although different AMF taxa can perform symbiosis, their ability to promote plant succession varies based on the matching of plant and fungal species (Nemec, 1979; Powell *et al.*, 1982; van der Heijden *et al.*, 1998). However, species-specific interactions do not always occur, and AMF could be more specific to plant functional groups than to individual plant species (Öpik *et al.*, 2009).

In addition, the interaction between AMF and plants generates feedbacks that influence species diversity in fungal communities and, eventually, in plant communities (Bever *et al.*, 2001; Bever, 2002). Therefore, the colonization level of an AMF community is more likely to be shaped by several factors, including abiotic (e.g. soil physico-chemical properties) (Chaudhary *et al.*, 2008; Dumbrell *et al.*, 2010a), and biotic (e.g. host species, local availability of inocula and by AMF–plant feedback). Thus, AMF have a profound determinative effect on plant community dynamics and diversity (Bever *et al.*, 2001; Smith *et al.*, 2003; Parniske, 2008b; Helgason & Fitter, 2009). The role of as a symbiotic support system that promotes seedling establishment and reduces recruitment limitation in grasslands has helped clarify whether seedlings are able to compete with adult plants in existing vegetation, and if there are any benefits for the seedlings when associating with AMF (Van Der Heijden, 2004). These benefits include: quick integration with AMF that are already associating with neighbouring plants species, expanding the root area that may allow seedling to gain excess C from

adult plants via AMF hypha (Grime *et al.*, 1988; Newman, 1988), and reducing the risk of infection by pathogenic soil fungi (Newsham *et al.*, 1995).

1.2. The ecology of AMF communities

AMF are common components of most natural and agricultural ecosystems that are considered to be relatively functionally equal (Lekberg *et al.*, 2007). AMF community composition may influence plant species composition, and productivity (van der Heijden *et al.*, 1998; Bever, 2002; Hart & Reader, 2002; Helgason *et al.*, 2002; Stampe & Daehler, 2003), as well as ecosystem functioning (Miller & Jastrow, 2000). AMF communities have also been shown to vary with plant communities (Helgason *et al.*, 1998; Öpik *et al.*, 2003), and other abiotic variables in the soil (Johnson *et al.*, 1992; Landis *et al.*, 2004; Dumbrell *et al.*, 2010a). However, the mechanisms generating differences among AMF communities remain understudied (Klironomos *et al.*, 2001).

Recent studies of AMF community dynamics have considered the effect of the spatial scale (Lekberg et al., 2007; Fitzsimons et al., 2008; Dumbrell et al., 2010a), and season on their assemblage (Dumbrell *et al.*, 2011). Due to the functional importance of AMF in terrestrial ecosystems, their community ecology has been widely studied. Many of these studies explain the diversity, structure and composition, either through 'niche-based' mechanisms as any other natural communities (Tokeshi, 1990), or niche-based theories that suggest an inter-specific variation in species' ecologies, due to the partitioning of limited resources between competing species, and the differentiation of niche space across all species within a community (Leibold & McPeek, 2006). Thus, the fundamental premise of niche theory explains the coexistence of AMF species and maintenance of their biodiversity based on their response to ecological traits

(Leibold & McPeek, 2006). Contrastingly, neutral theories assume all species to be ecologically equivalent and to have the same demographic rates including, birth, death, dispersal and speciation rates (Chave *et al.*, 2006). The structure of AMF as any other species community, comes only from stochastic processes and dispersal limitation (Hubbell, 2001).

Usually, AMF diversity is investigated using morphological methods that are recognised as being limited in their detection ability (Sanders, 2004; Chaudhary et al., 2008; Rosendahl, 2008). However, advances in molecular methods enable the detection of active AMF within the root. These more advanced methods in AMF diversity studies have, over the years, made AMF biogeography research more achievable (Hazard *et al.*, 2013). The capacity of high throughput sequencing (NGS) methods to reliably and accurately profile AMF communities at sufficient cost is evident. Insights into how AMF communities vary due to changes in the biotic and abiotic variables at certain scales have increased since the start of using NSG.

Although, AMF has proved to significantly influence the entire terrestrial ecosystem, their biodiversity is yet to be fully understood. Spore-based studies of AMF have described 214 species. This number has increased due to the DNA-based techniques, yet is believed to represent only a small fraction of the existing AMF diversity (Öpik *et al.*, 2008). There are difficulties in identifying AMF that lies hidden in a biotrophic lifestyle in the soil, with few distinct morphological characters, and little potential to produce dimorphic spores. This has resulted in placing many AMF species, phylogenetically belonging to different orders, into one genus (Glomus). On the other hand, those AMF individuals that form different spore morphs have been described as members of different orders. Furthermore,

AMF spores have great surviving capabilities, allowing them to remain in the soil until they are activated. Thus, the presence of spores may not reflect a symbiotically active organism community (Krüger *et al.*, 2009). Therefore, molecular methods have led to the preliminary understanding of the deterministic response of AMF communities to soil physico-chemical parameters, their function, and their demography in natural ecosystems (Redecker *et al.*, 2003; Dumbrell *et al.*, 2010a). As any newly introduced method, the use of DNA-based techniques in the field of studying AMF was rare until the discovery of the polymerase chain reaction –PCR (Saiki *et al.*, 1988). PCR later confirmed that variation within AMF communities mirrors the impacts of changes in the surroundings (Davison *et al.*, 2011), including changes in habitat range, green host, and possibility of interaction with seasons. These findings reveal that attempting to understand the mechanisms regulating AMF diversity at different scales is complex, and studies thus far have been insufficient (Davison *et al.*, 2011).

1.2.1. Niche, natural or stochastic theory

Despite a strong interrelationship between AMF and plant communities, we do not yet understand what governs AMF diversity or spatial distribution. The most common explanation for coexistence of multiple species in any system, however, is resource based niche-partitioning of species (Gause, 1936).The niche theory assumes that species differ in their traits, and often show trade-offs that enable them to coexist within communities for long periods of time (Chesson, 2000; Chave, 2004). Contrastingly, the neutral theory emphasizes the importance of stochastic events such as dispersal, local extinction and speciation (Chave, 2004). A negative correlation between distance among samples and

species similarity within soil types suggested that spatial structure may in fact be a good proxy for dispersal limitation (Lekberg *et al*, 2007). Both environmental and geographical variables together explained substantial portions of the variability in AMF community composition, compared with the environmental variables alone, which had a slightly stronger influence. Similarly, in a large-scale study of AMF communities from grassland and woodland habitats in England, both niche and neutral processes were shown to structure this community. Dumbrell *et al*., (2010b) indicated in that AMF communities are structured by environmental factors, and that they responded in an expectable deterministic means to changes in soil pH is evidence of the significant role of stochastic-natural processes, such as dispersal limitation.

Most recent studies have demonstrated that soil has a profound influence on the assembly of bacterial and AMF communities in the rhizosphere (Harpole & Tilman, 2007; Kraft *et al.*, 2008; Kembel, 2009; Dumbrell *et al.*, 2010a). Thus, occurrence of AMF symbiosis is influenced not only by the species of their host plant, but also by environmental variables including temperature, pH and soil fertility (Hu *et al.*, 2013). AMF are able to associate with plants with relatively low specificity and therefore the association is thought to be largely non-specific (Van Der Heijden, 2004; Smith & Read, 2008). Nevertheless, there is growing evidence of host-specific differences in plant responses to AMF, and of fungal responses to different plants species, indicating that the extent of plant growth promotion by AMF depends upon specific plant-fungal combinations (Adjoud *et al.*, 1996; van der Heijden *et al.*, 1998). Similarly, measures of growth of AMF species also depends on the associated host plant species (Bever *et al.*, 1996; Eom *et al.*, 2000). Moreover, AMF have been repeatedly shown to exhibit host-

specific growth responses (Bever, 2003) and to invoke differential growth responses to host plant species (van der Heijden et al., 1998; Klironomos, 2003). However, the proposed specificity in the plant–AMF association might occur at functional or ecological groups level of plants, and not necessarily at the level of plant species per se (Öpik *et al.*, 2009). Many of the recent observations, however, support the existence of low or very low partner specificity, and hence a wide choice of partners in plant–AM fungus combinations (Smith & Read, 2008). The degree of specificity or preference of these associations remains to be resolved (Öpik *et al.*, 2009), as recent studies showed the structure of AMF communities to defer over time according to their host phenology (Lauber *et al.*, 2013).

A distinct temporal variation assemblage of AMF may be observed in grassland habitats, due to a changed C supply. Further temporal variation in the structure of AMF communities could result of the seasonal cycle of niche and natural processes at the same time point. Although communities of AMF in forest soil show no significant changes in their composition between different time points in the growing season (May-September), and spatial structure in soil AMF communities was shown to be related to the heterogeneous vegetation of the natural forest study system (Davison *et al.*, 2012). However, there is evidence that distinct seasonal changes in the composition of AMF communities tends to develop late. AMF communities in a forest soil were the least distinctive in spring, but developed later in the season. Thus, predicting the composition and functions of AMFs within communities and ecosystems requires an understanding of these variables (Antoninka *et al.*, 2011). Öpik *et al*., (2009) concluded that methodological constraints in acquiring data on the natural diversity of AMF are

primarily due to the high labour demand and cost of molecular analyses. Introducing second-generation sequencing technologies to the studies of the ecology of AMF communities provide significantly improved throughput, at lower cost per sequence (nucleotide), enabling not only sequencing of genomes of nonmodel organisms (Ellegren, 2008) but also more powerful assessments of the complexity of natural populations by targeting specific genes (Dowd *et al.*, 2008).

1.2.2. Role of spatial and seasonal pattering of biotic and abiotic variables in changing the structure of AMF communities

Previous studies of AMF concluded that community structure and diversity of AMF is mainly governed by the level of water content (Wolfe *et al.*, 2006), soil texture (Lekberg *et al.*, 2007), nutrient availability (Fitzsimons *et al.*, 2008), along with host-plant preferences. However, species-specific interactions do not always occur, and AMF could be more specific to plant functional groups than to individual plant species (Öpik *et al.*, 2009). Additionally, the interaction between AMF and plants generates feedbacks that influence species diversity in fungal communities, and eventually, in plant communities (Bever *et al.*, 2001; Bever, 2002a). Therefore, the colonization level of an AMF community is more likely to be shaped by the host plant, local availability of inocula, and by AMF–plant feedback. Thus, AMF have a profound determinative effect on plant community dynamics and diversity (Bever *et al.*, 2001; Smith *et al.*, 2003; Parniske, 2008; Helgason & Fitter, 2009). The complexity of these properties in the ecosystem over space and/or time, known as environmental heterogeneity, is due to major factors affecting the composition, diversity and structure of soil microbial communities. However, the relative importance of the environmental heterogeneity is still poorly understood due to its dependence on spatial scales,

which also likely affects microbial communities (Ramette & Tiedje, 2007). Therefore, spatial variability in soil organism distributions is often regarded as random noise. Distributions of soil biota was considered at varying spatial scales, depending on the organism, the system studied, and the minimum and maximum spacing of the samples (Ettema & Wardle, 2002). Small scale heterogeneity in soil resources results in microhabitat diversity that promotes species coexistence through greater resource partitioning (Anderson, 1975; Giller, 1995). However, because of dispersal limitation and the complexity of the soil matrix, the importance of competition as a major structuring force is likely to be reduced to smaller scales, where potential competitors are using the same space (Ettema & Wardle, 2002).

Significant heterogeneity in distributions of soil organisms interacts with soil resource heterogeneity to form complex spatial patterns observed in soil communities, and these are, in turn, further altered by localized stochastic disturbances. For example, using spatio-temporal analyses on space–time species data, Dumbrell *et al*., (2011) described how limited dispersal, and soil pH led to complex spatiotemporal patterns of AMF, suggesting that stochastic dynamics contributed to the maintenance of AMF diversity at the landscape scale. The diversity of AMF communities differs in their seasonality as a consequence of many interacting factors, such as plant communities, soil site characteristics, and climate (Escudero & Mendoza, 2005; Dumbrell *et al.*, 2011). Different AMF communities resulted from changes in their host plants phenology over different seasons (Carvalho *et al.*, 2001), which in turn likely affected the C availability (Dumbrell *et al.*, 2011). Spatio-temporal patterns of soil glomalin and phosphatase activity in different managed semiarid Steppes also proved to have

significant effects on the dynamics of AMF communities (Wang *et al.*, 2014). However, studies of AMF communities at different spatial scales provided a more comprehensive view of whether geographical and/or environmental factors shape the distribution of AMF at various spatial scales (Ettema & Wardle, 2002; Hazard *et al.*, 2013). Our understanding of what drives soil biodiversity was hampered by the apparent contradiction of high species richness of soil communities and low degrees of resource specialization of constituent species (Anderson, 1975; Giller, 1995). A solution to the analysis of this problem lies in considering the spatial patterning of environmental factors and soil populations, which profoundly influence the nature of species interactions and their coexistence (Tilman & Kareiva, 1997). NGS is a powerful method that was suggested for assessing the complexity of natural populations by targeting specific genes (Dowd *et al.*, 2008).

1.2.3. Applying high-throughput next generation sequencing to studies of AMF communities

Until the early 1990s when DNA- based methods started to be applied in AMF studies, more than 200 species of Glomeromycota had been identified morphologically (Walker 1992; Krüger *et al*. 2009). However, this technique always suffered from limitations that prevented an accurate assessment of AMF diversity in the roots of individual plant species (Helgason *et al*. 1999). There are no unbiased methods for the identification of AMF at the species level, both for morphological identification, and other molecular techniques. Identifying AMF species is essential to reveal functional and ecological aspects of distinct AMF communities associated with different plants under different environmental conditions. This is mainly conducted by using DNA sequence based methods that are known to detect organisms at different community levels, depending on

reliable databases and tools (Krüger *et al.*, 2009). Molecular characterization of AMF is mostly achieved by PCR, using primers that target the rDNA regions as molecular markers that are AMF specific. This technique mostly results in the amplification of only a restricted number of Glomeromycotan taxa or DNA of nontarget organisms. The most comprehensive taxon sampling for the Glomeromycota covers the small subunit (SSU) rDNA region (SCHÜßLER *et al.*, 2001), for which a new, AMF specific primer pair was recently published, known as AML1 and AML2 (Lee *et al.*, 2008). Although, AM1 primer (Helgason et al., 1998), was proved to be unbiased (Cotton *et al.*, 2014) AML2 is perhaps suitable to amplify sequences from all AMF taxa (Krüger *et al.*, 2009). However, both primers target the SSU rDNA and are inadequate for species resolution of AMF (Krüger *et al.*, 2009). Therefore researchers have started targeting the internal transcribed spacer (ITS), and the large subunit (LSU) rDNA region, that is known to allow both robust phylogenetic analyses and species level resolution (Gamper *et al.*, 2009; Krüger *et al.*, 2009).

DNA-based techniques replaced the previous morphological methods that sever great limitations (Krüger *et al.*, 2009; Hazard *et al.*, 2013). These new techniques include the NGS that showed the AMF species richness was to be underestimated by other methods. For example, studies on AMF communities at a local scales reported that the numbers of AMF species and plant species in any community are often similar (Fitter et al. 2005). In another study of a boreonemoral forest ecosystem, only 32 AMF species were identified form the root of 57 plant species (Öpik et al. 2008). Vandenkoornhuyse *et al*. (2002) found that only one AMF species belonged to a morphologically defined species out of 24 taxa in a temperate grassland. Thus, using NGS in studies on AMF

communities showed the possibility that AMF species richness rivals that of plants within communities (Fitter *et al.*, 2005). This was recently supported by identifying 47 fungal operational taxonomic units (OTUs) within a 100m^2 boreal forest, that were hosted by an equivalent number of plant species (Öpik *et al.*, 2009). The majority of previously mentioned studies have focused on woodland and grassland habitats found mainly in Northern Europe, and research was generally conducted over small spatial scales. The role of AMF in other key habitats, such as saltmarsh habitats, remains unknown, and cannot be predicted based on the information from other habitats. Due to a paucity of studies on these habitats, and considering their great importance, our understanding of how such systems function, under different physical patterns, remains poor. Thus, understanding how spatiotemporal patterns of soil physiochemical properties drive heterogeneity of biodiversity of AMF communities in salt marshes is necessary.

1.3. Saltmarsh Habitats

A saltmarsh habitat is an intertidal area of fine sediment (Boorman, 2003) in most cases, providing an important nursery for small fish and invertebrates (Green *et al.*, 2012), and accommodating pioneer species that play critical roles in the earlier stages of saltmarsh formation (Boorman, 2003). These habitats usually result in fine sediment brought by water tides, and are stabilised by vegetation root nets that helps increase their size (Boorman, 2003). For stability salt marshes require protection from sea water, and therefore usually occur in lagoons. Under low frequency of sea waves, deposition of sediment creates extensive, gentle sloppy formations. Indeed, open coast salt marshes like Tillingham salt marsh on the east coast of England are to be significantly affected

by the delivery, erosion, and/or resuspension of sediment due to wave action (Möller., 2006). Once these formations are mature, plants modify the physical environment. For instance, plant species like *Spartina alterniflora* are able to withstand the full force of waves along the shore (Wiegert *et al.*, 1981). This process, known as the saltmarsh zonation, is a clear sign of the characteristic landscape, and reflects the strong environmental and topographic gradients from sea to land (Adam, 1993, Flowers et al., 1986). In such habitats, the degree of salinity governs the competitiveness of plants (plant zonation), and other parameters like soil texture, structure and pH are of minor importance for plant growth (Sherwood et al., 2000). Thus, the lower bounds of saltmarsh vegetation typically includes a thin pioneer community of glassworts (*Salicornia* spp.), and invasive common cord-grass (*Spartina anglica*) growing on otherwise bare estuarine mud.

Typical middle range saltmarsh plants include *Triglochin maritima*, *Juncus gerardii*, *J. maritimus*, and *Atriplex hastata* (Van Duin *et al.*, 1990). The upper salt marsh terrestrial plants can tolerate infrequent inundation by seawater. These include grasses such as Red Fescue (*Festuca rubra*), Creeping Bent (*Agrostis stolonifera*), tall-herbs like Corn Sow-thistle (*Sonchus arvensis*), Sea Rush (*Juncus maritimus*), coastal varieties of Common Couch (*Elytrigia repens*), and hybrid couches. Plant diversity starts to increasing landwards, where plants are irrigated by freshwater (Van Duin *et al.*, 1990). However, these areas may be colonised by tall vegetation to form reedbeds and other fen communities which occur alongside coastal species like Sea Club-rush (*Bolboschoenus maritimus*). Despite their role as major habitats, salt-marshes are well known as natural barriers that protect farmland from the sea water. In a country like the United

Kingdom, coastal salt marshes share the same ecological importance as mangroves, by serving as a nursery to fish, and providing increased food and shelter (Green *et al*., 2012). This was estimated to add £51.2 billion (1994-95 prices), to the total UK annual turnover by the marine-related sector, that reached a total of £27.8 billion (Turner *et al*., 1998). Moreover, in an important ecosystems like salt marshes, both fungi and bacteria are recognized as key components, providing decomposed organic matter from plant litter, transforming pollutants (Smith & Hollibaugh, 1993; Benoit *et al.*, 2003), and performing other ecosystem services (Harris, 1999). Among these microorganisms are the root colonisers AMF, that play an important role in improving plants succession under different stresses and habitats.

1.3.1. Availability of AMF in saline and water-logged habitats

AMF are obligate aerobes (Khan, 1993; Miller & Sharitz, 2000), thus, most wetland plants were thought of as non-mycorrhizal (Khan, 1974; Anderson *et al.*, 1984), and/or AMF have little significance under these conditions (Bohrer *et al.*, 2004). However, the presence of AMF colonisation of low marsh plants species is evidence that showed symbiosis of AMF under the water-logged conditions (Carvalho *et al.*, 2001). Although these conditions may cause slow AMF spore germination (Harley & Smith, 1983; Hildebrandt *et al.*, 2001), high symbiosis was reported in the Canadian saltmarshes (Van Duin *et al.*, 1990). AMF was found to be associating with the upper marsh plants *Aster tripolium*, *Plantago maritima*, *Glaux maritima* and *Festuca rubra* (Van Duin *et al*., 1990), as well as *Spartina patens* and *S.alterniflora* at the low marsh zone (Cooke *et al*., 1993, Rozema *et al*., 1986). The last two plants species grow in a nutrient rich soil which may cause AMF symbiosis to have a negative effect, due to unfair trade-off with their

host plant (Johnson *et al.*, 1997; Hart *et al.*, 2003). However, an improved biomass was reported in aquatic plants that formed an association with AMF (Andersen and Andersen, 2006). Given that most of the plants in these habitats are halophytes, salinity may not have a significant effect on their ability to grow at lower zones. The role of AMF might be to improve the water relationship under conditions of water-logging in many habitats (Mason, 1928; Khan, 1993; Al-Garni, 2006; Evelin *et al*., 2009).

A majority of AMF studies focus on woodland and grassland habitats found mainly in Northern Europe, and generally conduct research over small spatial scales. Thus, the role of AMF in other key habitats, such as the saltmarsh habitats, remains unknown, and cannot be predicted based on the information from other habitats. Due to a paucity of studies on these habitats, and in light of their great importance, our understanding of how such systems function under different physical patterns remains poor. Thus, understanding how spatiotemporal patterns of soil physiochemical properties drive the biodiversity is a key in understanding the functional significance of saltmarsh habitats. Most previous attempts have focused on habitats or organisms (Rountree & Able, 2007).

1.4. Aims& objectives

This study aims to examine whether biotic or abiotic based mechanisms best explain the composition and structure of natural AMF communities in saltmarshes. The collected data set is used to examine the diversity of AMF in saltmarsh habitats, and to investigate the relative importance of changes in these patterns by examining species abundance distributions. If niches regulate the composition of AMF diversity in this habitat I predict that:

1- The heterogeneity and physicochemical properties of saltmarshes are spatiotemporally dependent (Nedwell & Azni bin Abdul Aziz, 1980; Piceno *et al.*, 1999).

2- Patterns of dissolved nutrients, pH, temperature and salinity play an important role in structuring AMF communities (Öpik *et al.*, 2006) in saltmarsh habitats, as reported in grasslands and woodlands studies. Changes in these patterns over different seasons and at different scales cause significant effects on the diversity of AMF in saltmarshes.

3- Different AMF species create different alterations in their host's tolerances under water-logged and high-salinity conditions.

Theses hypotheses can be examined by addressing the following objectives:

1. To extract and preserve plant roots from each soil core for molecular analysis of AMF communities and microscopy-based (if possible) analysis of AMF colonisation.

2. To quantify the sediment's physicochemical factors likely to influence the community composition and diversity of AMF – this includes measuring sediment/soil PO_4^{---}, NH_4^+, NO_3^-, NO_2^-, organic C, salinity, pH, moisture, plants species, plants biomass and root density.

3. To use molecular methods in the investigations of AMF species in saltmarsh samples by extracting DNA from dry roots and profiling differences in AMF species using different sets of primers for NGS.

4. To examine how conditions of high salinity and water-logging, in the low and middle zones on the saltmarsh will affect the composition of AMF communities.

Chapter two

2. Material and Methods

2.1. Sampling area

All the sediment samples were collected using a soil corer, that provides 5cm × 15cm samples. Winter samples were collected in January, and summer sampling started in May, of 2013, in both Essex and Lancashire, UK (Table 2-1). The salt marshes of Essex are located in the East Anglian coastline (Figure 2-1). Sampling was carried out using the same sites selected for the study of coastal biodiversity & ecosystem service sustainability (CBESS).

Essex marshes consist of clays and silts, with high organic matter content, dominated by Salt Grass (*Puccinellia)* and Saltbush (*Atriplex)* communities (National Vegetation Classification SM13 and 14). Lancashire is a large embayment in the northwest of England, at the confluence for four principal estuaries, the Leven, Kent, Lune and Wyre, which drain through intertidal flats of sand and mud. The predominant sediment types are fine sands and silts with the finer particle sizes occurring in the sheltered innermost areas of the Bay, and at the top of the shores.

Lancashire marshes are underlain by fine sands and areas of higher organic content, associated with standing water in the areas where there is some freshwater influence on the landward margins. Except Cartmel Sands, Lancashire marshes are heavily sheep grazed, and have a long history of land use. Saltmarshes of Lancashire are found on predominantly sandy bedrocks and have a macrotidal regime (Davidson, 1990; Adam, 2002). These intertidal habitats are mainly dominated by grasses *Juncus gerardii Loisel, Agrostis stolonifra L* and *Festuca rubra* (Adam, 1976).

Table 2-1: the location and ecology of each sampling site used in this study.

Location	Site	Description
Essex	Fingringhoe Wick (51◦ 49'N, 0◦ 58'E)	A polyhaline estuarine fringing salt marsh of approximately 120 ha. The surface is dissected by creeks of a complex dendritic structure and saltmarsh pans in the upper regions. Tidal exposure in this site is minimal.
	Tillingham (51◦ 41'N, 0◦ 56'E)	An extensive euhaline open coastal marsh, approximately 11 km in length and up to 1 km wide covering an area of 409ha. The marsh is dissected by shore-normal linear drainage channels (which extend into mudflat 'mud mound' topography). Tidal exposure in this site is severe.
	Abbotts Hall (51◦ 47'N, 0◦ 52'E.)	A small (approx. 24 ha) restricted embayment saltmarsh, located in Salcott channel. The location is sheltered from winds and waves from the SW and NE, with maximum, but very limited, fetch under south easterlies (ca. 2 km from 121^{o} from N) Tidal exposure in this site is intermediate.
Lancashire	West Plain (54◦ 9'N, 2◦ 58'W)	A salt marsh located on the southern tip of the Cartmel peninsula, bounded by the river Kent to the east and the river Leven to the west, exposing it to the prevailing south westerly winds (maximum fetch is 264 km at 235^{o} from N). Tidal exposure in this site is minimal and intermediate.
	Cartmel Sands (54◦ 10'N, 3◦ 0'W),	A salt marsh situated at the mouth of the Leven estuary on its eastern bank, with exposure to the dominant south westerly winds (maximum fetch is 107 km at 210^{o} from N). The marsh is 3.2 km long and 1 km at its widest point. Tidal exposure in this site is minimal.
	Warton Sands (54◦ 8'N, 2◦ 48'W)	An extensive open coastal marsh, approximately 3 km long and up to 2 km wide. The marsh faces south east and is exposed to the prevailing south-westerly winds and waves (maximum fetch is 266 km at 237^{o} from N). Tidal exposure in this site is severe.

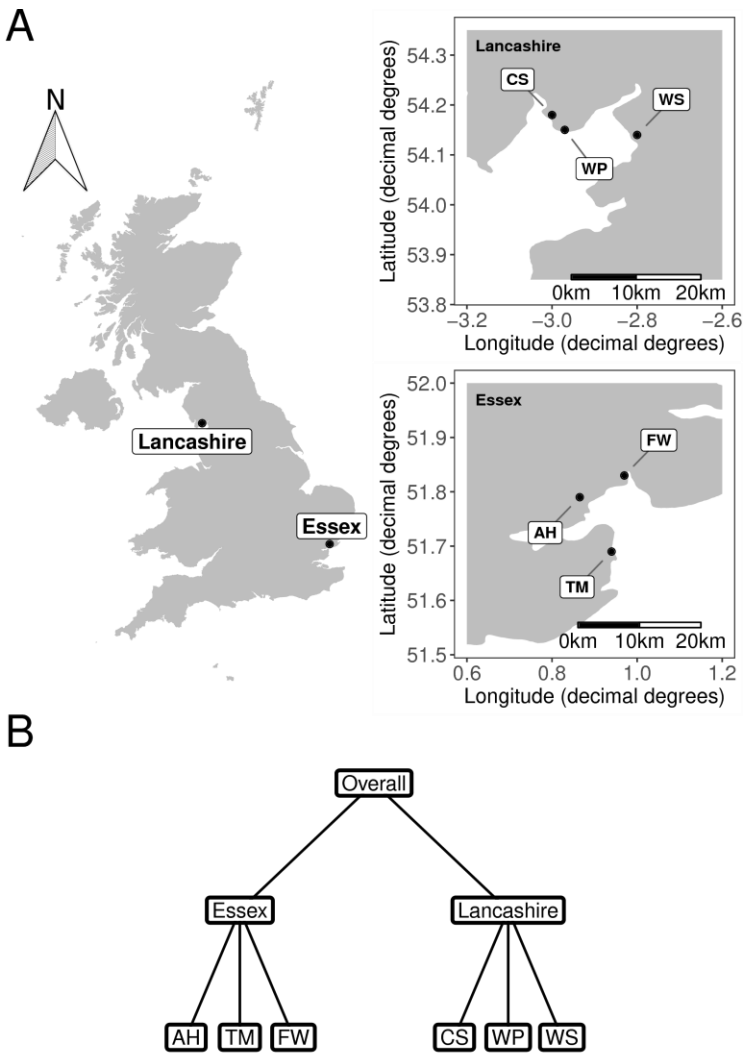

Figure 2-1: Sampling strategy. (A): UK map with sampling regions (left panel) and sampling sites (right panels). Site labels are as follows; AH = Abbott's Hall, FW = Fingringhoe Wick, TM = Tillingham Marsh, CS = Cartmel Sands, WP = West Plain and WS = Warton Sands. Coordinates are presented in a WGS84 coordinate system. (B): Schematic of fungal OTU richness modelling approach. Models were conducted at the site level (bottom row), regional level (middle row) and with all data pooled (top row).

2.1.1. Sampling design

At each site, 22 quadrates of $1m^2$ were randomly selected at for different spatial scale to form a nested design. This nested design resulted in 6 sampling sites (2 locations × 6 salt marshes × 2 seasons) that covered different zones on each site (Figure 2-2). This design allowed assessing the effect of spatiotemporal patterns including tidal, temperature and different plant species on nutrients fluxes that drove changes in species diversity.

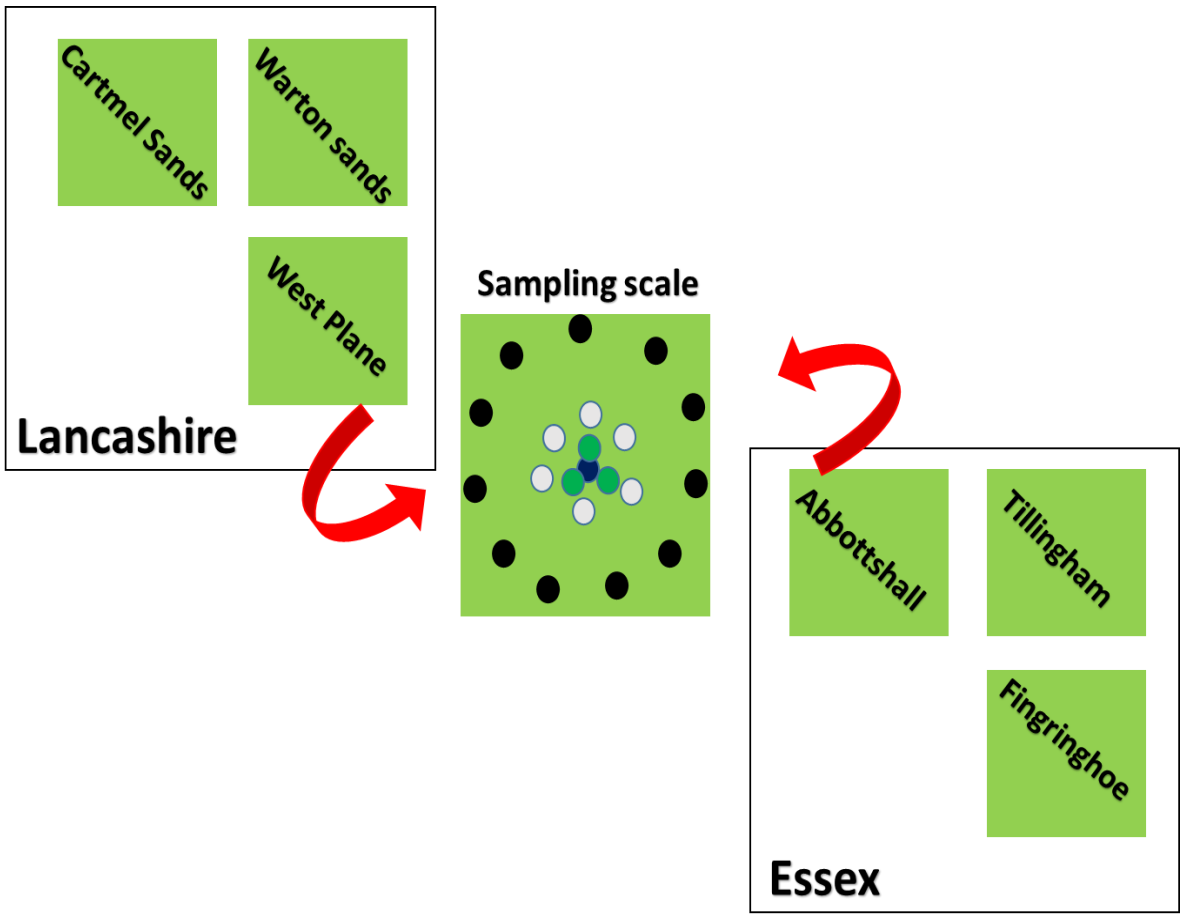

Figure 2-2: Summary of the orthogonal sampling design, identifying the 6 unique sampling points that were sampled during two seasons to represent biodiversity ecosystem service contexts. At each site 22 sampling points were randomly distributed over different scales ranging from 1(blue), 10 (green), 100 (white) and black for the 1000m.

2.1.2. Samples preparation

Fieldwork was conducted during winter (January) and summer (September) 2013 from 3 salt marshes in Essex and Lancashire, UK (Table 2-1, and Figure 2-1). A stratified random sampling design was used to place 22 quadrats in each marsh during each season, whilst maximising the range of spatial separation between samples (Raffaelli et al. 2014). From each quadrat, a sediment core (5 cm diameter, 15 cm depth) was collected during each season. Cores were transported from the field on dry ice before being frozen at -80℃. Plant roots were extracted and dried at 75℃ for 5 days, before being homogenised and stored in sterile Eppendorf tubes at -80℃ for molecular analyses. Pore water was extracted from 40 g of sediment from each core, by centrifugation at 15,000 rpm at 4℃. This pore water was used for salinity and pH analyses. The plant community in each quadrat was quantified as described by Ford *et al*. (2016) with the percentage cover of species, and above- and below-ground biomass measured.

2.2. Molecular Examination of AMF Diversity

DNA templates were obtained from 0.05 g homogenised dry root samples from 264 sediment cores using the MoBio PowerPlant DNA isolation kit, following the manufacturer's instructions (MoBio Laboratories Inc., Carlsbad, CA, USA). Clean roots were used to ensure only active fungi were included. Resultant DNA was then used to prepare the NGS libraries as follows (Figure 2-3):

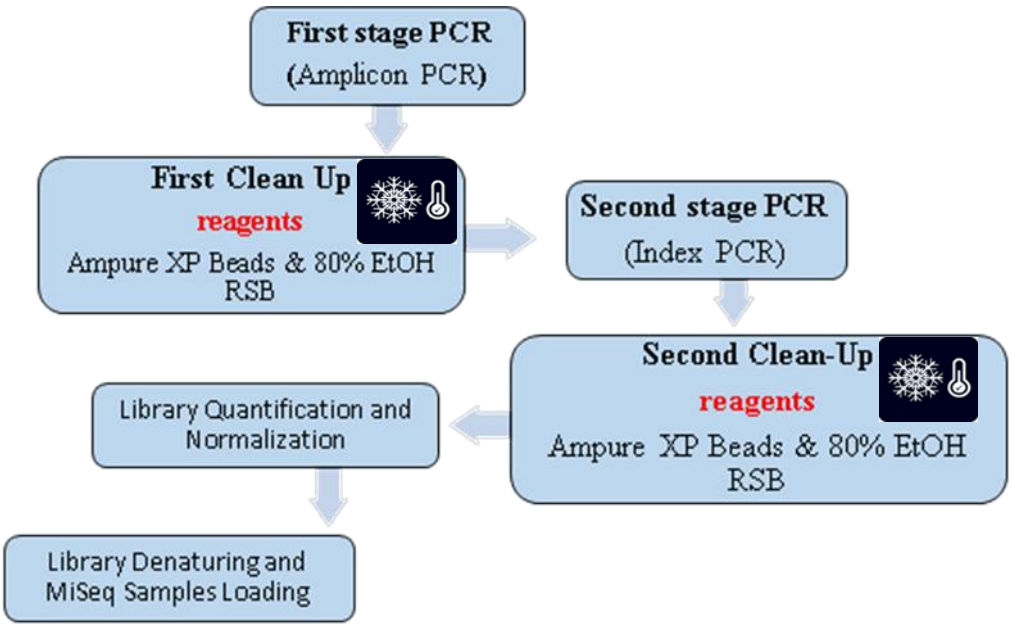

Figure 2-3: Illumina HiSeq Library Preparation Workflow. Save stop points are labeled after each cleaning step.

2.2.1. Amplifying and characterizing AMF DNA in plant roots

To profile the root, and associated AMF communities in these samples, a Nextera XT dual indexing approach was used. This approach requires two rounds of PCR amplification in which the first is used to amplify the gene of interest, and the second is used to add sample specific indices, allowing samples to be multiplexed. For the first PCR, the partial small-subunit ribosomal DNA fragments

of about 550 base pairs were amplified using AM1 primers. These primers were designed to amplify fungal and exclude plant DNA sequences (Helgas*on et al*, 1998), and the universal eukaryotic primer NS31 (Simon *et al.*, 1992). The 18S primer set amplifies most AMF species, except *Archaeospora*, *Paraglomus,* and some *Glomus* group B species (Redecker *et al*., 2000). Thus, the first-round PCR, was repeated using the primers ITS1f and ITS2 (Gardes & Bruns, 1993; White et al., 1990 respectively), which were modified to contain Illumina specific sequencing adaptors, a commonly used pair of primers in molecular studies of fungi (e.g. Buée et al. 2009; Pellissier et al. 2014; Dini-Andreote et al. 2016; Vannette et al. 2016). The ITS region was amplified in 25µl reactions using 12.5µl REDTaq® ReadyMixTM (Sigma-Aldrich Co.), 5µl of each (1µM) primer and 2.5µl of DNA template. Where necessary, 0.05 µl of T4 Gene32 protein (Roche Diagnostics lid, W. Sussex, UK) was added to PCR reactions to prevent inhibition from humic acids or other inhibitors (Kreader 1996). REDTaq ReadyMix DNA polymerase contained MgCl2 20mM Tris-HCl, pH 8.3, with 100mM KCl, 3mM MgCl2, 0.002 % gelatin, 0.4mM dNTP mix (dATP, dCTP, dGTP, TTP), stabilizers, and 0.06unit/mL of Taq DNA Polymerase. The PCR program was carried out in Eppendorf Mastercycler® personal, and consisted of an initial DNA melting step of 3 min at 95°C, followed by 32 cycles each of 30 s at 94°C, 40 s at 58°C for annealing and 45 s at 72 °C for extension. After a final extension step of 10 min at 72°C, PCR products were kept at 4°C.

2.2.2. Amplifying and characterizing fungal DNA in plant roots

To profile the root associated fungal communities in these samples, a Nextera XT dual indexing approach was used. This approach requires two rounds

of PCR amplification in which the first is used to amplify the gene of interest, and the second is used to add sample specific indexes, allowing samples to be multiplexed. For the first PCR, the fungal internal transcribed spacer region of the rRNA gene was amplified. The ITS region has become the standard barcode of choice for molecular studies on fungal communities, as it is often easier to amplify than other marker genes, and has good species level discrimination (Schoch *et al.* 2012). For the first-round of PCR, the primers ITS1f and ITS2 (Gardes & Bruns, 1993; White et al., 1990 respectively) were modified (Table 2-2) to contain Illumina specific sequencing adaptors, which are a commonly used pair of primers in molecular studies of fungi (e.g. Buée et al. 2009; Pellissier et al. 2014; Dini-Andreote et al. 2016; Vannette et al. 2016). The ITS region was amplified in 25µl reactions using 12.5 µl REDTaq® ReadyMixTM (Sigma-Aldrich Co.), 5µl of each (1µM) primer and 2.5µl of DNA template. Where necessary, 0.05µl of T4 Gene32 protein (Roche Diagnostics lid, W. Sussex, UK) was added to PCR reactions to prevent inhibition from humic acids or other inhibitors (Kreader 1996). The PCR program consisted of an initial DNA melting step of 3 min at 95°C, followed by 32 cycles each of 30 s at 94°C, 45 s at 59°C for annealing and 1 min at 72 °C for extension. After a final extension step of 10 min at 72°C, PCR products were kept at 4°C.

Table 2-2: both 18S and ITS primer sequences in the direction from 5' to 3' with Illumina adapter overhangs in bold.

Region	Primer name	Illumina overhang adapter	Primer sequence
18S rDNA	AM1 (Forward primer 5'-3')	TCGTCGGCAGCGTCAGATG TGTATAAGAGACAG	TTGGAGGGCAAGTCTGGTG CC
	NS31 (Reverse primer 3'-5')	GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAG	GTTTCCCGTAAGGCGCCGA A
ITS	ITS1-F (Forward)	TCGTCGGCAGCGTCAGATGTGTATAAG AGACAG	CTTGGTCATTTAGAGGAAGT AA
	ITS2 (Reverse)	GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAG	GCTGCGTTCTTCATCGATGC

2.2.3. First DNA purification

The DNA gel below shows the amplicon PCR product, with a size of ~600bp in length, which confirms the successful attachment of the Illumina overhang (Figure 2-4). PCR products were cleaned up following Illumina library preparation protocol by using Agencourt AMPure XP - PCR Purification beads (a carboxyl-coated magnetic particle that can reversibly bind DNA in the presence of polyethylene glycol (PEG) and salt) by (Beckman Coulter (UK) Ltd). This step should remove the primers and any products below 100bp. To ensure the right size was obtained in this step, random samples were selected to run on Agarose gel aganiset DNA marker (Figure 2-4).

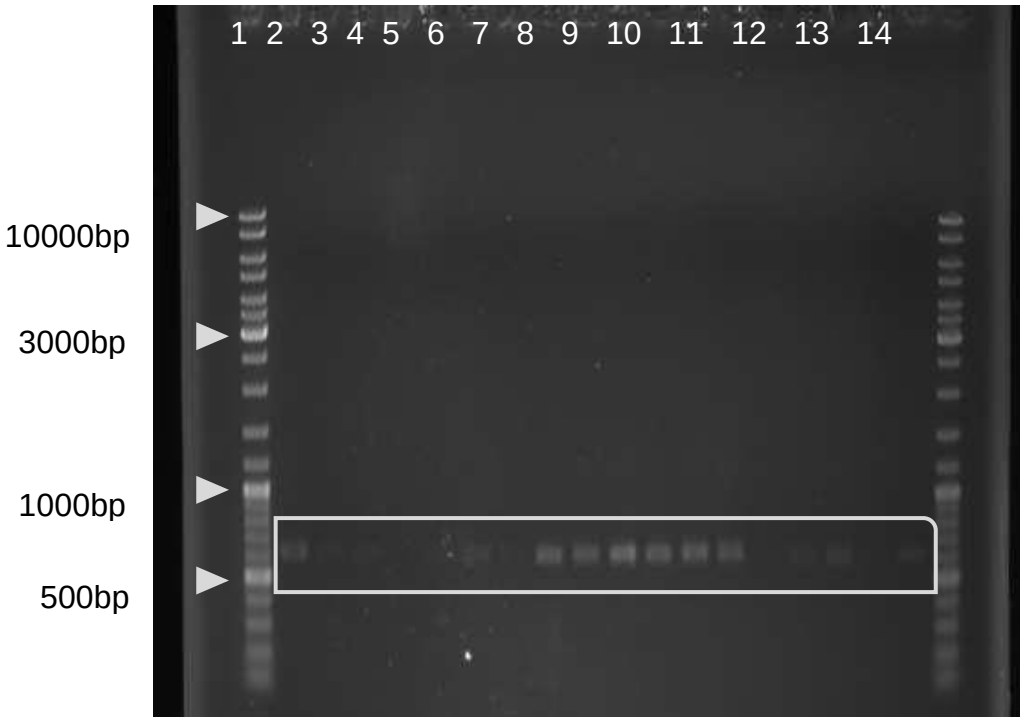

Figure 2-4: Agarose gel image for amplicon of PCR product, showing the size of 11 amplified samples at 600bp against DNA marker that loaded into the lines number 1 and 14. The band clarity may vary as a result of different DNA abundance in each sample.

2.2.4. Second stage PCR; Index PCR

The indexing (second) round of PCR was carried out using 5µl of bead purified PCR product as a template. Samples were added into a new PCR mix of 5µl of Nextera i5 and i7 index, 25µl of REDTaq® ReadyMix™ (Sigma-Aldrich Co.) and 10 µl of PCR water (Bioline Reagents Ltd, UK). The PCR program consisted of an initial DNA melting step of 3 min at 95°C, followed by 8 cycles each of 30s at 95°C, 30 s at 55°C for annealing and 30 s at 72°C for extension. After a final extension step of 5 min at 72°C, PCR products were kept at 4°C. PCR products were purified using Agencourt AMPure XP PCR Purification beads (Beckman Coulter (UK) Ltd, High Wycombe, UK) as before. Random samples were then selected to be run on Agarose gel to ensure that a successful indexing was done (Figure 2-5).

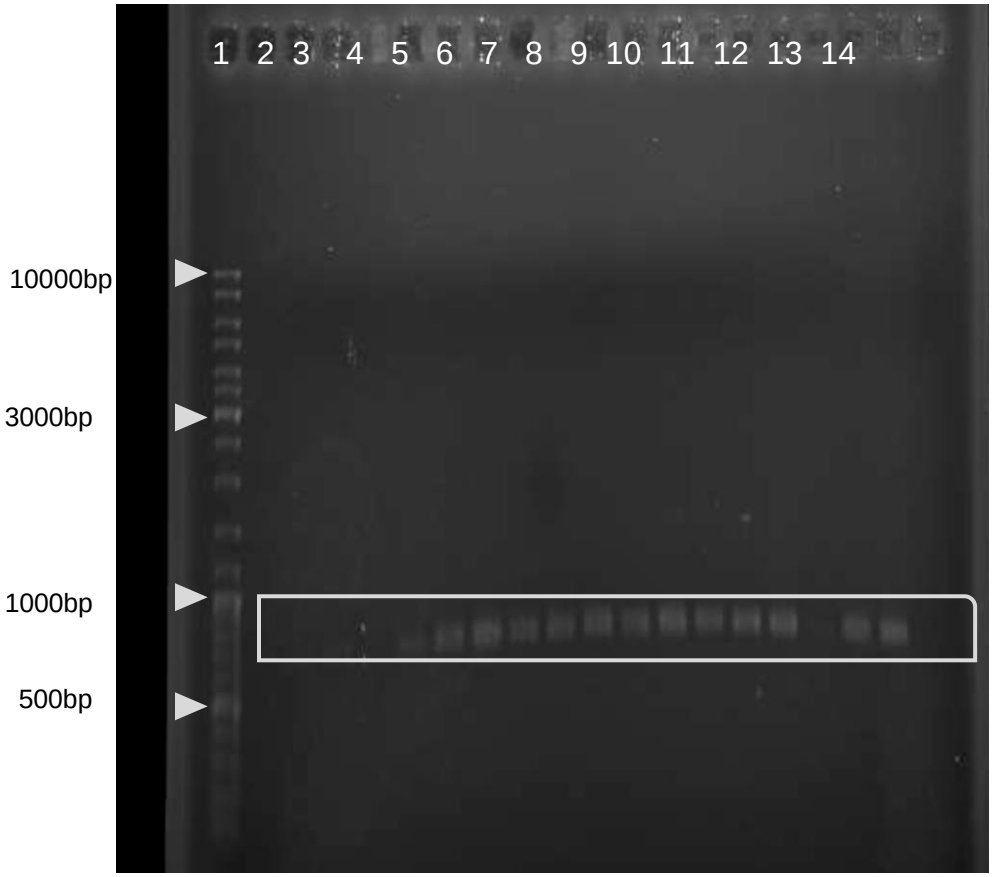

Figure 2-5: Agarose gel image of index the PCR product, showing the size of 12 indexed samples at 700bp against DNA marker loaded into the first line. Increased size proves that the attachment of Illumina index to these samples was successful. Band clarity may vary as a result of different DNA abundance in each sample.

2.2.5. Library Quantification and sequencing

Quantification of PCR products was done using the PicoGreen® dsDNA quantification assay (Thermo Fisher Scientific Inc. USA), on a POLAR star Omega (BMG LABTECH GmbH, Germany) plate reader. All PCR products from the second PCR samples were pooled in an equimolar concentration. The concentration and length of these pooled amplicon were verified using an Agilent 2100 Bio-analyser compliance solution. ITS sequencing was done on an Illumina HiSeq 2500 at the Earlham Institute (Formerly the Genome Analysis Center, Norwich Research Park, Norwich, NR4 7UH, UK), and the 18S sequences were

done on Illumina MiSeq V3 by the same provider. Different platforms were used due to the reported problem with the V3 kit from Illumina.

2.3. Pore water extraction and colorimetric determination of soil nutrients

264 soil cores were cut vertically to allow access to water at different depths. Then, 50g of each sample was mixed with 15ml of MQ in a 50ml Falcon tube. All samples were placed into an ultrasonic bath for 20 min, with continuous water changing to keep the low temperature. Sediment samples were placed in centrifuge for 20 min at 13,000 rpm and 5°C for water extraction. Pore water samples were then syringe filtered through sterilized 0.2µm Minisart® Filters.

Determination of dissolved inorganic nutrients (PO_4^{---}, NH_4^+, NO_3^-& NO_2^-) in pore water samples was done using Seal analytical continuous-flow Auto Analyser (AA3 from SEAL Analytical, Ltd.), following the methods described by (Hager & Atlas, 1972; Gordon, 1993). 10ml of each sample was diluted using Millipore water, with salinity that made up to match the average salinity of the samples. The previous step ensured that nutrients were within the range of the AA3 machine (20mM).

AA3 passes filtered pore water samples through a cadmium reduction column, where nitrate is quantitatively reduced to nitrite. Sulphanilamide is introduced to the sample stream followed by N-(1- naphthyl) ethylenediamine dihydrochloride which couples to form a red azo dye. The stream is then passed through a flowcell and the absorbance measured at 520nm. A similar technique is employed to analyse the nitrite, except no cadmium column is used. This is an automated procedure of the Armstrong (1967), in which the nitrate concentration is calculated by subtracting the nitrite value from the combined Nitrate + Nitrite (N+N) value (Armstrong *et al.*, 1967).

AA3 employs an automated method of Murphy and Riley to measure phosphate. The addition of the acidic solution of ammoniummolybdate to the water sample produces phosphor-molybdenum, which is later reduced to phosphor-molybdenum acid (a blue colour compound), following the addition of ascorbic acid at pH<1. The reaction product is automatically heated to ~37°C to enhance colour development and passed through a flow cell to measure the absorbance at 880nm (Murphy & Riley, 1962).

Finally, the method of Kerouel, R and Aminto, A (1997) was used to analyse ammonium by forming fluorescent species that are proportional to the ammonia concentration. In this method samples react with ophthaladehye (OPA) in the presence of borate buffer and sodium sulphite at 75°C. All samples were then passed through a flow cell and measured at 460nm, following an excitation at 370°C (Kérouel & Aminot, 1997).

2.3.1. Reagents preparation

All reagents were prepared using Millipore water with an adjusted salinity that matched the average salinity of the samples. Salted water was prepared in large 10 litre containers by adding sodium chloride to Millipore water.

2.3.2. Phosphate Analysis

Reagents for this analysis were prepared in the lab according to the following protocol: 3g of ammonium molybdate was dissolved in 500ml of Millipore water containing 32ml of concentrated sulphuric acid and 11ml of potassium tartrate, then brought up to 1 litre volume with cooled Millipore water and stored in a dark PE bottle in a refrigerator.

Ascorbic acid solution was made by adding 2g of ascorbic acid, dissolved into 600ml of Millipore water. Then, 10ml of acetone and 1.8g of sodium dodecyl

sulphate (SDS) were added before the solution was brought up to 1 litre volume using Millipore water and refrigerated. Finally, to ensure no P remained in the channels between samples during the analysis, a solution of 10g of the sodium dodecyl sulphate (SDS) was dissolved in 1 litre of Millipore water.

2.3.3. Standards

Every nutrient measured against a standard curve that was prepared in the lab, according to the following protocol: A stock standard of 10mM of nitrate (Na_2NO_3), nitrite ($NaNO_2$), potassium phosphate (KH_2PO_4) and ammonium sulphate (NH_4SO_4) were made in a volume of 250 ml. Then 25ml of nitrate and nitrate were added to 12.5ml of potassium phosphate and 6.25ml of ammonium sulphate in a single flask, and made up to 250ml using Millipore water, used to make serial dilutions (Table 2-3).

Table 2-3: Composition of a standards solution for nutrients analysis in pore water. The range of each standard was adjusted based on the highest read for each nutrient, which was investigated by running random samples from each site and season.

Compound	Standard rang in µM					
Na_2NO_3	1.162	1.196	3.981	5.95	8.175	9.893
KH_2PO_4	2.304	4.146	7.983	11.734	15.798	20.251
$NaNO_2$	1.146	2.105	3.996	5.677	7.934	10.194
NH_4SO_4	2.085	4.154	8.3	12.129	16.031	19.719

2.3.4. Salinity and pH measuring

To determine salinity, a hand-held refractometer (Bellingham & Stanley Ltd. Registered in England and Wales) was used using the extracted pore water. Salinity is required for accurate determination of the pH of sea water using a glass / reference electrode cell (Whitfield *et al.*, 1985). In this automated analysis, the values of pH were determined experimentally from sequential measurements of the (*E*) of the cell

Reference Electrode	concentrated KCl solution	Test solution	glass (H^+) electrode

In a standard buffer (S) of known (defined) pH and in the sea water sample (×) using the following stat:

$$pH(x) = pH(S) + \frac{Es - Ex}{Rt\ 1n10/F}$$

Where *F* is the free concentration of hydrogen ion in sea water.

2.3.5. Moisture and total organic carbon measurements

To determine the moisture content in the sediment, 5g of each frozen sample was oven dried at 75°C for 72 h, then the following were measured to calculate the final water content:

i) Weight of the clean ceramic container (Weight 'W_1').

ii) Weight of the wet soil specimen in the container (Weight 'W_2').

iii) Weight 'W_3'of the container with the lid and the dry soil sample.

Thus, the water content (w) was calculated as follows:

$$w = [W_2\text{-}W_3] / [W_3 - W_1]*100\%.$$

Dry soil samples were also analysed for total organic C on a TOC-5000 (Shimadzu Corporation, Kyoto, Japan). The organic C component was combusted to CO2 at 680°C and detected on an infrared gas analyser. Extractable OC was determined as total organic C in extracts from marsh soil.

Chapter three

3. The role of biotic and abiotic variables in determining community composition of arbuscular mycorrhizal fungi

3.1. Summary

- Understanding the dynamics of AMF communities is essential for predicting their important role in any ecosystem. Yet, most previous AMF studies cover grassland and woodland, and little is known from other habitats. This includes salt marsh habitats, where AMF is thought to be rare, or to have less important due to both high salinity and water-logged conditions.
- Using the NGS techniques, AMF was surveyed in 66 individual root samples of 32 plant species, along a soil physio-chemical gradient. Regardless of the high level of soil moisture and salinity, plants in Lancashire salt marshes were heavily dominated by AMFs belonging to *Glomeraceae* family, along with few species from other AMF families at the middle and upper zones. However, only a low number of species were recorded in the roots of pioneer zone halophytes like *Salicornia.europaea*.
- Results revealed a significant role of abiotic variables (such as salinity and pH) in changing the composition of AMF communities in these salt marshes. Soil pH and salinity significantly influenced the abundance of different AMF species, causing the number of predominant species to rise, especially in the pioneer zone. The lower stressed conditions at other zones enhanced the number of plant and thus AMF diversity by maintaining the level of competition within the rhizosphere area. AMF composition showed a strong response to soil physical factors in these salt marshes, confirming the high sensitivity of soil microbes toward changes in their surrounding environments.

3.2. Introduction

Understanding the relative importance of changes in different abiotic and biotic controls over species diversity has become a central aim in ecological research (Preston, 1948; Peet, 1974; Hamilton, 2005; Ieno *et al.*, 2006). Biodiversity has been considered as the main driver of the ecosystem functioning. Thus, changes in species diversity can directly influence ecosystem processes (Pimm *et al.*, 1995; Loreau & Hector, 2001), resilience and the resistance to environmental change (Van Cleve *et al.*, 1991; Wolters *et al.*, 2000).

Morphological studies of microbial communities showed their distribution to follow the Baas-Becking hypothesis, which predicted that “everything is everywhere, but, the environment selects” (Baas-Becking, 1934). However, a new interest in understanding microbial biogeography has emerged since the involvement of molecular-based approaches in the studies of microbial diversity (Martiny *et al.*, 2006; Prosser *et al.*, 2007; Ramette & Tiedje, 2007).

Recent studies suggest that complexities in microbial spatial patterns are greater than the Baas-Becking hypothesis predicts. The effect of spatial patterns on microbial diversity remains detectable with and without environmental heterogeneity (Ramette & Tiedje, 2007; Wang *et al.*, 2008; Martiny *et al.*, 2011; Van Der Gast *et al.*, 2011; Hazard *et al.*, 2013). Indeed, both spatial scale and environmental heterogeneity can be an important contributor to community structure (Tilman, 1997). Although spatial patterns of soil biota, and the factors that determine them can influence spatial patterns of decomposition, nutrient supply and root herbivory ultimately affect the spatial structure of plant communities. Most empirical and modelling studies have concentrated on aboveground biota (Ettema & Wardle,

2002). For example, organisms that are present in the rhizosphere microbiota can have profound effects on the growth, nutrition and health of plants, directly and/or indirectly affecting the composition and biomass of plant communities in natural ecosystems (Kardol *et al.*, 2007; Schnitzer *et al.*, 2011). Numerous organisms contribute to these processes, leading to countless interactions between plants, antagonists and mutualistic symbionts, both below and above the ground (Bennett & Bever, 2007; Vannette & Rasmann, 2012).

An example of one group of functionally important rhizosphere microorganisms is the arbuscular mycorrhizal fungi AMF. Arbuscular mycorrhizal fungi(AMF) (phylum Glomero-mycota; Schüßler et al., 2001b) form an important symbiosis with 80% of all terrestrial plant species (Kennedy & Smith, 1995; Smith & Smith, 1997). By developing a specialised area called the symbiotic mycorrhizal interface that allows interaction with the host plant, AMF facilitates the horizontal transfer of a range of beneficial impacts including increased P and N uptake (Jin *et al.*, 2005; Hodge & Fitter, 2010). Although different AM taxa are capable of colonization, their ability to promote plant succession varies based on the matching of plant and fungal species (Nemec, 1979; Powell *et al.*, 1982; van der Heijden *et al.*, 1998). Therefore, anything that will affect the functional diversity of AMF will influence the plant species, which can be an important tool in the restoration of plant communities in disturbed habitats (Smith *et al.*, 1998). Thus, AMF have a profound determinative effect on plant community dynamics and diversity (Bever *et al.*, 2001; Smith *et al.*, 2003; Parniske, 2008; Helgason & Fitter, 2009). As an obligate biotroph, AMF depend on green plants to supply the carbon compounds essential for tissue

production and survival (Ho & Trappe, 1973), and their populations are thus entirely dependent on the formation of mycorrhizas.

Given that most of the AMF extra radical hyphae are in direct physical contact with the surrounding soil, they help to increase the surface area for nutrient exchange (Hart & Reader, 2002). As in any other rhizosphere, microbial changes in the soil physical properties should influence the structure of AMF communities. Ecosystem type, soil pH, soil moisture, total soil C and N and disturbance regime are all factors that influence AMF community structure (Johnson *et al.*, 1991; Dodd *et al.*, 2000; Egerton-Warburton & Allen, 2000; Carvalho *et al.*, 2001; Dumbrell *et al.*, 2011). AMF colonization decreases as a result of phosphorus fertilization in agriculture studies (Barea, 1991; Antoninka *et al.*, 2011), and has been shown to mediate the effect off increased N (Johns*on et al*, 2004). The enrichment of N can affect AMF symbioses by changing the availability of limited resources, and by changing plant community composition (Fitter *et al.*, 2000). Thus adding limited N to the soil affects AMF symbioses because mycorrhizal trading value are based upon the exchange of fixed carbon (C) for mineral elements (Antoninka *et al.*, 2011). Carbon is one of the main limiting resources controlling AMF communities, and many other microorganisms in the soil (Smith & Read, 2008; Helgason & Fitter, 2009; Dumbrel*l et al*, 2010b). Decreases in this resource typically reduce both diversity, and community evenness, and increases the overdominance of strong competitors that are able to capture higher amounts of carbon. This serves as evidence of the role of niches in structuring soil microbial communities (Dumbrell *et al.*, 2011), since AMF can only obtain carbon directly from their host plants. Thus, many reports mention host-plant preferences as being key component in this

relationship. However, species-specific interactions do not always occur, and AMF could be more specific to plant functional groups than to individual plant species (Öpik *et al.*, 2009).

Additionally, the interaction between AMF and plants generates feedbacks that influence species diversity in fungal communities and, eventually, in plant communities (Bever *et al.*, 2001; Bever, 2002a). Thus, AMF have a profound determinative effects on plant community dynamics and diversity (Bever *et al.*, 2001; Smith *et al.*, 2003; Parniske, 2008; Helgason & Fitter, 2009). Yet, most previous studies of the AMF community in the UK have been conducted in woodlands and grasslands. Thus, the importance of the biotic and abiotic drivers of AMF community structure have remained unclear in many habitats including the salt marshes. Salt marshes are key habitats in the UK, where AMF are subjected to large spatial variations due to variations in soil properties (Armstrong *et al.*, 1985), high levels of salinity, and soil flooding. Such factors are known to significantly reduce the extraradical mycelium length of AMF (Carvalho *et al.*, 2003).

The current chapter investigates the AMF distribution at the landscape scale, under the hypothesised role of the host plants and local environment that were proved to have determinative effect on their community composition in different habitats. Data from 66 sediment cores were used to determine the effect of both spatial scale and seasonality on these variables. The community composition of AMF in plant roots was assessed for three sandy salt marshes in the north-west of England. Pore water from these sediment samples was used to quantify the physicochemical properties that are likely to influence the community composition and diversity of AMF – this includes measuring sediment NH3, NO_3^-, PO_4^{--}, total C,

salinity, pH, moisture and root density. Plant data were obtained from Dr Hilary Ford, a member of the coastal biodiversity and ecosystem service sustainability (CBESS). AMF community composition in plant roots was identified using next generation high-throughput sequencing (NGS) of 18S rRNA, a method that has previously been shown to be useful for characterising AMF communities of plant roots (Öpik *et al.*, 2009; Dumbrell *et al.*, 2011; Cotton *et al.*, 2014).

3.3. Methodology

Sampling design and site description with Sediment chemistry analysis and AMF determination were explained in detail in the methodology chapter two. Sixty six soil cores from West Plain, Warton Sands and Cartmel Sands salt marshes in Lancashire were collected to study the structure of AMF communities in relation to the different biotic and abiotic variables across these sites (

Table 3-1).

3.3.1. Sequence analysis

Sequencing analysis was conducted on QIIME pipeline and associated modules (Caporaso *et al.*, 2010). Sequences were then de-multiplexed using the Nextera Indexes and quality filtered to remove sequences below Q20 or that contained any errors in the primer region, above 6 ambiguous bases, and chimeras. The quality filtered reads were clustered into operational taxonomic units (OTUs) using the USEARCH algorithm (Edgar, 2010) at the 0.97 level. 18S rRNA representative sequences from each OTU were extracted and assigned taxonomic identities using the 18S AMF database MaarjAM (Öpik *et al.*, 2010), and NCBI

databases at a 97% similarity threshold. The phylogenetic tree was inferred using the Neighbor-Joining method (Saitou & Nei, 1987) on MEGA7 (Kumar *et al.*, 2016). The resultant bootstrap consensus tree obtained was a result inferred from 1000 replicates (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed, and *Geosiphon pyriformis* (Bilger *et al.*, 1994) was used as a specific outgroup to the AMF.

3.3.2. Data analysis

To determine the quality of the sampling method in estimating the diversity of AMF species in each host plant, site, and location, a rarefaction curve was produced using the 'exact' method within the *specacum* function (Ugland *et al.*, 2003), using the package *vegan* in R 2.15.3 (R Development Core Team, 2013) (Oksanen *et al.*, 2016).

Differences in the abiotic (NH3, NO_3^-, PO_4^{--}, total C, salinity, pH, moisture) and biotic (plant type, percentage cover, biomass) properties across these salt marshes were computed using an ANOVA in statistical software R (R Development Core Team, 14/04/2016). Further, species richness was calculated based on the number of the virtual taxa (VTX) in each site (Öpik *et al.*, 2009). To examine which biotic factors better predicted the abundance of VTX, a multivariate generalised linear modelling approach was followed, using the *Mvabund* package (Wang *et al.*, 2012). Data were first split by site and VTX; data that occurred fewer than 50 times were removed to improve the test quality. In this model, an offset of log (n sequences in sample) was included to account for random variability in the number of sequences generated for each sample. Biotic models were written as:

VTX ~ Number plant species + Root Biomass + % cover of forbs + % cover of grasses

And

VTX ~ Site+ NH_3+ NO_3^-+ PO_4^{--}+ % total C + salinity (ppt)+ pH+ % moisture

A negative binomial error distribution with a log link function was used, as sequence data is often too over dispersed for other count distributions such as the Poisson. An analysis of deviance using a Likelihood ratio test as implemented in the *anova.manyglm (Analysis of Deviance for Multivariate Generalized Linear Model Fits for Abundance Data)* function was used to test which model better described VTX abundance, followed by 999 montecarlo permutations were used to assess the significance of each model on the abundance of different AMF species. Finally, plant and AMF associations were examined in replicate matrices constructed for each combination of host species (Table 3-2). Network structures were calculated using the package *bipartite* in R. (Dormann *et al.*, 2009).

3.4. Results

A total of 28 AMF species were identified successfully in the current study of three salt marshes in Lancashire from the root samples of 21 plants species, the majority of which belonged to the *Glomeraceae* followed by *Paraglomeraceae*, *Diversisporaceae* and *Ambisporaceae* families. Of the 66 soil cores, AMF were identified in 42 samples associating with 38 separate plant roots in the downstream analyses. MiSeq high-throughput sequencing yielded 28 AMF OTUs that were identified against the MaarjAM (Öpik *et al.*, 2010) and NCBI database. Of these AMF 22 members belonging to the genus *Glomus* were dominant in these salt marshes, followed by 3 *Paraglomus*, by 2 *Diversispora*, 1 *Acaulospora* and 1 *Ambispora* respectively. Of these *Glomus* species, the *Goomaral 13a Glo9* was dominant in most of the amplified samples. The accumulation curve of the rarefied AMF species reached an asymptote after ~32 soil quadrates of the 66 that were used in this work (Figure 3-2), indicating that the number of samples was sufficient to represent AMF diversity. Visual inspection of rarefied VTX accumulation curve showed that further sampling would have added few additional data and would be unlikely to qualitatively affect the results (Figure 3-2).

Table 3-1: Number of samples that have been used in the analysis of both biotic and abiotic variables in this chapter. This also includes the total number of dry root samples that were used for DNA extraction.

Measurement	Number of samples
Plant roots extracted for molecular analysis	66
pH	66
NO_3	66
NO_2	66
NH_4	66
PO_4	66
Total Carbon (TOC)	66
Sediment Salinity	66
Water Content	66

Table 3-2: Typical species for Lancashire saltmarshes, the percentage cover for each plants species is presented separately followed by their type and weather they are annual or perennial and the abundance of different AM fungal species. Plants data provided by Dr Hillary Ford (CBESS Member) and detail for each species was obtained from the UK plants atlas website whenever it possible, otherwise different resources were used.

Plant Species	Type	Lifespan	Cartmel Sands	West Plain	Warton Sands
Agrostis.stolonifera	grass	perennial	1.428571429	12.93333333	6
Armeria.maritima	forb	perennial	0.633333333	0.066666667	0.833333333
Aster.tripolium	forb	perennial	0.095238095	0	0
Atriplex.prostrata	forb	annual	0	0	0
Atriplex portulacoides	forb	annual	0	0.873333333	0.833333333
Carex extensa	forb	perennial	0	2.14	3.333333333
Cochlearia.anglica	grass	perennial	0	0	0
Elytrigia.atherica	grass	perennial	0	0	0
Elytrigia.repens	grass	perennial	0	4.333333333	0
Festuca.rubra	grass	perennial	9.666666667	32.33333333	26.66666667
Glaux.maritima	grass	perennial	13.52380952	5	8.333333333
Inula.crithmoides	forb	perennial	0	0	0
Juncus.gerardii	grass	perennial	3.095238095	6.466666667	8.333333333
Juncus.maritima	grass	perennial	0	12.93333333	16.66666667
Leontodon.autumnalis	forb	perennial	0	1.866666667	0.016666667
Limonium.vulgare	forb	perennial	0	0	0
Oenanthe.lachenalii	forb	perennial	0	1.333333333	0.833333333
Potentilla.anserina	forb	perennial	0	9.333333333	0
Plantago.coronopus	forb	perennial	0	0	5
Plantago.maritima	forb	perennial	1.047619048	0.946666667	1.5
Puccinellia.maritima	grass	perennial	52.0952381	6.333333333	8.666666667
Salicornia.europaea	forb	annual	2.447619048	0	0
Sarcocornia.perennis	forb	perennial	0	0	0
Seriphidium.maritimum	forb	perennial	0	0	0
Spartina.anglica	grass	perennial	0	0	0
Spartina.anglica	grass	perennial	0	0	0
Spartina.maritima	grass	perennial	1.866666667	0.533333333	0.333333333
Suaeda.maritima	grass	perennial	0	0	0
Trifolium.repens	forb	perennial	0.761904762	9.4	12.5
Triglochin.maritima	grass	perennial	0	0.673333333	0
Urtica.dioica.nettle	forb	perennial	0	0	0
OTUs number for the associated AMF Species					
Glomus			6025094	600032	74843
Paraglomus			2492	3686	236028
Diversispora			1014	3917	2
Acaulospora			57	47	0
Ambispora			5733	2	1
AMF total species Abundance			6034390	607684	310874

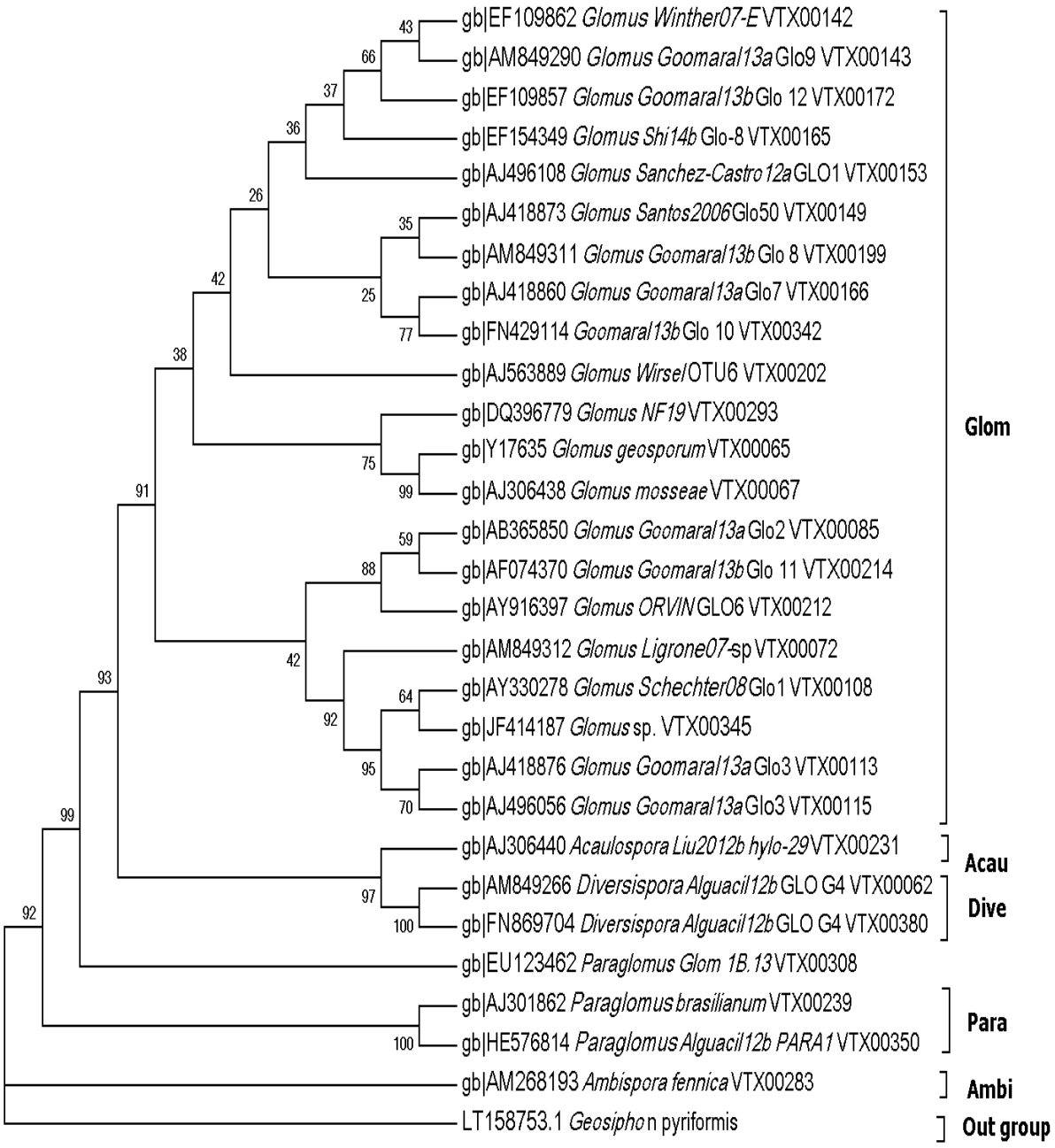

Figure 3-1: Neighbour-Joining tree showing AMF taxa from three salt marshes at Lancashire, UK. The bootstrap consensus tree is inferred from 1000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. All branches were rooted to the out-group.

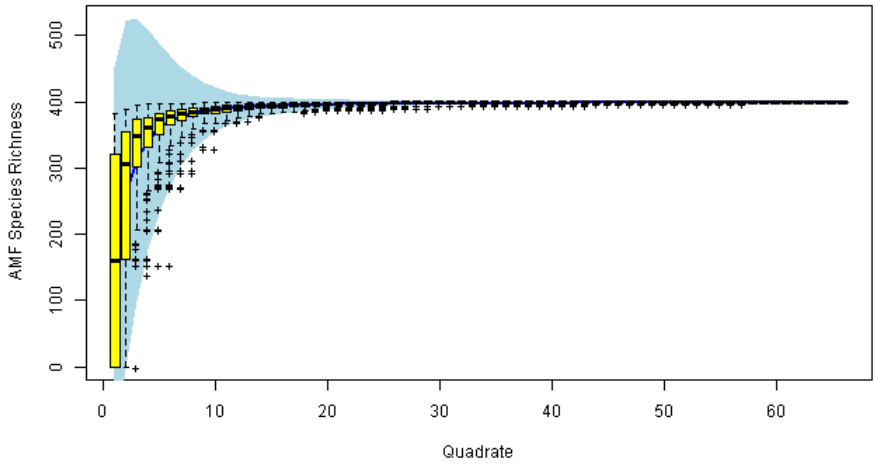

Figure 3-2: MiSeq expected species richness of AMF VTXs is highlighted in blue and its standard deviation that is represented by yellow boxes for each sampling quadrate. This includes 22 sediment quadrates from the three salt marshes in Lancashire.

3.4.1. Environmental heterogeneity patterning on both AMF and plants communities structure

The soil variables PO_4^{3-}, NH_4^+ and NO_3^- where all significantly different across sites (Figure 3-3; ANOVA; $F_{2,63}$= 12.9, P= 0.001 in all cases). However, total organic carbon and soil moisture did not significantly differ across sites (Figure 3-3; ANOVA; $F_{2,63}$ = *0.423*, P > 0.05 in all cases). The sampled rhizosphere soil was generally pH neutral (ave; 6.71±1.07), with a few samples from West Plain salt marshes that were slightly acidic compared with other sites (ave; 7.03±6.71). Soil salinity ranged between (1–15‰), with highest values recorded at Cartmel Sand. Water content (33–58.2 %) had the highest measures recorded at West Plane, that also had high levels of both phosphate and ammonia (ave; 84.49±73.59 and 138.64±83.49 respectively) compared with Cartmel Sand (ave; 41.54±62.6 and 78.22±53.74) and Warton Sands (ave=30.26±28.91 and 76.31±56.57). The level of nitrate in the soil was higher in West Plain (ave; 7.49±1.59) compared with Cartmel

Sands and Warton Sands (ave; 1.38±1.05 and 1.35 ±3.367, respectively). The number of plant species significantly differed between these sites (ANOVA; $F_{2,63}$=*349.8, P=0.001*). The highest plant species richness was recorded in West Plain (ave; 8.133±2.38), which accommodates high numbers of both types of grass and forb (Figure 3-4), followed by Warton Sands (ave; 5.66±0.81) and Cartmel Sands (ave; 5.055±1.34) that are dominated by grass species.

The redundancy analysis of AMF communities showed plants to be far better explained by changes in these properties at ~80% compared with ~40% on AMF (Figure 3-5). Most AMF VTXs richness was positively related with plants species richness, and was also affected by abiotic properties including (ANOVA; moisture $F_{2,63}$=1.39, P=0.005 and pH $F_{2,63}$=0.921, P=0.04).

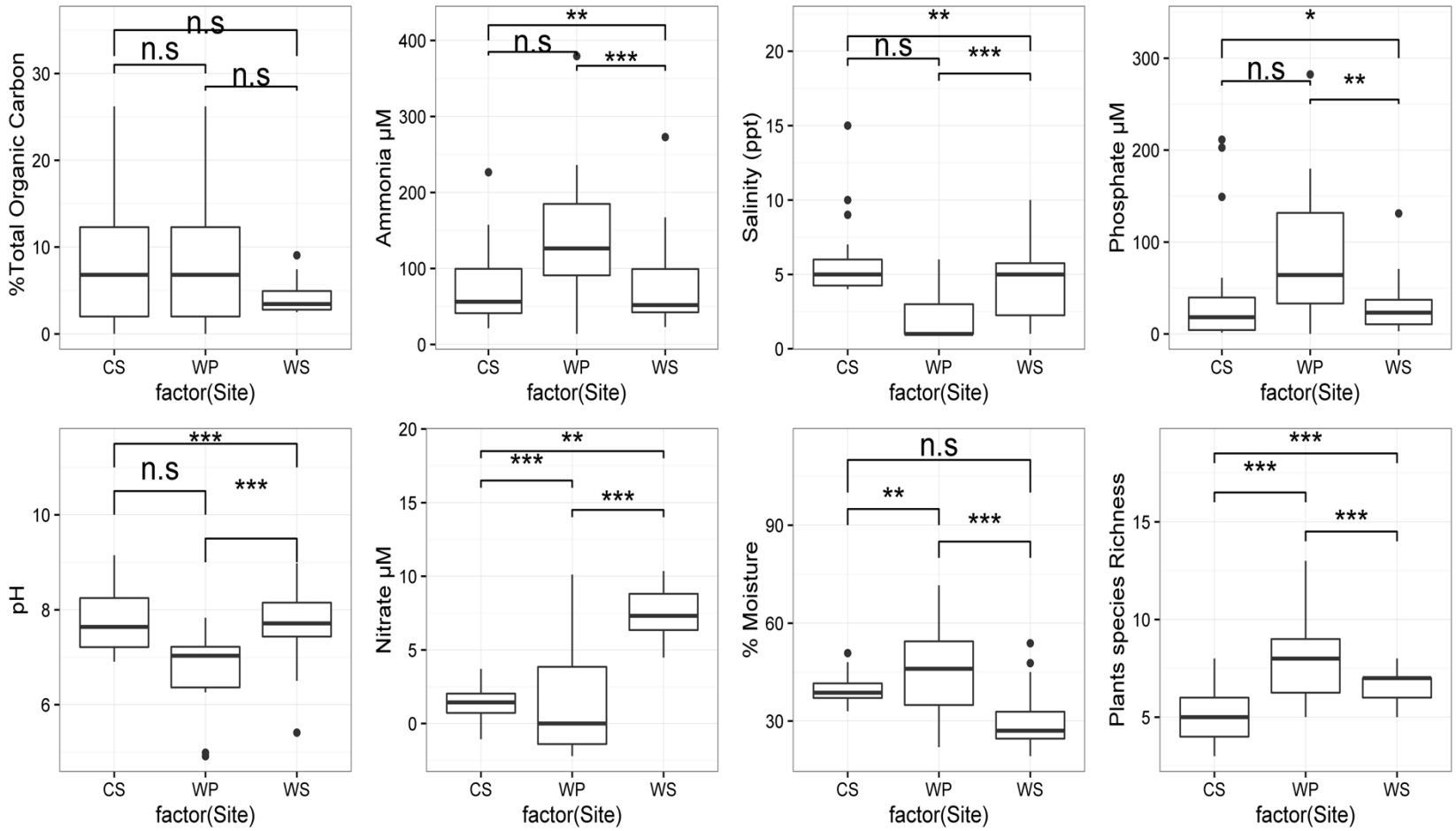

Figure 3-3: regression analysis of the biotic and abiotic variables between sites at Lancashire. The p value of the ANOVA test is included, along with significance levels (*0.05, **0.01, ***0.001 and no significance n.s). Sites were labelled by their first letters as follows: Cartmel Sands (CS), Warton Sands (WS) and West Plain (WP). Sample points out side of the box plot are outliers.

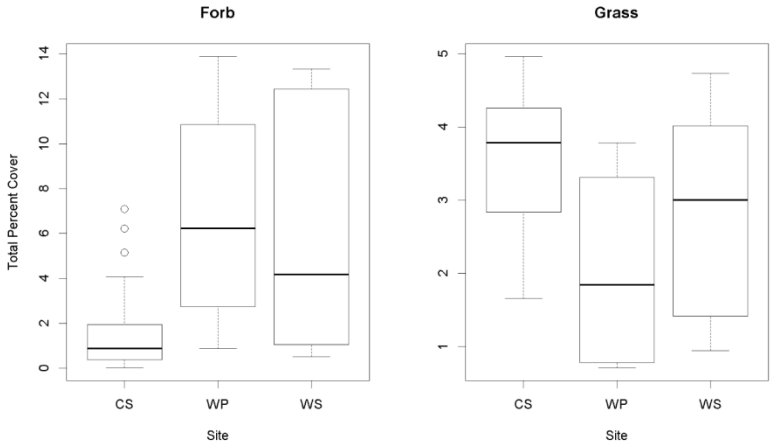

Figure 3-4: Regression analysis of plant percentage cover per type at each site in the Lancashire salt marsh. Sites are labelled using their first letters as follows: Cartmel Sand (CS), West Plain (WP) and Warton sands (WS). Sample points out side of the box plot are outliers.

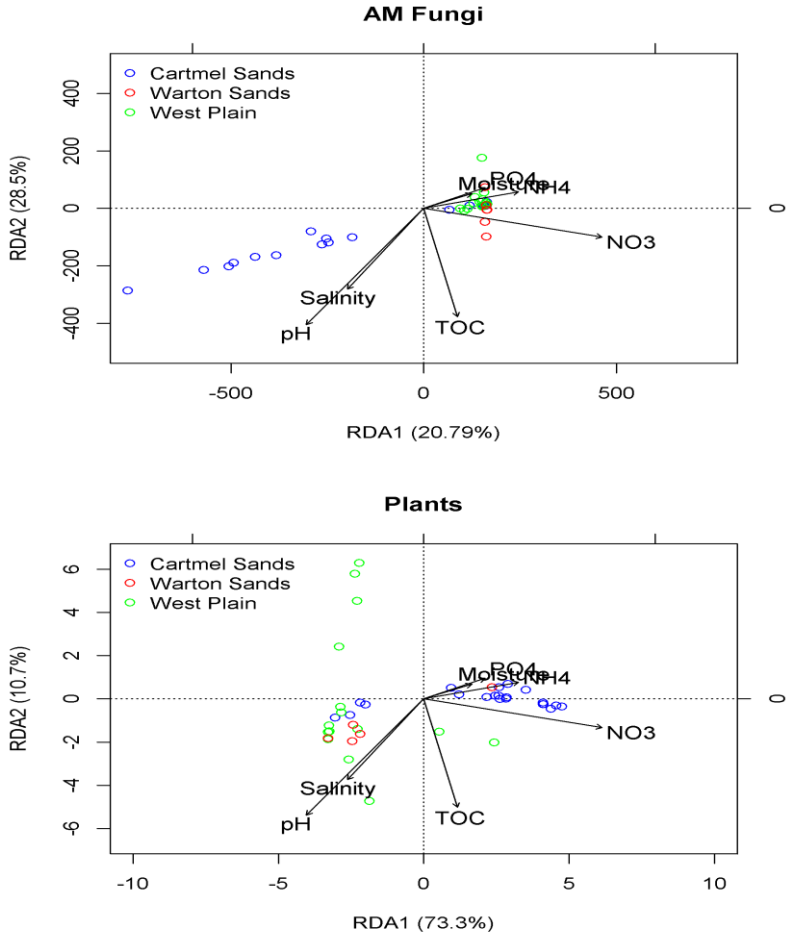

Figure 3-5: Redundancy analyses of plants and AMF samples collected from the Lancashire salt marsh. Percentage values of variation, explained by consecutive axes are included on each axis. Both plant and AMF VTXs richness of each sample is significantly related to soil salinity, TOC, Moisture and pH.

3.4.2. The Relative Roles of the Biotic and Abiotic Environment on abundance of AMF VTXs

At the site level, AIC based comparison of biotic and abiotic models suggested a significant influence of the biotic variables to most OTUs at Cartmel Sands (ANOVA; $F_{2,1}$= 323.8, P=0.001), compared with little effect that has been recorded at other sites (Figure 3-6). Few OTUs had large **Δ**AIC between biotic and abiotic models and both West Plain and Warton Sands that did not favour the biotic model. Within these sites, OTUs abundances were better described by biotic variables with 57.14% in Cartmel Sands, and abiotic 100% in West Plain and 70.8% in Warton Sands.

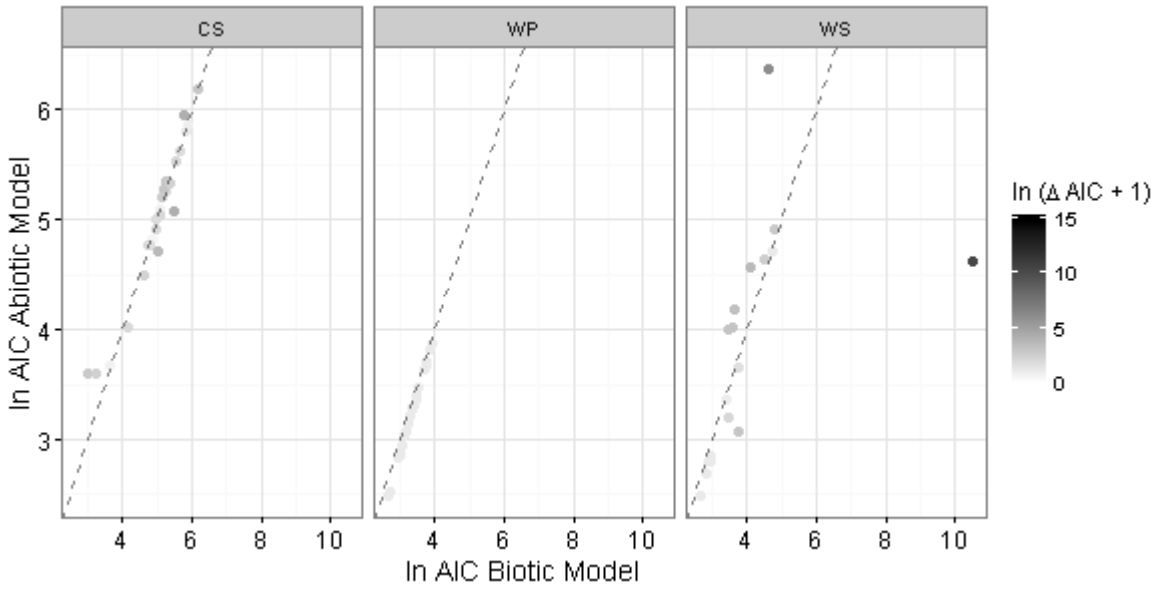

Figure 3-6: AIC scores from multivariate models with biotic or abiotic variables for each OTU in each site. The dashed 1:1 line represents equal AICs for the two models. OTUs below this line have a lower AIC for abiotic variables, indicating abiotic variables better described the OTU's abundances, whereas OTUs above this line are better described by biotic variables. Hollow points indicate OTUs for which the difference in AIC (ΔAIC) between the two models is less than 2, indicating insufficient support for one set of variables over the other.

3.4.3. Correlation between the abundance of AMF species and surrounding environment

A Spearman test of correlation between the abundance of AMF VTXs and abiotic variables indicated that AMF VTXs defer in their responses to changes in their surroundings (Figure 3-7). At the local scale, AMFs defer in their response to the soil pH, where they had been shown to be negatively correlated in Cartmel Sands (Spearman; R=-0.39, P=0.09). In both West Plain and Warton Sands AMF correlation with soil pH differs based on their species. A positive effect of soil salinity in Cartmel Sands and West Plain on the abundance AMF VTXs was noted (Spearman; R=0.892, P=0.01 and R=0.966, P=0.001), compared with Warton Sands where salinity negatively correlated with the abundance of almost all AMFs. A mixed type of correlation was recorded between AMFs, and changes in ammonia in both West Plain and Cartmel Sands. In Warton Sands, the level of ammonia negatively correlated with AMFs and this was significant with AMF *Glomus_Goomaral13b_Glo_11* (Spearman; R=-0.97, P=0.001). Nitrate negatively correlated with different AMF species in West Plane. This effect however, was random in Cartmel sands and Warton Sands. The levels of both moisture and total organic carbon had no significant effect, and were randomly correlated with different AMF VTXs.

The OTUs number of different AMF VTXs was negatively correlated root biomass, plant height, and plant richness in Cartmel Sands (Figure 3-8). The negative effect of plant biomass was significant with AMF species *Glomus_Ligrone07.sp* (R=-0.55, P=0.01). At Warton sands, AMF was positively correlated with both root biomass and plant richness, and was significant with AMF

Glomus_Goomaral13a_Glo3 (*R*=0.94, *P*=0.004). The AMF at West Plain were randomly correlated with the biotic variables. In this site, the abundance of AMF *Glomus_Goomaral13b_Glo_12* was significantly correlated with the percentage cover of grass species (*R*=-0.99, *P*=0.02). AMF *Glomus_Goomaral13b_Glo_11* significantly correlated with the percentage cover of forbs in West Plain (*R*=0.99, *P*=0.027).

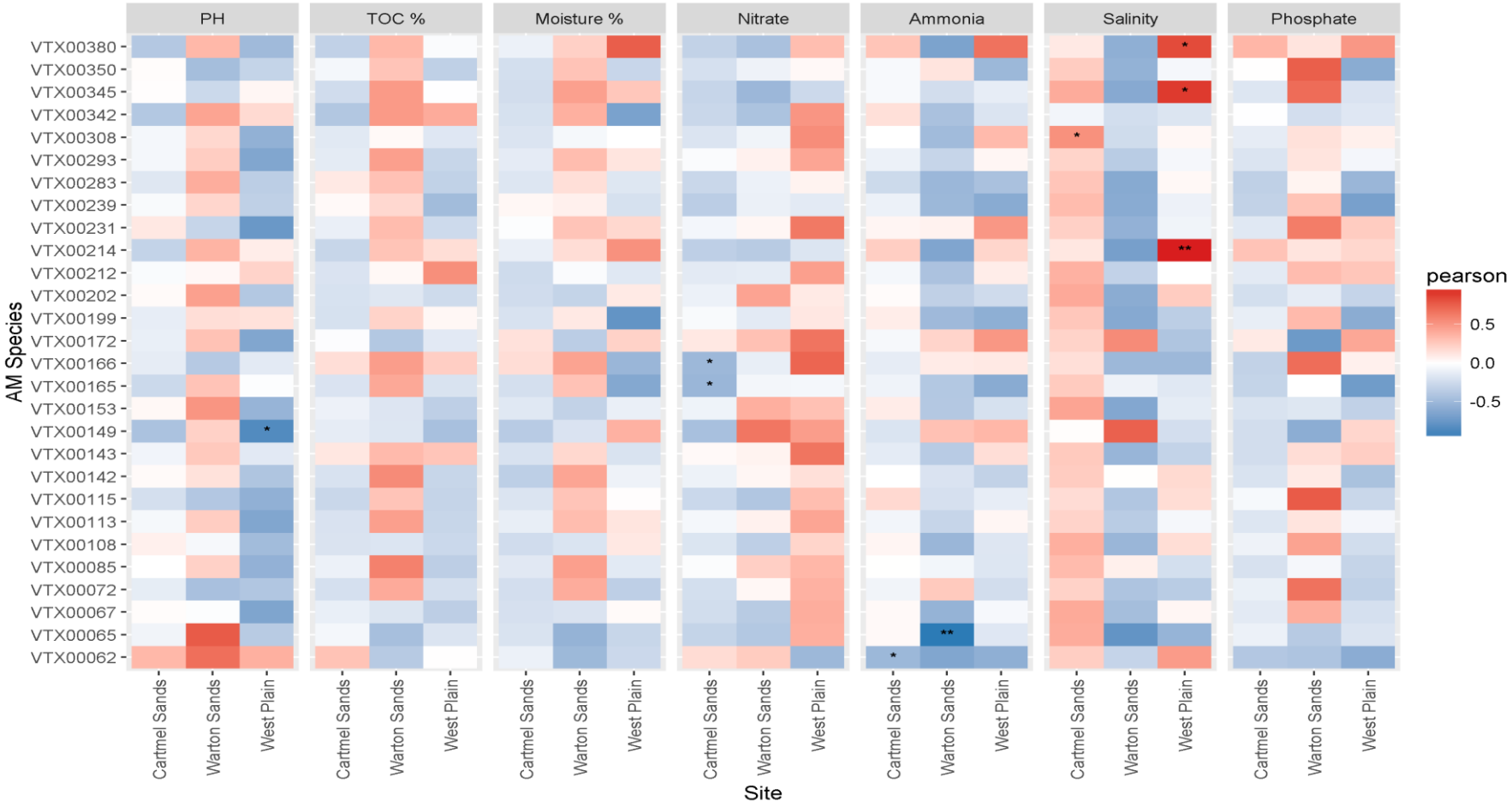

Figure 3-7: Multiple subplots showing the result of the Pearson correlation test between different abiotic variables and the abundance of AMF species at each site. The correlation (R) value is represented by different colours as follows: Blue=negative, red=positive and white for normal. P values represent any significant correlation (levels include *0.05, **0.01, and ***0.001).

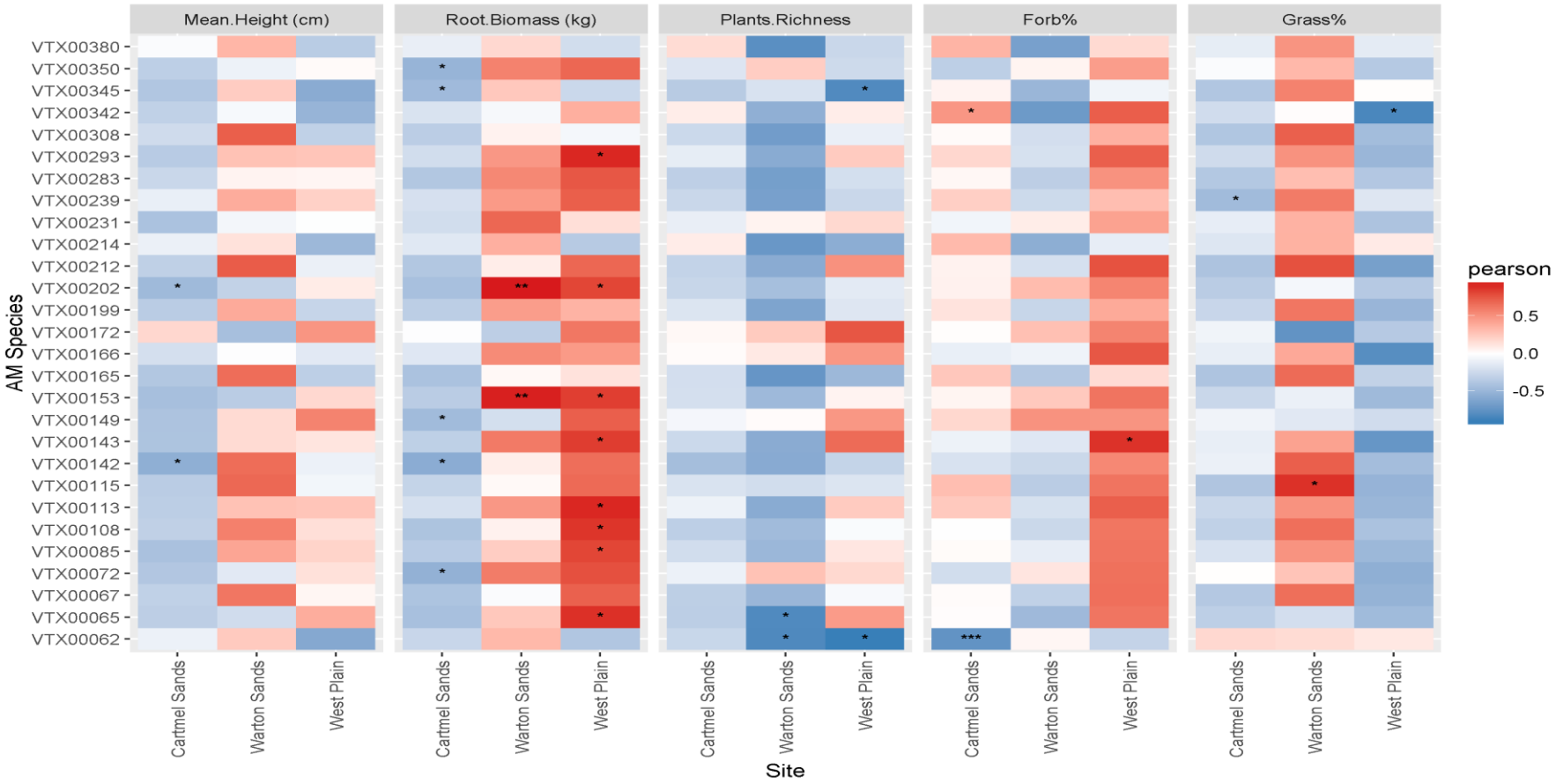

Figure 3-8; Multiple subplots showing the result of Pearson correlation test between different biotic variables and the abundance of AMF species at each site. The correlation (R) value is represented by different colours as follows: Blue=negative, red=positive and white for normal. P values represent significant correlation (levels include *0.05, **0.01, and ***0.001).

3.4.4. Measuring the effect of the local environment on the composition of AMF in different zones within a signal salt marsh

Model coefficients estimated from overall biotic, and soil physical and chemical properties suggested that there was considerable variation in environmental responses both within and between AMF VTXs (Figure 3-9). The ANOVA test of a GLM model created to measure the effect of different soil physical properties on the community composition of AMF confirmed that there was no significant change in the OUT number of AMF VTXs ($F_{15,16}$=18, P=0.08) with changes in the host plant conditions at the site level. This potentially results in differences in plant species richness, that had a significant effect (ANOVA; $F_{15,16}$=323.8, P=0.001) in this site, compared with other sites. A regression analysis of these coefficients showed that changes in the number of plant species across different zones on a single salt marsh had a significant effect on the abundance of the AMF OTUs ($F_{15,16}$=21.16, P=0.01) in Cartmel sands. The overall effect of changes in plant richness and root biomass had a non-detectable influence on the OUT abundance of AMF VTXs. The ANOVA of the biotic model showed these variables to have no significance ($F_{15,16}$=6.18, P=0.075).

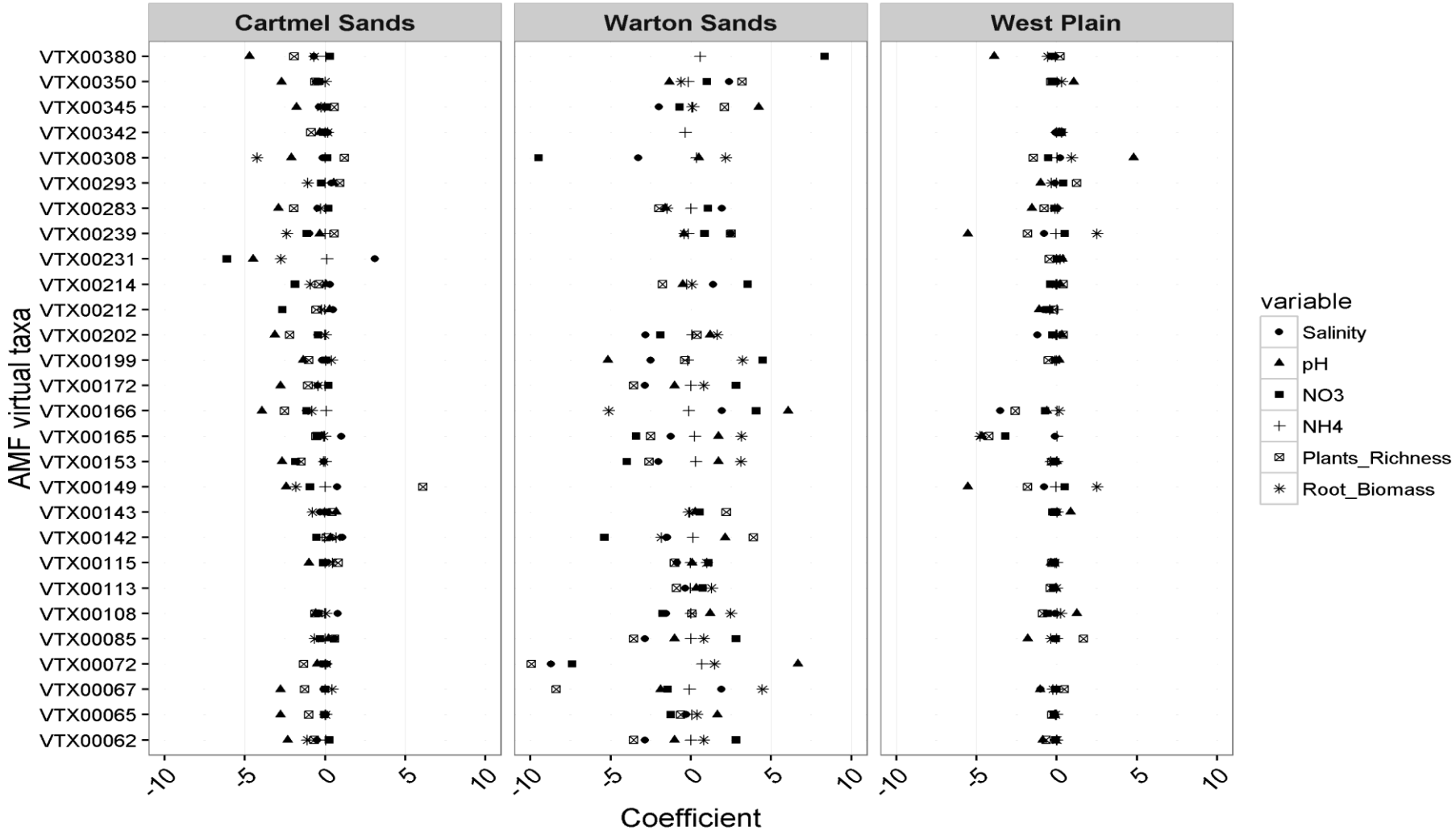

Figure 3-9: AMF virtual taxa (VTX) show significantly different responses to environmental variables. Coefficient values of 0 indicate little relationship between the given variables and the abundance of VTXs. Outliers outside the range -10:10 were removed for easy visualisation.

3.4.5. Measuring the AMF association in each plant at different zones on the salt marsh

The abundance of AMF VTXs was shown to be different among plants types (grass or forb), with the highest abundance recorded in the root samples of grass species. Root samples of annual plants species accommodated higher abundance of AMF VTXs compared to perennial ones (Figure 3-10). The abundance of AMF VTXs was significantly different ($F_{25,30}$=3.97, P=0.02), especially in plants species growing in low-middle and pioneer zones of the marsh, such as *Salicornia europaea*. A network was generated to assess AMF and plant interaction, to detect host specificity if it exists. However, the AMF-plant relationship was shown to be random. The number of AMF seems to increase as a result of zonation effects on these plants species. Although these salt marshes are dominated by Glomeraceae species, this network shows that they represented <1% of the population of *Ambispora* showed to be associated with a range of plant species growing on different zones.

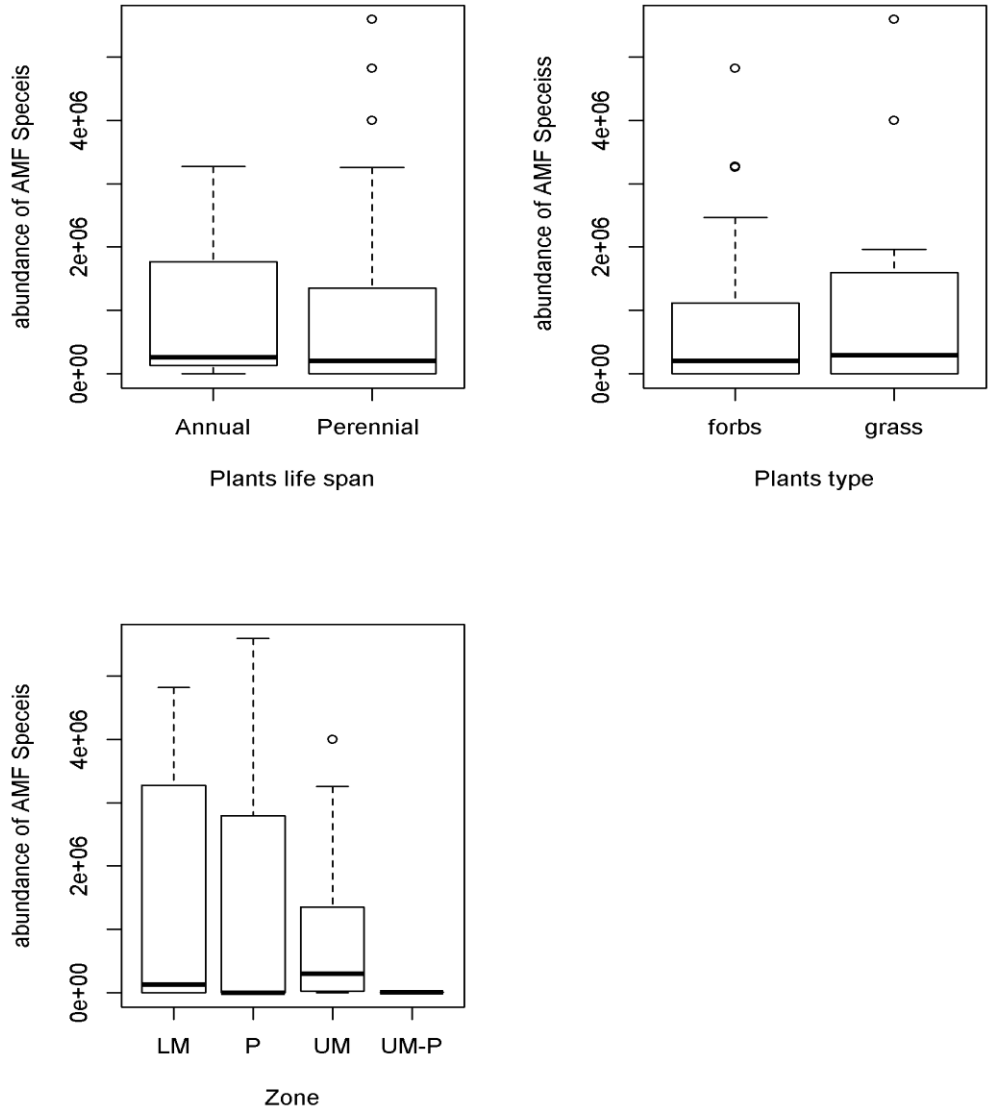

Figure 3-10: AMF response to changes in plants type, life span and position on the salt marsh. Different plants species were identified based on the literature and classed to species growing on the pioneer zone are in direct contact with the seawater (P), where the lower marsh (LM) and upper marsh (UM) zones are landward.

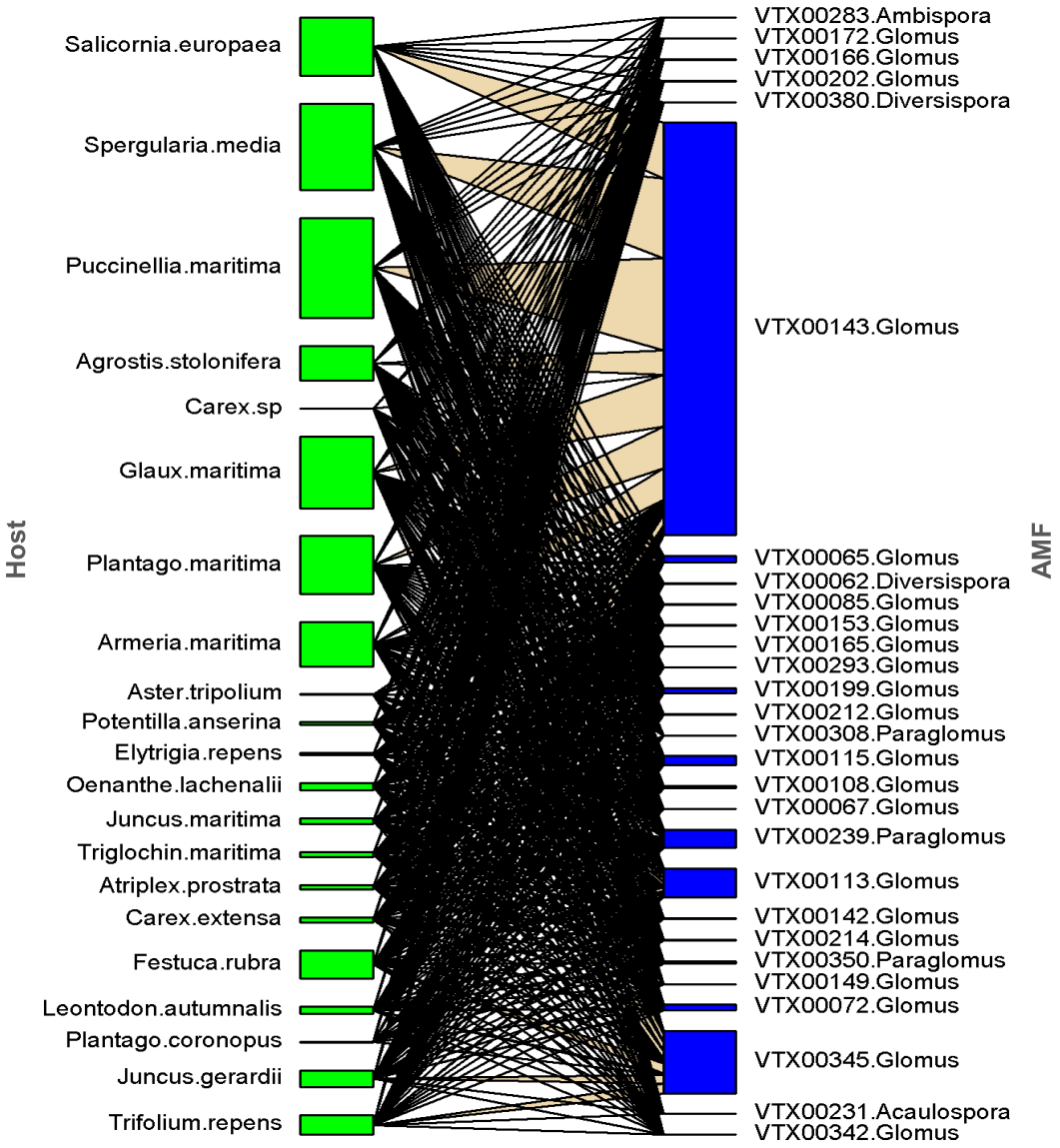

Figure 3-11: Bipartite plot web mapping the relationship between different plant and AMF species . The green colour marks the percentage cover of each plant species, and the AMF virtual taxa (VTX) and their family are labelled in blue colour. Species area increases following the abundance of each species in the matrix; larger area indicates higher number of AMF OTUs and high percentage cover of their host plant.

3.5. Discussion

For the AMF phylotypes obtained in this study, those of *Glomus spp.* dominated the salt-stressed environments. The AMF species *Glomus intraradices*, *Glomus versiforme*, and *Glomus etunicatum* were the most

predominant AMF species in Iranian saline soils (Aliasgharzadeh *et al.*, 2001), while a single AMF species, *G. geosporum*, was reported to be frequent in European salt marshes and saline soils (Carvalho *et al.*, 2001; Wilde *et al.*, 2009). Examination of the diversity of AMF spores in the rhizosphere of five wild plants in saline-alkaline soils of the Yellow River Delta, China, confirmed that *G. caledonium* was dominant (Wang *et al.*, 2004).

The AMF *Glomus* were reported to be dominant under conditions of high salinity in costal habitats and other ecosystems (Van Der Heijden *et al.*, 2008; Wang *et al.*, 2011; Guo & Gong, 2014). Species belonging to family *Paraglomeraceae* are known to develop an association during floods in rice crops (Wang *et al.*, 2015) and wetland grass, along with AMF species belonging to *Diversisporaceae* (Wirsel, 2004; Hempel *et al.*, 2007). However, both families have not been reported in the salt marsh ecosystems. The AMF *Ambispora* is a new taxon belonging to *Archaeosporales,* and had been recorded in both the saltmarsh of Schreyahn, Northern Germany (Wilde *et al.*, 2009), and in other Italian salt marshes (Carvalho *et al.*, 2001; Calvo-Polanco *et al.*, 2014). In this study *Archaeosporales* was identified in the root samples of plants species growing in both pioneer and low-middle marshes like *Salicornia. europaea* and *Puccinellia. maritima*. These are halophyte species with the ability to grow under conditions of high salinity, thus, it is more likely that such associations with AMF might improve water relation in these regularly flooded zones.

3.5.1. Linking AMF community structure with their local environment

At the site level, the comparison of biotic and abiotic models suggested little difference in the relative influence of these variables to most AMFs. Environmental variables explained variations in AMF communities, and were

correlated with the variation of the AMF species abundance, unlike their host plants species that only showed an effect in Cartmel Sands. My current results indicate that soil salinity and pH are the primary abiotic factors influencing the distribution patterns of AMF in Lancashire saltmarshes. Soil salinity was confirmed as a global pattern influencing soil microbial distribution (Lozupone & Knight, 2007). The pattern of high salinity has been confirmed to reduce both germination of AMF spores, and hyphal extension (Juniper & Abbott, 2006). However, the increased stress of soil salinity in Cartmel Sands was positively correlated with the increasing abundance of AMF species compared with both West Plain and Warton Sands, where lower salinity was recorded. High salinity along with low phosphate and nitrate in Cartmel Sands have created stressful conditions for most plants species, resulting in lower plant species richness compared with other sites in this study. Such stressful conditions likely enhanced the demands of the AMF association, and helped increase the root area and the plants ability to obtain these limited elements from different parts of the soil.

Unlike soil salinity, the abundance of AMF was negatively correlated with soil pH in both West Plan and Cartmel Sands. Both sites had a significantly different range of the soil pH compared with Warton sands, that was narrower and almost natural. Soil in West Plain was acidic and P:N rich - factors that are known to influence the AMF community composition (Johnson *et al.*, 1991; Dodd *et al.*, 2000; Egerton-Warburton & Allen, 2000; Carvalho *et al.*, 2001; Dumbrell *et al.*, 2011). Thus, the low number of AMF obtained in West Plain confirms the role of these abiotic variables in changing the composition of AMF communities in salt marsh ecosystems. Morphological studies of AMF have shown soil pH to influence sporulation level (Lovelo*ck et al*, 2003), spore density and richness

(Tchabi *et al.*, 2008), extraradical mycelium growth (Van Aarle *et al.*, 2002) and spore community composition (Coughlan *et al.*, 2000; Fitzsimons *et al.*, 2008). Soil pH is also widely known to reduce up to 50% of soil carbon solubility (Clar*k et al*, 2005), and this normally results of decreased rates of decomposition, as well as the solubility of phosphate compounds that heavily influence the ratio of organic carbon and other abiotic factors (Chapman and Reiss, 1999).

N and P are limited elements in the soil and were shown to have a negative effect on the abundance of AMF in several studies (Fitter *et al.*, 2000; Gamper *et al.*, 2004). In low-P (high N:P) soils, plant dependency on AMF for P acquisition increases (Hetrick *et al.*, 1990), but not in the high-P soils elsewhere (Bever *et al.*, 2001). Contrastingly, in soil that is N limited and relatively P rich, N fertilization decreases AMF abundance and species richness (Johnson *et al.*, 2003). All other abiotic variables including soil moisture that were thought of as a limiting variables of the obligate aerobic microbes in the soil were not as significant as soil pH and Salinity. Although the highest level of moisture was recorded in West Plane, it is unlikely to have affected the abundance of the AMF, which was higher in Cartmel sands regardless of the higher soil moisture levels compared with Warton Sands.

3.5.2. Host plant species effects on Arbuscular Mycorrhizal Fungal communities in salt marshes

AMF are obligate symbionts that can only obtain carbon directly from their host plants (Smith & Read, 2008; Helgason & Fitter, 2009). The only significant effect of biotic variables was obtained in Cartmel Sands. Unlike their correlation with the abiotic variables, AMF species within each site were showed to be

affected by plant biomass, richness and type in a similar way. For example, the correlation between the richness of plants species and the abundance of AMF species in Warton Sands was almost positive compared to the negative correlation that was recorded in Cartmel Sands. These AMF species only differed in their correlation in West Plane, however, this is the site where the lowest number of AMF species was recorded, due to the previously mentioned effect of soil pH. The higher percentage of grass coverage in Cartmel sands was negatively correlated with the abundance of the predominant species, due to the high number of AMF species that favoured grass species over forbs. Grasses are known to perform higher root biomass that also was shown to affect the abundance of AMF species in a similar way in this site, by allowing more AMF species to coexist. Higher root biomass can enhance AMF species richness by reducing the soil acidity. Greater root biomass helps absorb more nitrogen in the form of nitrate, which increases the soil pH (Nye, 1981) and the area of the rhizosphere, which in turn creates a niche effect on the AMF diversity in these salt marshes, as in in other habitats (Dumbrell *et al.*, 2010a).

Occurrence of the same AMF species within the root samples of more than one plant species suggests that partner specialisation contributes little to the structure of AMF communities in these salt marshes. My results indicate that AMF are able to infect a wide range of plants under different types of stress. Previous studies of AMF in other habitats showed that species-specific interactions do not always occur, and AMF could be more specific to plant functional groups than to individual plant species (Öpik *et al.*, 2009). Moreover, the structure of both AMF and their host community may be influenced by differential effects between individual AMF–host partners, causing similar

variations to what were recorded at different zones. In such types of relationships, the composition of AMF communities may be strongly influenced by the host species, through differential effects on hyphal growth and/or sporulation (Bever et al. 1996; Daniels Hetrick and Bloom 1986; Eom et al. 2000; Johnson et al. 1992; Sanders and Fitter 1992). In return, AM-plant community structure may be strongly influenced by the specific composition of the associated AMF and the effectiveness of each of the fungal species in promoting growth of each host (Grime *et al*. 1987; Hartnett *et al*. 1994; Streitwolf-Engel *et al*. 1997; van der Heijden *et al*. 1998a, 1998b). This confirms that AMF colonization does not depend on position within the salt marsh nor, consequently, on the tidal flooding regimes which create different levels of anoxia around the roots, nor on salt gradients. This suggests that AMF distribution does not coincide with the zonation pattern of vegetation.

3.6. Conclusions

The current study of AMF in 28 dominant plant species dwelling in Lancashire salt marshes reveals a previously unrecognized diversity of these root colonizers in salt marsh habitats. My results show that the influence of plant species on the composition of the AMF communities may undergo a significant change due to high environmental heterogeneity from one zone to another across a salt marsh. These results provide compelling evidence that the role of soil physico-chemical properties in determining the composition and distribution of AMF in salt marsh ecosystem is superior. This study shows that AMF can have considerable influence on different plants species under different environmental stresses. However, AMF species response to changes in their local environments is phylogenetically dependent. This alone can explain how an understanding of

the AMF diversity is important to predict their role in improving ecosystem functionality.

Chapter four

4. Structure of Arbuscular Mycorrhizal Fungal communities in salt marshes across space and time

4.1. Summary

- AMF have a major role in directing the functioning of many ecosystems. However, the mechanisms regulating the diversity and community structure of AMF remain poorly studied across many habitats. This is particularly true for salt marshes, which are key conservation priority habitats in many countries, including the UK.
- In this chapter, I quantify plant-root associated AMF communities from six UK saltmarshes across two distinct geographic locations (Essex and Lancashire) during winter and summer seasons of 2013, using NGS. These six study sites provide a gradient of ecological and environmental contexts, differing in the composition of plant species present and severity of tidal exposure where distribution of AMF at the site level was driven by local environmental variables.
- The use of GLM-based models to assess the relative importance of biotic and abiotic variables in regulating AMF communities across these contexts revealed that, within the local scale, soil pH and salinity best predicted AMF distributions, but at larger scales, the plants' role was superior, indicating a degree of context dependency. The recorded role of the abiotic variables highlights the susceptibility of AMF to environmental change over the previously reported role of plants.

4.2. Introduction

Understanding what drives the distribution of organisms is one of the central aims of ecology. While describing the niches of macro organisms has dominated research for a long time, an interest in microbial niches has recently started. With a growing interest in the extensive diversity present in the soil, and the many influences that these microorganisms have on aboveground dynamics, and their role in the ecosystem functioning, microbial niches are now being investigated with greater interest (Fitter *et al.*, 2005; Lekberg *et al.*, 2007; Dumbrell *et al.*, 2010a). This current chapter investigates the major niche requirements of a particularly important group of rhizosphere microorganisms, arbuscular mycorrhizal fungi (AMF), by using a gradient approach represented by six salt marshes over two locations in England.

Plants in salt marsh ecosystems experience stressful conditions due to tidal flooding with seawater that causes partial or total submergence of vegetation, high soil salinity and soil anoxia. Water-logged soil creates anaerobic and chemically reduced conditions around plant roots (rhizosphere), leading to oxygen deficiency and accumulated phytotoxic substances (Armstrong *et al.*, 1991). Thus plant growth and survival, species composition, and zonation patterns are strongly influenced by flooding and the concentrations of salt and oxygen (Armstrong *et al.*, 1991; Pennings & Callaway, 1992). These stressful conditions are known to decrease landward, creating a zoned pattern in the marsh vegetation. Although salt marsh plants exhibit biochemical, morphological and physiological adaptations to waterlogging and salinity (Armstrong *et al.*, 1991), microorganisms in the rhizosphere, particularly AMF, enhance ecological adaptation of these plants, including pioneer zone colonizers, in salt marsh

environments (Khan & Belik, 1995). Previous studies showed that AMF improved plant tolerance to salinity (Feng *et al.*, 2002), though other results showed that AMF spore production was suppressed by both high soil salinity (Juniper & Abbott, 2006) and waterlogging (Harley and Smith 1983). However, AMF association with salt marsh plant species was confirmed in the central European salt marshes (Rozema *et al.*, 1986; Carvalho *et al.*, 2001; Hildebrandt *et al.*, 2001; De La Peña *et al.*, 2006), United States (Cooke *et al.*, 1993) and China (Wang *et al.*, 2004). Indeed, plants in these habitats were shown to differ in AMF colonization. Only few AMF species were shown to associate with plants growing in the pioneer zone (McHugh & Dighton, 2004), compared to those in other zones (Carvalho *et al.*, 2001). This includes smooth cordgrass *Spartina alterniflora*, a common dominant species in the pioneer zone (Bertness, 1999) that was reported as non-mycorrhizal (McHugh & Dighton, 2004). Other species that occupy the upper edge of salt marshes are often associated with AMF as well (Daleo *et al.*, 2008).

Regardless of the factors that shape the vegetation distribution in each zone of a marsh, plant zonation remains heavily dependent on the relative long-term stability of these factors. This includes stability of short-term fluctuations that are known to occurr between different seasons. Moreover, the ability of each plant species to grow in a zone of the marsh proves that it can adap to these fluctuations, including possible extreme physical conditions in less favourable seasons (Rogel *et al.*, 2000; Álvarez-Rogel *et al.*, 2006). These dramatic variations in physical factors occur and are maintained in time, and thus patterns of plant zonation change in response to the new conditions (Álvarez-Rogel *et al.*, 2007). Being an obligate symbiont with an extensive extra radical mycelium, AMF

are in direct contact with the physicochemical properties of the soil. Morphological studies showed that both the percentage of root colonization (De Oliveira & De Oliveira, 2005; de Oliveira & de Oliveira, 2010) and the community composition (Gemma *et al.*, 1989; Bever *et al.*, 2001), of AMF are highly dependent on the seasonal pattern of these properties (Maček *et al.*, 2011; Su *et al.*, 2011).

Recent developments in molecular methods have allowed the study of AMF diversity at larger sampling scales, that can be repeated over different seasons (Dumbrell *et al.*, 2011; Su *et al.*, 2011; Zangaro *et al.*, 2013). Yet the dynamics of the AMF colonization of root systems has not been fully determined in many important habitats. This includes salt marshes in countries like the UK, where AMF association has been reported many years ago (MASON, 1928; Read *et al.*, 1976). AMF diversity in other salt marshes were shown to be subjected to plant phenology, as well as other abiotic stresses such as high moisture and salinity (Rozema *et al.*, 1986; Van Duin *et al.*, 1990; Carvalho *et al.*, 2003), all of which are known to vary between different zones (Brown & Bledsoe, 1996).

AMF associated with the roots of halophyte species have been primarily investigated using 18S primers (Toju *et al.*, 2012), that are known to amplify most AMF species, except some of those belonging to *Archaeospora*, *Paraglomus* and *Glomus* group B species (Redecker *et al*., 2000). Thus, these primers will be used in this current chapter, to assess the response of AMF community composition to the spatial and temporal patterns of different biotic and abiotic factors that were evaluated in the previous chapter (Chapter 3). The composition of AMF communities in plant species subsequently posed two main questions: (1) did the incidence of AMF occurrence vary spatially at different scales; (2) as AMF

occurrence related to phenological events of plant species, or to edaphic conditions? To answer these questions, AMF from 78 sediment cores along the salt marsh were evaluated from summer to winter, to assess the response of mycorrhizas to temporal variation in salinity, soil moisture, soil organic carbon, salinity, pH, P and N as well as to plants conditions.

4.3. Materials and methods

Sampling design and site description with Sediment chemistry analysis and AMF determination were fully explained in the methodology Chapter (Chapter 2). This current chapter includes data from all sediment cores that have AMF association successfully amplified by the 18S primer.

4.3.1. AMF compositional analysis

The dissimilarities of AMF communities (β diversity) among locations and between seasons were computed by nonmetric multidimensional scaling (NMDS) with Bray–Curtis distance using the function 'NMDS' in the r package *vegan* (Oksanen *et al*, 2016).

To examine whether spatial scale and/or season affects the role of these abiotic and biotic factors in predicting the abundance of OTUs within each site and location, models with either abiotic or biotic variables were compared for their smaller AIC. Biotic models included the variables plant species richness (Hiiesalu et al. 2014; Tedersoo et al. 2015; Kivlin & Hawkes 2016), total root biomass (Hiiesalu et al. 2014) and % cover of forbs, grasses, herbs and sub forbs (Tedersoo et al. 2013; Chagnon et al. 2015; Nguyen et al. 2016). Plant species were grouped into grasses and forb, rather than using the percentage cover of individual plant species to predict fungal community structure. These groups

reflected the broad differences in root morphologies and life history strategies (e.g. Perennial vs annual) that I expected to affect AMF communities (Hawkes et al. 2006; Tedersoo et al. 2015; Kivlin & Hawkes 2016).

4.3.2. Measuring the response of OTUs abundance in relation to biological vs physical factors at the different scale

In order to examine whether abiotic or biotic factors better predicted the abundance of AMF OTUs within each site, a multivariate generalised linear modelling approach was used in the *Mvabund* package (Wang *et al.*, 2012). Data were first split by site and OTUs which occurred fewer than 5 times were removed. In both the biotic and abiotic models an offset of log (n sequences in sample) was included to account for random variability in the number of sequences generated for each sample. This approach assigns a covariate (log (n sequences)) and assigns it a value of 1, inferring that OTU abundances are proportional to the number of sequences in each sample. Abiotic (physical) models could be written as:

OTUs ~ Season + Salinity + pH +Soil Moisture+ Ammonia+ Phosphate+ Nitrate+ Carbon

Biotic models could be written as:

OTUs ~ Number plant species + Root Biomass + % cover of grasses + % cover by forb

A model selection was performed to test which group of variables better explained OTU abundance rather than which specific variables were the best predictors. A negative binomial error distribution with a log link function as sequence data is often too over dispersed for other count distributions such as the Poisson. To test which model better described OTU abundance, an analysis

of deviance was performed using a Likelihood ratio test as implemented in the *anova.manyglm* function. 999 montecarlo permutations were used to assess significance.

4.4. Results

In this chapter a total number of 78 root samples were analysed and revealed 29 AMF virtual taxa (VTX) based on the MaarjAM database (all were reported in Chapter 3). These samples were collected during the summer and winter seasons of 2013, from both locations of this work at Lancashire and Essex according to the following table:

Table 4-1; the number of samples included in the analysis. Each site was sampled during summer (S) and winter (W) of the year 2013. Samples that contain no AMF were excluded from the current data.

Variables	**Location**											
	Essex						**Lancashire**					
sites	Abbottshall		Fingringhoe		Tillingham		Cartmel Sands		Warton Sands		West Plain	
seasons	S	W	S	W	S	W	S	W	S	W	S	W
AMF	9	0	4	3	0	0	21	3	6	5	15	12
pH	9	0	4	3	0	0	21	3	6	5	15	12
Ammonia	9	0	4	3	0	0	21	3	6	5	15	12
Phosphate	9	0	4	3	0	0	21	3	6	5	15	12
Nitrate	9	0	4	3	0	0	21	3	6	5	15	12
Salinity	9	0	4	3	0	0	21	3	6	5	15	12
Moisture%	9	0	4	3	0	0	21	3	6	5	15	12
TOC%	9	0	4	3	0	0	21	3	6	5	15	12

4.4.1. Spatial-seasonal variation in soil properties

All the measures of the soil variables significantly differed between saltmarshes, which also differed in different seasons. Values of soil variables for each site in this chapter are summarized in (Table 4-1). Generally, these measures showed the mean soil salinity to be higher in Essex (~56ppt) compared with Lancashire (~6ppt). On the other hand, the soil in Lancashire had a higher pH (7.71 ±0.8), phosphate and moisture compared with Essex. Both locations had higher salinity during summer compared with winter of the same year. Moister levels in the soil of these salt marshes were in the range of 30-60%

during summer, with the highest recorded in Essex compared with 54-14% during winter, with the highest recorded in Lancashire.

The percentage of the organic carbon in the soil significantly differed between seasons in both locations. This was shown to change from 8.45 to 0.08% in Lancashire, and from 7.23 to 4.62% in Essex. The seasonality had different effects on both the nitrate and ammonia in the soil. High levels of nitrate during the summer significantly decreased during the cold season, and the opposite was recorded for ammonia. Measures of nitrate were generally higher in Essex compared with Lancashire, whereas, the soil in the latter had higher levels of ammonia. Finally, the level of phosphate in these salt marshes was high, and was higher in Essex compared with Lancashire; both places had high levels of P during the summer compared, with winter of the same year.

Table 4-2; Seasonal measures of sediment properties per site are shown by Mean ±SD. ANOVA results are shown as F, with their significant values labelled as follows: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 ' ', 1. Sites are labelled as follows: AH (Abbottshall), TM (Tillingham), FW (Fingringhoe) for sites at Essex and CS (Cartmel Sands), WS (Warton Sands), WP (West Plain) for Lancashire salt marshes, followed by W (Winter) or S (Summer), depending on the season. All summer measures are shaded in grey, and the median for pH values are shown in red.

Soil Variables	AH-S	AH-W	FW-S	FW-W	CS-S	CS-W	WP-S	WP-W	WS-S	WS-W	(DF) F
TOC %	7.23 ±2.3	4.62±1.81	7.09±2.3	8.14±4.9	8.45 ±7.72	0.07±0.03	8.45 ±7.72	0.08±0.04	4.11±1.76	0.17±0.2	(1,262) 53.4***
pH	6.86 ±0.2	7.33±0.19	7.12±0.61	7.29±0.2	7.63 ±0.69	8.33±0.60	7.03 ±0.80	7.25±0.76	7.71±0.8	7.2±0.55	(1,262) 9.01**
Salinity ppt	56±14.5	25 ±2.69	48.04±6.1	19.8±4.08	5.86 ±2.58	5.54±1.71	2.04 ±1.58	4.72±1.03	4.5±2.5	4.2±0.9	(1,262) 38.4***
Nitrate µM	40.8 ±45	2.73±3.86	28.27±21.5	8.91±5.7	1.47 ±0.90	0.4±0.44	2.79 ±2.24	0.96±0.5	7.49±1.5	0.4±0.3	(1,262) 18.2***
Moisture %	56.9 ±5.1	17.1±3.14	60.6±10.1	19.2±5.47	39.5 ±4.23	25.2±2.82	44.5 ±14.0	25±9.98	30.1±9.08	22.7±8.	(1,262) 374***
Plants Richness	5±1.11	4.27±1.24	4.7±1.5	4.3±1.52	4.14±1.27	2.7±1.1	6.93±2.43	3.1±1.2	4.6±0.81	4.4±1.0	(1,262) 30.3***
Ammonia µM	55.5±47	154.5±89	68.8±43.3	205±115	78.2 ±53.7	212.2±85.	138.6 ±83.4	135.4±67	76.3±56	171±84	(1,262) 91.5***
Phosphate µM	75.7±60	15.8±27.8	62.4±65.9	14.6±18	41.5 ±62.6	46.3±59.6	84.49 ±73.9	32.42±34	83.1±88.6	30±28.9	(1,262) 12.7***

4.4.2. Are AMF species randomly distributed?

A different percentage of grass and forb species was recorded in these sites - Essex salt marshes were dominated by forbs that were rare in Lancashire (Figure 4-1). Both locations showed different plant species richness (ANOVA; $F_{4,73}$=14.3.42, P=0.001), where Lancashire was dominated by annual species, with numbers fluctuating between seasons. Essex plant species were more perennial and had almost similar abundance in different seasons. No significant differences were recorded in the number of plant species between the two locations (ANOVA; $F_{1,76}$= 1.348, P=0.24). However, the level of seasonal turnover of these species significantly differed (ANOVA; $F_{1,76}$=49.69, P=0.001). Environmental heterogeneity at both site and location level resulted in different AMF β diversity associated with their vegetation (Figure 4-2). Regardless of the temporal patterns of these factors, there were more AMF VTXs associated with the marsh plants at Lancashire compared with Essex.

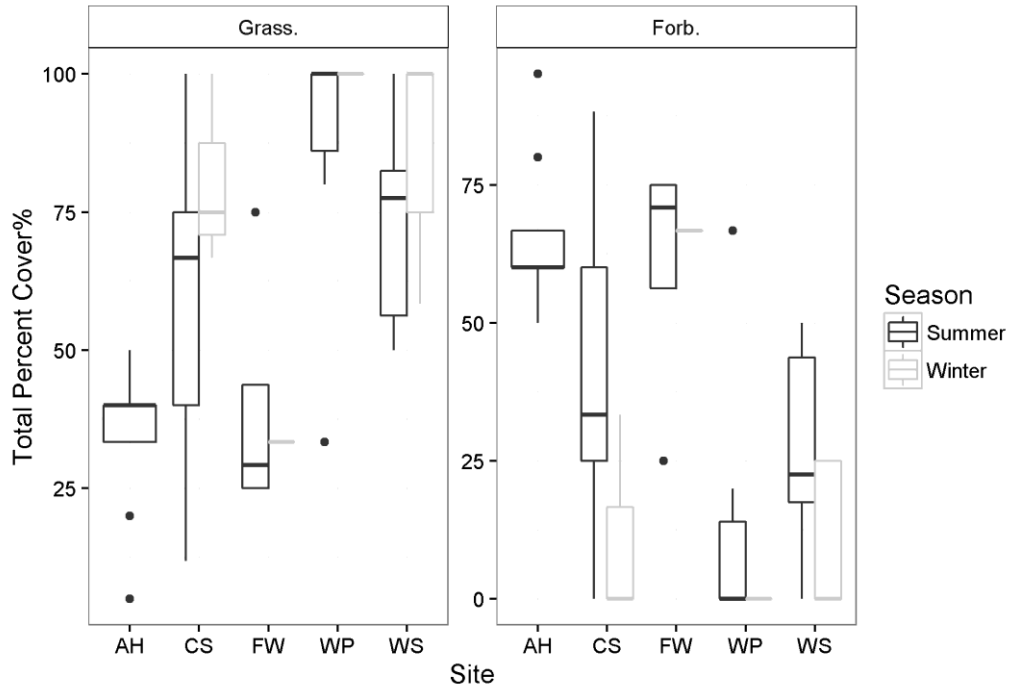

Figure 4-1: The differences in the percentage cover of different plant types at each site in both Lancashire and Essex. Sites are identified by abbreviated letters as follows: Abbottshall (AH), Tillingham (TM) and Fingringhoe Wick (FW) for Essex sites, and Cartmel Sands (CS), West Plain (WP) and Warton sands (WS) for Lancashire.

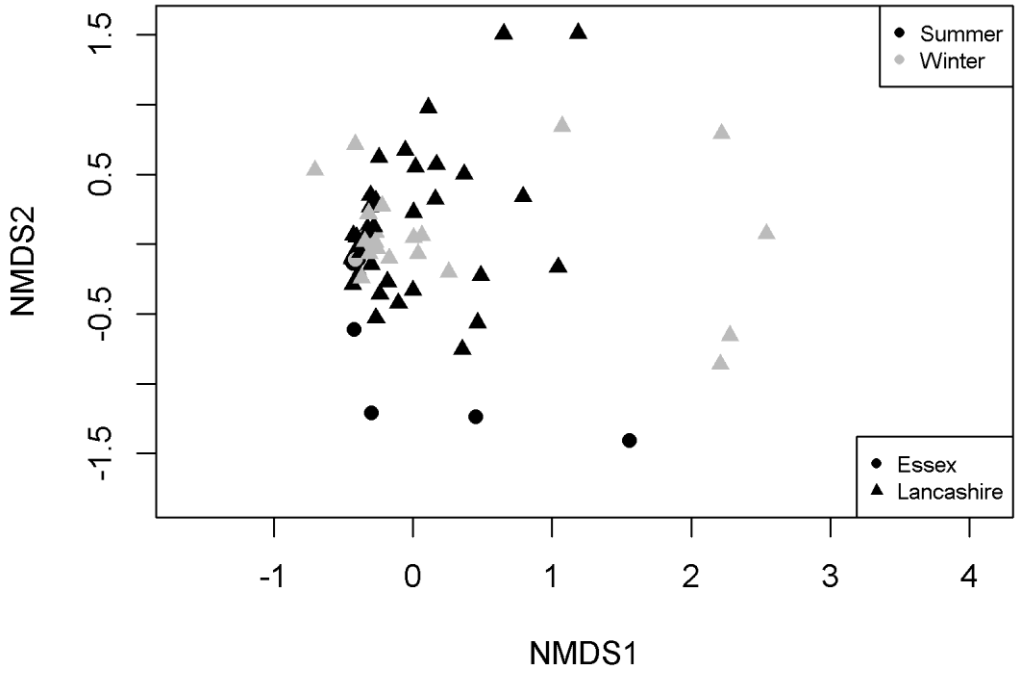

Figure 4-2: NMDS analysis based on a bray dissimilarity index showing that in terms of community composition, Essex and Lancashire communities were significantly distinct ($P<0.001$). The colours of the plot also show the seasonal distribution of AMF OTUs over summer and winter for each location, and with significant differences ($P<0.02$). The larger spread of both points indicates more variation in community composition.

4.4.3. Measuring the effect of different biotic and abiotic variables on the OUT abundance of AMF VTXs across salt marshes

Using a GLM model to estimate patterns of different biotic, abiotic, spatial and temporal variables on various AMF VTXs, at different spatial scales, I showed that spatial scale, season and soil physical properties (pH, moisture and salinity) have a greater effect on the distribution of AMF VTXs in salt marsh habitats. At the site level, AIC based comparison of these biotic and abiotic models suggested little difference in the relative influence of these variables to most OTUs. Few OTUs had large **Δ**AIC between biotic and abiotic models and those that did tended to favour the abiotic model (Figure 4-3).

The salt marshes of Lancashire and Essex accommodate different composition of AMF associating with their vegetation during summer and winter (Figure 4-2). Thus, dividing the data set based on locations enabled the investigation of large scale patterns on AMF distribution, by assessing the variation in biotic, abiotic and soil physical properties with and without the effect of seasonality (Table 4-3). The AIC scores for each model also supported this trend. Cartmel Sands was the only site in which the abiotic model better explained OTU richness, with an AIC of 87.73 vs an AIC of -42692.3 for the biotic model. Significant predictors of OTU richness were also found to be different between sites (Table 4-3). None of the abiotic variables were found to be significantly in Abbott's Hall, whereas a significant increase in OTU richness was found in the winter at Fingringhoe Wick. Within the Lancashire sites, a marginally significant negative relationship was found for pH at Cartmel Sands. Both Warton Sands and West Plain had significant changes in OTU richness in the Winter, with Warton Sands showing increased OTU richness and West Plain showing

decreased richness. For biotic models, few of the predictors were significant, particularly within the Essex sites. In Cartmel Sands, small but significant positive relationships were found for percentage cover by grass and forbs.

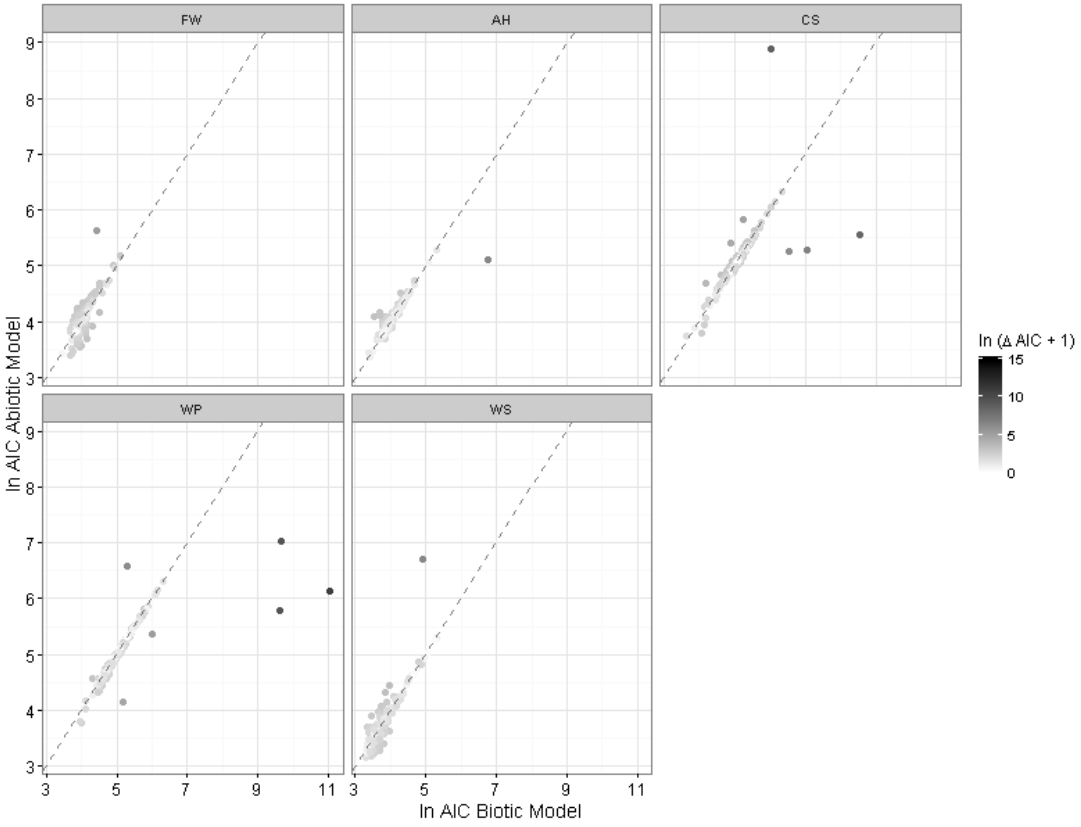

Figure 4-3: AIC scores for biotic and abiotic models for each OTU within each site. The dashed line shows a 1:1 relationship (if the two models had equal AIC scores) and darker points are those with a larger ***Δ***AIC between models. Note that the natural log of AIC scores is shown for ease of visualisation.

Table 4-3: Results of likelihood ratio tests between models with and without season. Significant values are labelled as follows: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' and 0.1, indicating a result in which inclusion of season significantly improved the model. Missing AMF data in any season will result in a Null result, as shown for both Tillingham and Abbottshall.

Site/Location	Abiotic Model with season - abiotic model without season				Biotic model with season- biotic model without season			
	Residual degrees of freedom	Degrees of freedom	Test statistic	P value	Residual degrees of freedom	Degrees of freedom	Test statistic	P value
AH	-	-	-	-	-	-	-	-
FW	32	1	65455	0.001***	21	1	1	0.25
TM	-	-	-	-	-	-	-	-
CS	2019	1	450009	0.001***	1716	1	465845	0.001***
WP	2322	1	416.9	0.04*	2120	1	139.8	0.08
WS	65	1	812.5	0.001***	32	1	371.3	0.001***
Essex	1110	1	628.4	0.002***	109	1	55160	0.092
Lancashire	5554	1	1591	0.148	5453	1	78539	0.28
Overall	7268	5	2320	0.001***	7066	5	2227	0.001***

4.4.4. Spatial pattern of AMF diversity; response to changes in the environmental at different spatial scales

Salt marshes of both Lancashire and Essex are significantly heterogeneous in terms of their biotic and abiotic variables (Table 4-3). Measurements of the response of different AMF VTXs showed that their abundance was affected by changes in plant richness, root biomass and pH. However, the composition of AMF in the salt marsh of West Plain was an exception to this effect, and most of the recorded AMF were significantly influenced by changes in soil pH. The effect of spatial changes in root biomass on the OUT abundance of different AMF VTXs was also different. Reduced

abundance of AMF VTXs was recorded at Essex salt marshes and Cartmel Sands in Lancashire salt marshes. No significant changes were recorded in West Plane, and the abundance of different AMF VTXs differed in Warton Sands. At this level (differences between sites), both root biomass and plant type (grass, Forb) had significant effects on the abundance of the AMF OTUs. Although the influence of soil abiotic variables was less significant compared with biotic ones, soil pH recorded significant effects in Lancashire salt marshes. An increased number of different AMF VTXs in these sites seems to result of the low soil acidity that is known to be favored by many plants species. This was clear in West Plane, where the influence of soil pH overcame all other factors including biotic ones. Other abiotic factors had less effect on the abundance of the AMF OTUs at this scale, thus they have been dropped from the abiotic model. At larger spatial scale (between locations), the heterogeneity of soil salinity and moisture level had low effect on the OTU abundance of different AMF species in Essex and Lancashire salt marshes. These AMFs were almost affected by changes in the soil pH, resulting in higher abundance. Abiotic factors like plant richness and root biomass cause reduced abundance. The GLM model showed that at large spatial scales, biotic variables explained 65.68% and abiotic variables explained 34.31% of the recorded OTUs.

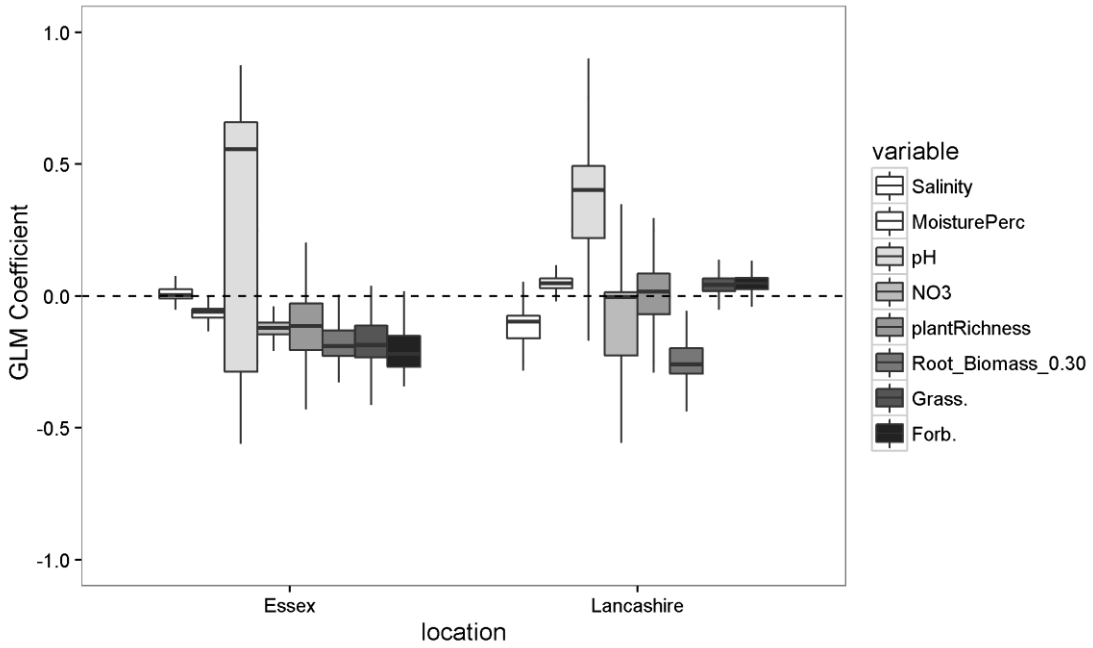

Figure 4-4: The numbers of OTUs for each AMF virtual taxa show markedly different responses to the large spatial variation in the environmental variables. Coefficient values of 0 indicate little relationship between the given environmental variable and the abundance of OTUs within a VTX.

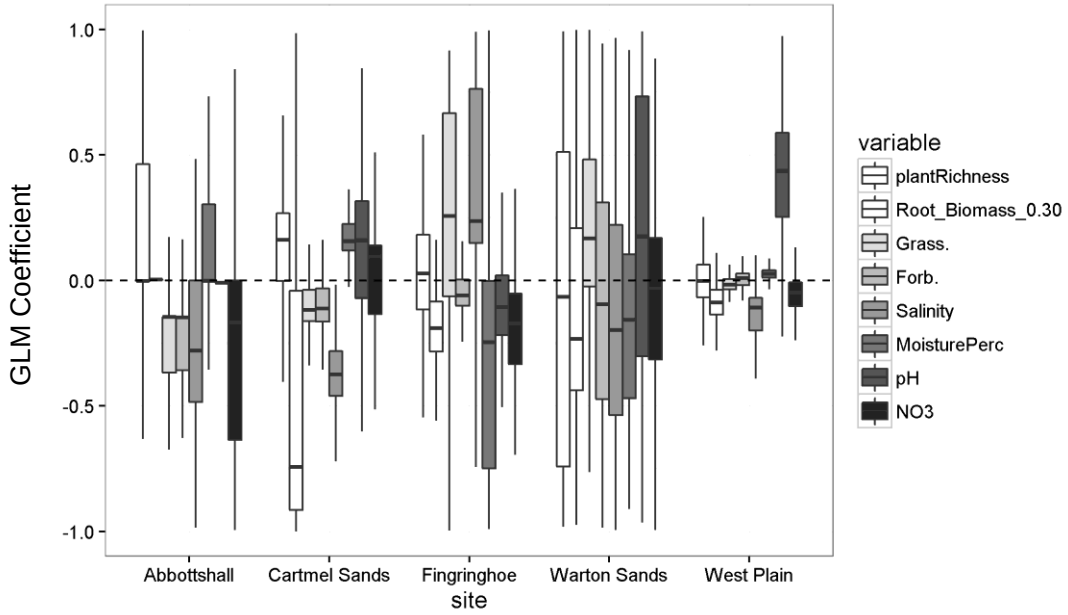

Figure 4-5: The numbers of OTUs for each AMF virtual taxa show markedly different responses to the spatial (at site level) variation in the environmental variables. Coefficient values of 0 indicate little relationship between the given environmental variable and the abundance of OTUs within a VTX.

4.4.5. Response of AMF composition to the seasonal changes of biotic and abiotic factors

Temporal variation in the AMF composition at these salt marshes was shown to be significant at large spatial scale (Figure 4-2). The GLM model showed the temporal variation of the soil conditions at these salt marshes to have low effect on the OTUs number of different AMF VTXs compared with the biotic variables (Figure 4-6). Increased abundance was showed to result in changes in the plants' condition during winter. A reduced abundance of AMF species resulted in changes in these biotic variables during the summer season, as a result of changed biotic factors.

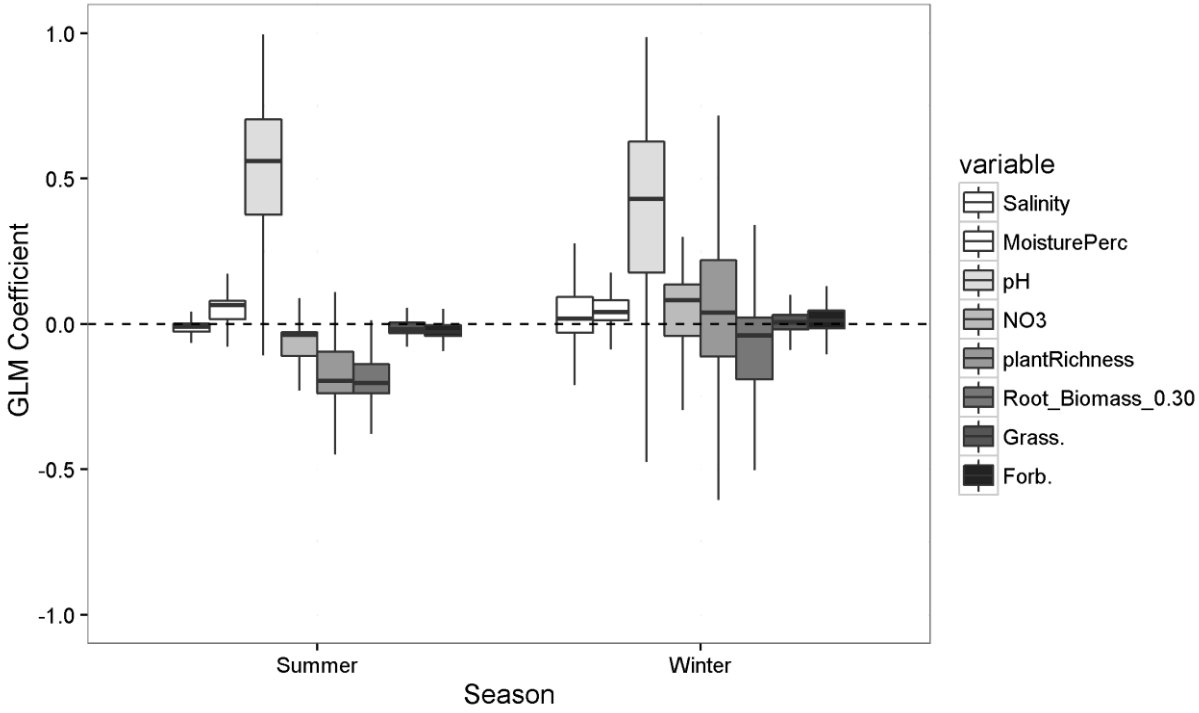

Figure 4-6: The numbers of OTUs for each AMF virtual taxa show markedly different responses to the temporal variation in the environmental variables. Coefficient values of 0 indicate little relationship between the given environmental variable and the abundance of OTUs within a VTX.

4.5. Discussion

4.5.1. Spatial differences in AMF community structure

The AMF community in these salt marshes mirrored the plant community in two important aspects - both had a low diversity and species composition that resulted due to plant zonation. The large difference between the AMF communities at the two locations may reflect variations in soil factors such as pH, nutrient content, moisture and temperature, which are known to influence spore distributions (Koske 1987; Porter *et al*. 1987; Johnson *et al*. 1991; Cuenca & Meneses 1996). The results of this study showed that the mechanism maintaining the associations between different hosts and AMF were spatially different. Although it has been reported that fungal sporulation rates vary under different host species, implying a host effect (Sanders & Fitter 1992; Bever *et al*. 1996; Eom *et al*. 2000), other studies confirmed that individual AMF exhibit a preference for specific hosts (Giovannetti & Hepper 1985; McGonigle & Fitter 1990; Talukdar & Germida 1994; Helgason *et al*. 2002).

The results from the previous chapter (Chapter Three) suggested that at the local scale the composition of AMF communities resulted in changes in their surrounding environment rather than their host plant. Nevertheless, this pattern was different at larger scales (between sites) where the role of host plants became more apparent. The role of the biotic variables in determining the community composition of AMF has only been detectable over a large scale of 100km (Green *et al.*, 2004). The salt marshes of Essex and Lancashire are representing two contrasting soil types; clay soil in Essex and sandy soil from the Lancashire (Ford *et al.*, 2016). Thus, it is highly likely that the differences in the composition of AMF communities in this chapter are due to the heterogeneity of both biotic and abiotic factors between

the two locations. AMF community composition differed significantly between soil types, with Gigasporaceae predominating in sandy soil, and Glomeraceae predominating in clay soil (Lekberg *et al.*, 2007). Similarly, the composition of AMF communities in these salt marshes showed the clay salt marshes of Essex to be dominated by Glomeraceae whereas Paraglomeraceae formed ~22% of the AMF community in the sandy salt marshes of Warton Sands, Lancashire. These soil types co-occur within an otherwise fairly large spatial scale, potentially confounding factors such as climate and host plant (Johnson *et al.*, 1992; Bever *et al.*, 1996). Other studies have shown that AMF α-diversity can be maintained through seasonal differences (Figure 4-1), differentiation among host plant species, and spatial separation (Stukenbrock & Rosendahl, 2005). The observed divergence of AMF communities between sand and clay in this study suggests that a regional mosaic of soil types may be important for maintaining high AMF β-diversity.

The significant role of biotic factors seems to be a result of changed salinity between these two locations. The role of abiotic factors remains significant regardless of spatial scale. In these salt marshes, the abundance of the AMF species was significantly affected by the soil acidity especially in West Plane, Lancashire. Morphological studies of AMF have shown soil pH to influence sporulation level (Lovelo*ck et al*, 2003), spore density and richness (Tchabi *et al.*, 2008), extraradical mycelium growth (Van Aarle *et al.*, 2002) and spore community composition (Coughlan *et al.*, 2000; Fitzsimons *et al.*, 2008). Soil pH is also widely known to reduce up to 50% of soil carbon solubility (Clark *et al.*, 2005), and this normally results in decreased rates of decomposition, as well as the solubility of phosphate compounds that heavily influence the ratio of organic carbon and other

abiotic factors (Chapman and Reiss, 1999). This was also apparent in Abbottshall and Fingringhoe Wick, Essex. Another abiotic factor that has significant effect on the abundance of AMF species in these salt marshes was the changed level of nitrate. High levels of nitrate in the soil of Essex salt marshes may result in the reduced number of AMF species compared with those of Lancashire. N is a limited element in the soil and was shown to have a negative effect on the abundance of AMF in several studies (Fitter *et al.*, 2000; Gamper *et al.*, 2004). Under the condition of low-P (high N:P) soils, plant dependency on AMF for P acquisition increases (Hetrick *et al.*, 1990), but not in the high-P soils elsewhere (Anderson *et al*. 1994, Schultz *et al*. 2001). Contrastingly, in soil that is N limited and relatively P rich, N fertilization decreases AMF number and species richness (Johnson *et al.*, 2003). Compared to both Cartmel sands and Warton Sands, the acidic soil in West Plain was showed to have different effect on the abundance of AMF species. Along with P:N rich soil, low pH was confirmed to enhance the number of AMF species by reducing the number of species (Johnson *et al.*, 1991; Dodd *et al.*, 2000; Egerton-Warburton & Allen, 2000; Carvalho *et al.*, 2001; Dumbrell *et al.*, 2011)

4.5.2. Temporal changes in the composition of AM community

Although many of the recorded AMF species in these salt marshes exist in both seasons, AMF response to seasonality was shown to be phylogenetically related. The recorded AMF compositions in the salt marshes of Fingringhoe Wick, Essex and all salt marshes of Lancashire between summer and winter were distinct. Unlike Lancashire salt marshes where seasonal variability of AMF composition might be attributed to the phenology of the annual grass species. Essex salt marshes are dominated by perennial plants species which rarely change their relative abundance

during the year. This is another confirmation of the existence of plant niches, that have a deterministic effect on the abundance of the AMF species (Dumbrell., *et al* 2010a) in grasslands, showing seasonal differences in the AMF composition, which result in changes in the local soil chemistry. Given that AMF are obligate symbionts that can only access carbon directly from their host plant, clearly carbon is one of the main limiting resources regulating AMF communities (Smith & Read, 2008; Helgason & Fitter, 2009). Increased plant richness and root biomass during summer resulted in lower number of AMF species, which in turn may be an increased limiting resource. This typically reduces both diversity and community evenness, as strong competitors are able to capture disproportionate amounts of resources and so dominate the environment (Tilman, 1987). This in turn directly affects the available habitats of AMF, and therefore the capacity of AMF species to coexist with available resources. This is likely to affect the species-specific niche space that normally results from different soil physical properties in the surrounding area between different plant species. In this case these properties are most likely to in be the soil salinity and pH, which were shown to vary from one scale to another in the current study.

4.6. Conclusions

To my knowledge, this is the largest study of AMF associating with plants from six salt marshes in the UK. Here I apply a multivariate finite-mixture model strategy, and the latest NGS technique to assess the relative importance of both biotic and abiotic variables in regulating the composition of AMF communities between different seasons, at different spatial scale. Current results revealed that within the site level, soil pH and salinity best predicted AMF distributions, but at larger scales the effect of the biotic variables was superior, indicating a degree of

context dependency. Following the changes in the conditions of these biotic variables over the seasons, the pattern of AMF community compositions appears to be seasonally dependent. By and large the significant role of soil pH in changing the abundance of the AMF may indicate a scale dependency of these biotic and abiotic factors. Thus ranking soil variables, and plants in order of importance on niche axes might be possible, however, they each appear to have a significant influence on AMF community composition. Although this chapter demonstrates an important scale dependency, our knowledge about the mechanisms that regulate the diversity, structure and composition of microbial communities still lag behind those for plants. Such knowledge is crucial to help conserve ecosystem functioning, and protect biodiversity from ongoing environmental change.

Chapter five

5. Ecological Drivers of Fungal Diversity are Context Specific

5.1. Summary

- Plant-root associated fungi are one group which are known to influence individual plants and plants community dynamics. A spectra of plant-fungal interactions have been documented ranging from highly beneficial though to highly detrimental to the plant. Despite this, our knowledge about the factors that drive community structure is taxonomically biased towards plants and other higher organisms whilst microorganisms are still relatively under-studied
- Using NGS techniques, AMF were surveyed in 264 individual sediment samples of 32 plant species, along a soil physio-chemical gradient from the salt marshes of Essex and Lancashire, UK. Multivariate generalised linear models were used to examine whether abiotic or biotic factors better predicted the richness and abundance of OTUs within each site and if OTUs in different functional groups responded differently to environmental gradients.
- Result showed the ability of abiotic and biotic variables to predict fungal richness and abundances differed greatly between sites, suggesting that drivers of fungal community structure may be site-specific to some extent. Indeed, fungal richness in relation to abiotic or biotic factors explained considerably different amounts of variation in each site, and generalised poorly to other sites. Therefore, it is possible now to argue against deriving general environment-diversity relationships without empirical validation in multiple sites. Finally, functionally similar fungi responded similarly to the environmental gradients, suggesting a possible disconnect between functional redundancy and resilience in microbial communities.

5.2. Introduction

Ecosystem multi functionality has long been understood to be influenced by the identity and numbers of species present in a given ecosystem (Tilman *et al.* 1997; Díaz & Cabido 2001; Mittelbach *et al.* 2001; Maestre *et al.* 2012; Fraser *et al.* 2015). It therefore follows that, changes in species richness or community structure may have significant effects on ecosystem processes and functionality (Chapin III *et al.* 1997; Schmid *et al.* 2001; Hooper *et al.* 2005; Balvanera *et al.* 2006). Elucidating the factors that modulate the richness and structure of communities is consequently of paramount importance if we are to maintain and manage the range of services that ecosystems provide.

Despite this, our knowledge about the factors that drive community structure is taxonomically biased towards plants and other higher organisms whilst microorganisms are still relatively under-studied. This is surprising as microorganisms are numerous, taxonomically and functionally diverse and extremely widespread, making them key drivers in most ecosystem processes (van der Heijden *et al.* 2008; Graham *et al.* 2016; Delgado-Baquerizo *et al.* 2016). In particular, microorganisms that associate with plants can have a large effect on ecosystem processes by altering plant community dynamics (Zak *et al.* 2003). Plant-root associated fungi are one such group which are known to influence individual plants and plants community dynamics. Spectrums of plant-fungal interactions have been documented ranging from highly beneficial though to highly detrimental to the plant. These effects are due to fungal roles in promotion of productivity and nutrient uptake (Van Der Heijden et al. 2006), alleviation of environmental stresses (Al-Karaki *et al.* 2004; Rodriguez & Redman 2008; García-Sánchez *et al.* 2014), and protection from

plant pathogens (Sikes 2010; Veresoglou & Rillig 2012), but also themselves as plant parasites and pathogens (Johnson *et al.* 1997; Klironomos 2003; Veiga *et al.* 2013). However, the ecological drivers of root associated fungal diversity and abundance are not fully understood.

For long time, studies of microbial diversity have mainly relied on morphological taxon identification; however, the limitations of such methods are well known (Bass *et al.*, 2007). Thus, the rapid progress of molecular approaches has significantly improved our understanding of how the structure of root associated fungal communities is altered by various environmental factors (Dumbrell *et al.,* 2010, Cotton *et al.,* 2015; Dumbrell *et al.,* 2011; Peay *et al.,* 2010; Rudgers *et al.,* 2014, Kivlin & Hawkes, 2016). Continues developmening of these methods has contributed to the coast reduction and allows studies to address these factors at larger spatial scales (Tedersoo *et al.,* 2014; Kivlin *et al.,* 2011; Davison *et al.,* 2015). A variety of abiotic ecological drivers of community structure have emerged from such studies, including edaphic factors such as pH, salinity and soil moisture (Dumbrell *et al.* 2010; Tedersoo *et al.* 2014) and climate related variables such as seasonality (Dumbrell *et al.* 2011; Cotton *et al.* 2015; Kivlin & Hawkes 2016). Biotic factors related to plant diversity, identity and traits have also been identified to modulate fungal community structure and diversity (Comas *et al.* 2014; Tedersoo *et al.* 2015; Kivlin & Hawkes 2016). A growing body of research also suggests that drivers of community structure may be dependent on the taxonomic or functional group in question (Tedersoo *et al.* 2014; Glassman *et al.* 2015; Nguyen *et al.* 2016). However, the predominant drivers detected by such studies are not consistent suggesting that they are, to some extent, context specific between sites, systems or

seasons. Moreover, whilst the fungal communities of certain habitats, such as grasslands or agricultural land (Johnson *et al.* 2004; Hiiesalu *et al.* 2014), are well studied, other important habitats remain under-explored. This is including both the coastal marshes and wetlands that are globally important and widespread habitats. They provide valuable ecosystem services such as coastal protection (Möller *et al.,* 2014) and sequestration of CO_2 (Mcleod *et al.,* 2011) as well as harbours of biodiversity and commercially important grazing land. The diversity of microbial communities and especially the root-associated fungi of these habitats are still poorly understood.

Fungi like the AMF are obligate aerobes (Khan, 1993; Miller & Sharitz, 2000) thus, most wetland plants were thought of as non-mycorrhizal (Khan, 1974; Anderson *et al.*, 1984), and/or AMF have little significance under these conditions (Bohrer *et al.*, 2004). However, Carvalho et al. (2004) found evidence that arbuscular mycorrhizal fungal (AMF) spores isolated from a salt marsh were specially adapted to the saline conditions of a salt marsh, and indeed were able to germinate in salinities that non salt marsh AMF spores couldn't. Mohamed & Martiny (2011) studied the sediment fungal communities of three tidal marshes representing a gradient in salinity. They found that fungal diversity peaked in the intermediate salinity marsh and was also promoted by the presence of plants in all three marshes, suggesting that the biotic environment could be important in regulating the fungal communities.

Most of the previous works on fungal communities were carried on the US or central European salt marshes, and many other systems, have demonstrated that both biotic and abiotic factors may be important drivers of fungal community

structure. Yet, the relative importance of these variables is rarely addressed (e.g. Bainard et al. 2014; Davison *et al*. 2015). It is therefore unclear how this balance between biotic and abiotic drivers scales between multiple sites, spatial scales and seasons in salt marsh systems. At the site level, local scale current results showed factors such as severity of tidal inundation may determine the steepness of abiotic gradients in marshes. Therefore, the importance of abiotic factors may vary at small spatial scales due to the high dependency on the extremity of the abiotic gradients present. Whereas at larger spatial scales, broad differences in vegetation type and structure, possibly caused by dispersal limitation of plants, may be more potent drivers of root-associated fungal communities and differences in abiotic factors may be less important. Given that fungi within taxonomic or functional groups are likely to be differentially effected by such abiotic gradients, thus the relative importance of biotic and abiotic factors to change between groups is also highly expected. This is evident for salt marshes in these countries and many others including UK, where salt marshes considered as key habitats that cover over 45,337 ha of the coastal line (Boorman, 2003).

Therefore, quantified root-associated fungal communities in 219 plant root samples, representing six salt marshes in two regions of UK, were collected over summer and winter to assess (I) the relative importance of biotic and abiotic drivers to fungal community structure at different scales and seasons. To evaluate (II) the responses of different fungal taxonomic groups to the spatio-temporal pattern of these drivers will be different. To examine (III) wither different functional groups will show different responses to abiotic and biotic variables.

5.3. Materials and methods

Sampling design and site description with Sediment chemistry analysis and AMF determination were fully explained in the methodology Chapter. Current chapter includes data for all sediment cores that have AMF association recorded within the rot samples within each of them. The fungal ITS region was amplified using the fungus-specific primers (ITS1F; Gardes & Bruns, 1993) and universal (ITS2; White *et al*., 1990). For the sake of accuracy and robustness any 18S data were not included in the analysis of between AMF and other fungi. Most of the surveyed studies had utilized the primer pair NS31–AM1 covering a *c.* 500-bp central fragment of the SSU rRNA gene. The primer pair is known to amplify most taxa of the *Glomeromycota*, but to exclude the basal families *Archaeosporaceae* and *Paraglomaceae* (Daniell *et al.*, 2001).

Bioinformatic analyses

Due to the length of the amplicon sequenced, paired-end overlapping of reads was not possible; therefore all analyses were conducted on the forward reads only. The use of single read sequence data instead of paired-end reads has been shown to have minimal effects on ecological conclusions (Werner *et al.* 2012) and is already in use in molecular studies of fungal communities (e.g. Dini-Andreote *et al*. 2016). The bioinformatics pipeline Qiime (Caporaso *et al.* 2010) was used to quality filter sequences with minimum quality threshold of Q20. Only high quality full length reads were used which were then stripped of the forward primer using Linux shell commands. VSEARCH (version 2.1.1; Rognes *et al*. 2016) was used to de-replicate reads, remove singleton sequences and then cluster sequences at a 97% similarity threshold. OTUs were reference checked for chimeras with VSEARCH against the

UNITE database (Kõljalg *et al.* 2013). VSEARCH is an open source implementation of the popular USEARCH pipeline (Edgar 2010), and has been shown to produce similar results and to perform well compared to other OTU clustering algorithms (Westcott & Schloss 2015; Jackson *et al.* 2016). Taxonomy was assigned to OTUs using the RDP classifier (Wang *et al.* 2007), trained on the UNITE database with a minimum confidence threshold of 0.7. , FUNGuild was used to assign fungal OTUs to a functional guild (Nguyen *et al.* 2016). FUNGuild annotates OTUs with functional traits by matching taxonomic terms with a database of known fungal functional groups. Both functional guilds and trophic modes are assigned to OTUs. Here, trophic modes were used to define the functional groupings for two reasons:

1. The number of functional guilds present in our dataset was large (19) with most of them represented by relatively few OTUs (Fig. S2). The presence of many low abundance groups may lead to downstream statistical problems such as over-fitting during finite-mixture modeling and unreliable results from Chi-Squared due to many groups with low expected values.
2. Functional groups that reflect groups of fungi having broadly similar effects or contributions to plant communities and ecosystem functions, especially as such groups have previously been shown to differ in their ecologies (e.g. Tedersoo *et al*. 2014). All analyses were performed using the BioLinux 8 operating system (Field *et al.* 2006).

5.3.1. Statistical analyses

Models with either abiotic or biotic variables were compared to examine whether abiotic or biotic factors better predicted the richness and abundance of OTUs within each site. Variables were selected for each model if they had been

shown in previous studies to be drivers of environmental fungal diversity and community structure. Variables included in the abiotic model were site (when analysing data at the location or overall scales), season (Dumbrell *et al.* 2011; Cotton *et al.* 2015; Kivlin & Hawkes 2016), salinity (Mohamed & Martiny 2011; Maciá-Vicente *et al.* 2012), pH (Dumbrell *et al.* 2010; Hazard *et al.* 2013; Tedersoo *et al.* 2015; Barnes *et al.* 2016) and soil moisture (Pellissier *et al.* 2014; Deepika & Kothamasi 2015; Erlandson *et al.* 2016). Biotic models included the variables plant species richness (Hiiesalu *et al.* 2014; Tedersoo *et al.* 2015; Kivlin & Hawkes 2016), total root biomass (Hiiesalu *et al.* 2014) and % cover of forbs, grasses, herbs and sub forbs (Tedersoo *et al.* 2013; Chagnon *et al.* 2015; Nguyen *et al.* 2016). Plant species were grouped into grasses, herbs, forbs or forbs, rather than using the percentage cover of individual plant species to predict fungal community structure. These groups reflect the broad differences in root morphologies and life history strategies (e.g. Perennial vs annual) that were expected to affect fungal communities (Hawkes *et al.* 2006; Tedersoo *et al.* 2015; Kivlin & Hawkes 2016).

5.3.2. Environmental drivers of OTU richness

OTU richness was modelled as a function of biotic and abiotic variables using negative binomial GLMs, as the highly over-dispersed nature of microbial count data can mean that distance based methods are unreliable (Warton *et al.* 2012). The spatial scaling of the relative influence of biotic and abiotic variables was investigated by conducting our modeling in a spatially hierarchical manner. Separate models were conducted for communities within sites (site level), within locations (location level) and for overall (all pooled data).

Random variation in the number of reads obtained for each sample was accounted for by including read numbers as a model covariate as used by (Bálint *et al.* 2015). This method avoids the undesirable statistical properties introduced by rarefaction (McMurdie & Holmes 2014). Akaike's information criterion (AIC) was used to compare the relative ability of the models to describe OTU richness. Adjusted D^2 was calculated as a measure of goodness of fit as described by Guisan and Zimmermann (2000).

5.3.3. Environmental drivers of OTU abundances

OTU abundances were modelled using multivariate negative binomial GLMs (Wang *et al.* 2012; Warton *et al.* 2015). The only included OTUs were occurred in more than 4 samples, as OTUs with fewer occurrences are unlikely to reveal much information about the drivers of community structure in fungal communities, and may in fact obscure patterns by adding noise. AIC scores were calculated for each OTU in each model and these were used to compare the fit of abiotic and biotic models. The total AIC of each model (sum of AICs from all OTUs) was used to compare models at the community level. The effects of accounting for temporal variation were investigated by adding and removing season to both abiotic and biotic models. Likelihood ratio tests with 999 monte-carlo permutations were used to determine whether the addition of season resulted in a significantly better model fit. As with the OTU richness models, the OTU abundance models were conducted in a spatially hierarchical manner with models for communities within each site, location and overall. The responses of different taxonomic groups to the environmental variables presented were investigated by examining the estimated model coefficients from all OTUs.

5.3.4. Consistency of environmental responses within functional groups

To investigate if OTUs in different functional groups responded differently to environmental gradients, finite mixture models were used. These models can be considered a form of multivariate generalised linear model, with the extension that response variables (OTUs) are clustered by their regression coefficients into a user determined number (referred to as *G*) of groups called “Archetype species” (Dunstan *et al.* 2013b). In our study, archetypes therefore represent groups of OTUs which have similar responses to either the biotic or abiotic variables. Setting G (number of archetypes) equal to the number of functional groups, allow a maximum association whereby the OTUs in each functional group fell into their own archetype. This method provides an elegant model based approach to dimension reduction in highly multivariate data such as OTU tables and has also been shown to improve the predictive ability of the model for low abundance species (Hui *et al.* 2013), of which there are usually many in microbial datasets (Sogin *et al.* 2006). Importantly, it also allowed testing hypothesis that OTUs in the same functional category would have similar environmental responses. By calculating a contingency table of the number of OTUs in each functional category and each archetype, Chi-Squared test was used to test for associations between functional groups and archetypes. Chi test P values were simulated with 10,000 permutations. This procedure was repeated independently for biotic variables and abiotic variables e.g. archetype G-1 from the biotic model has no relationship with archetype G-1 from the abiotic model.

5.4. Results

An initial ~99.6 million sequences were reduced to ~ 55.8 million reads after quality filtering. These reads clustered into 4,638 non-singleton OTUs. Most OTUs could be assigned a phylum, whilst ~25% were identified to species level (Figure 5-1).

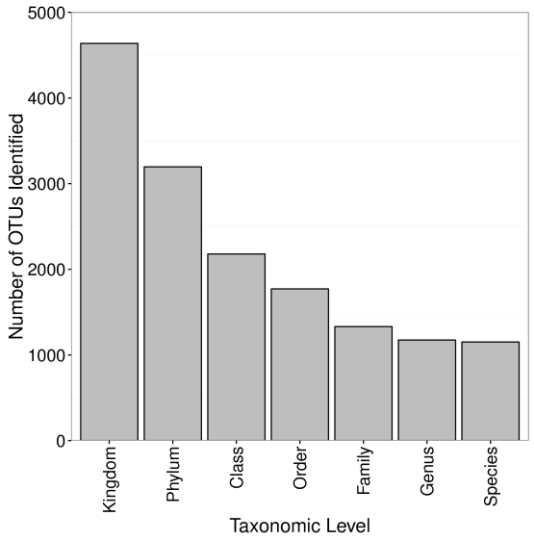

Figure 5-1: The number of fungal OTUs identified to each taxonomic level using the RDP classifier trained on the UNITE database. Of the 4 641 OTUs, 24.8% could be identified to species level at a 0.7 confidence level.

Taxonomic assignments showed that most sites were dominated by 3-4 fungal classes, with the Sordariomycetes, Dothideomycetes and Agaricomycetes particularly abundant (Figure 5-4). Functional assignments of the fungal OTUs revealed that the vast majority of fungal OTUs could not be assigned to a functional guild (Figure 5-2).

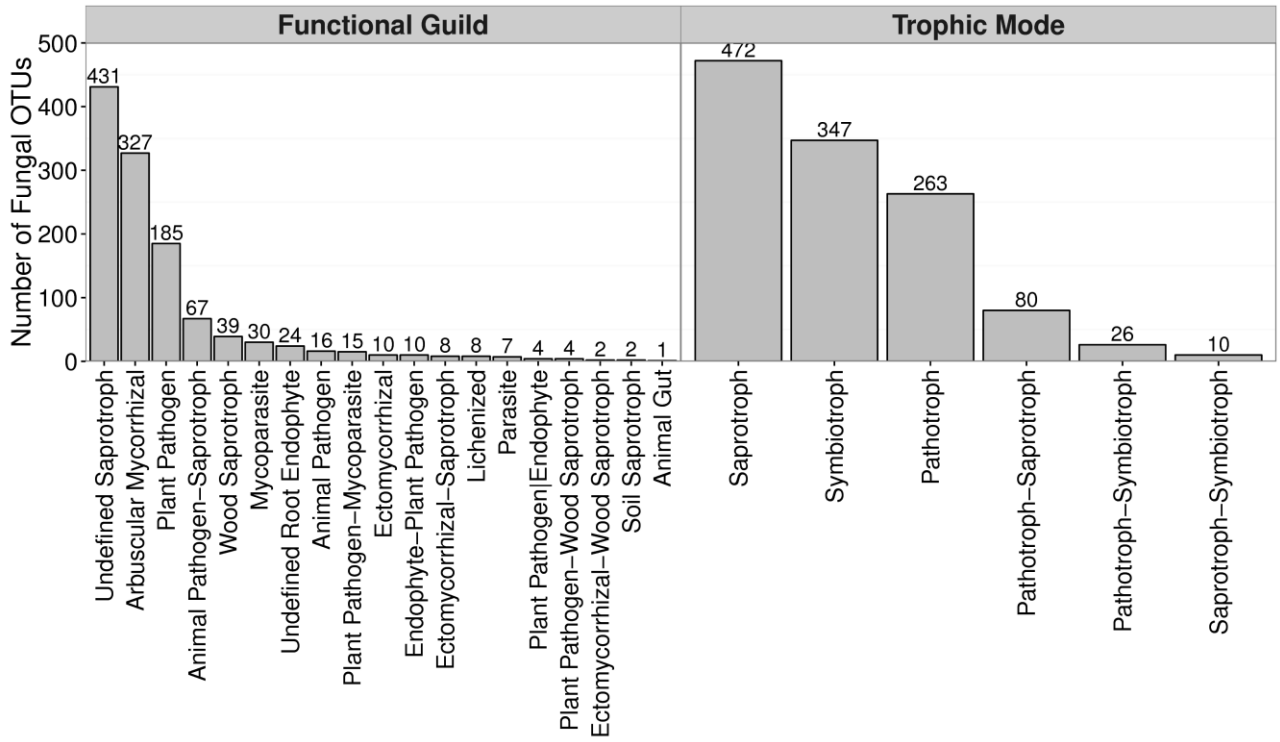

Figure 5-2: The number of OTUs assigned to functional guilds and trophic groups according to FUNGuild assignments using UNITE taxonomic assignments. Fungal OTUs which were not assigned a function were removed for visualisation purposes. 74.4% of all OTUs were not assigned to a functional guild and 74.2% were not assigned to a trophic mode. Undefined fungi represent OTUs which were not identified to a high enough taxonomic resolution and fungi whose functional traits may not be known/recorded.

Of those that were assigned to a guild, indeterminate Saprotrophs were the most abundant, followed by AMF and plant pathogens. Saprotrophs and symbiotrophs were the most abundant trophic groups of those fungi that were assigned a function (Figure 5-3).

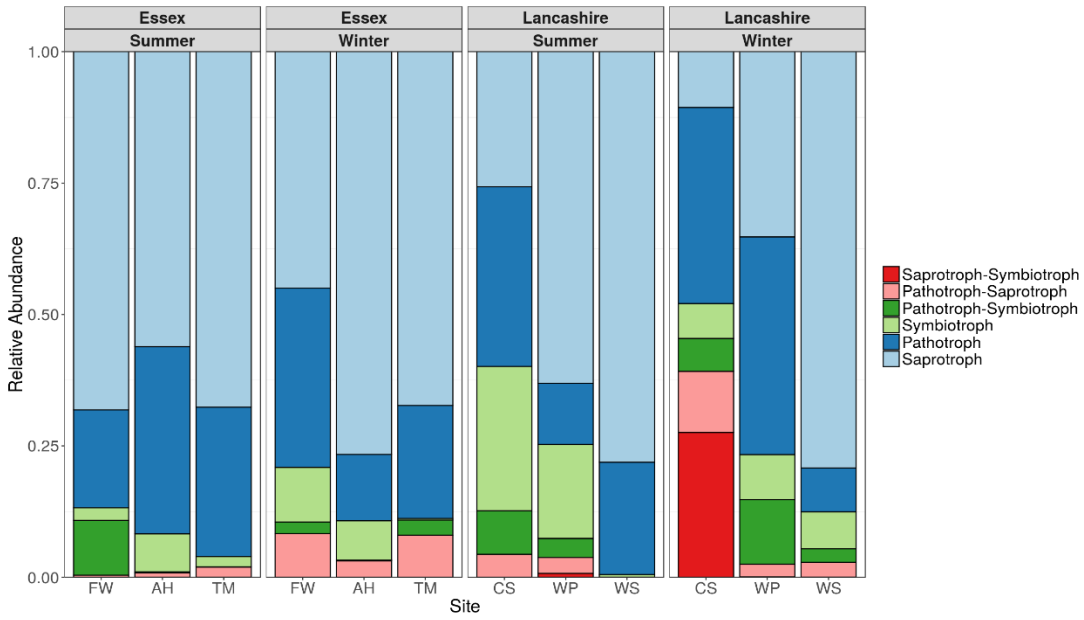

Figure 5-3: The relative abundance of different trophic groups between sites and seasons. Note that these relative abundances are scaled to total 1 after removing “undefined” fungi. 74.2% of OTUs were not assigned to a trophic mode.

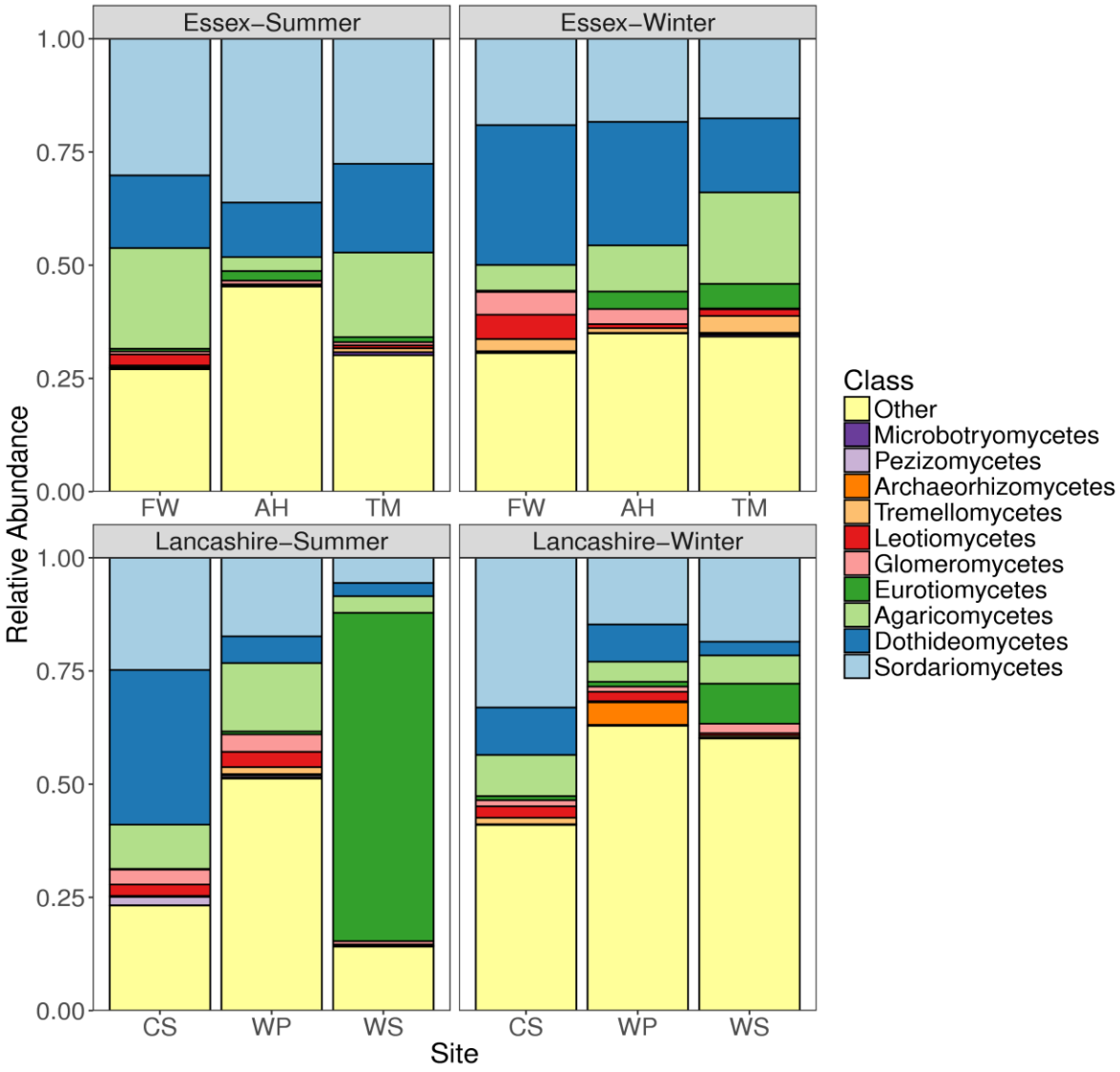

Figure 5-4: The relative abundance of the 10 most abundant classes overall. Sites are presented in order from least (left) to most (right) tidally inundated. Class “Other” represents binned low abundance classes and OTUs not identified to class level. Only the most abundant 10 classes are shown for visual simplicity.

Approximately 33% of all the OTUs found were shared between Essex and Lancashire sites. Lancashire however, contained far more OTUs unique to Lancashire sites than Essex (Figure 5-5). Non-metric dimensional scaling (NMDS) analysis of the fungal communities showed that, compositionally, Essex and Lancashire communities were distinct (Figure 5-6). Essex sites tended to vary less than Lancashire sites as illustrated by the tighter cluster of points representing Essex communities. There was no clear divide between summer and winter communities in terms of community composition.

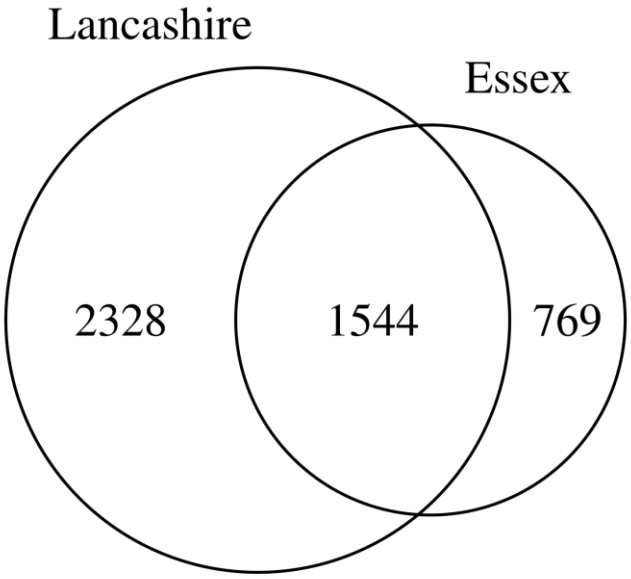

Figure 5-5: Venn diagram showing the number of OTUs (Richness) found in each sampling location. Approximately 50% of all the OTUs found were only found in Lancashire sites, compared to 16.5% found only in Essex sites.

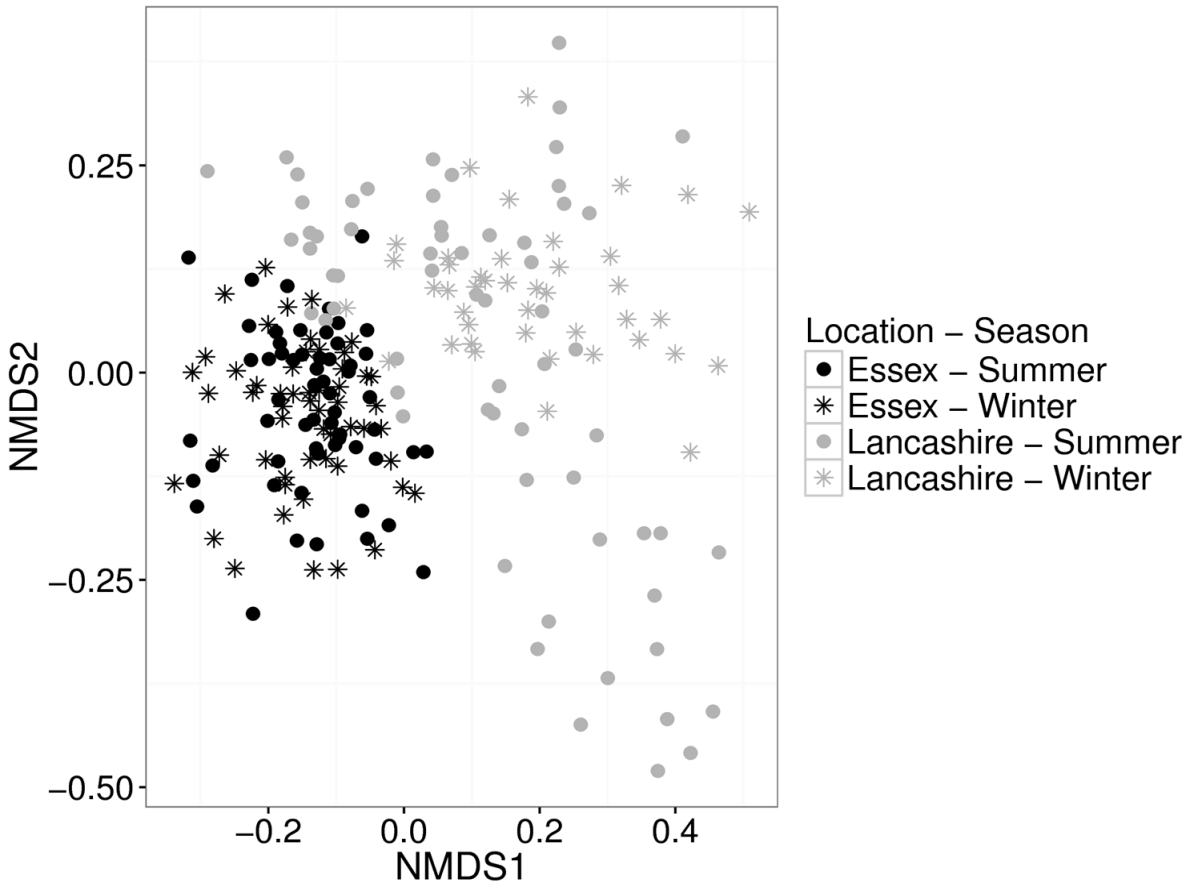

Figure 5-6: NMDS analysis based on a Jaccard dissimilarity matrix shows that in terms of community composition, Essex (black points) and Lancashire (grey points) communities were largely distinct. The larger spread of Lancashire points indicates more variation in community composition compared to the tightly clustered Essex communities.

5.4.1. Biotic and Abiotic Drivers of Fungal Richness

Generalised linear models were used to model the OTU richness within each site, location and overall using biotic and abiotic models. At the site level, abiotic models tended to better predict OTU richness compared with biotic models (Figure 5-7). Model fit varied considerably between sites, with abiotic variables having a D2 value of between 0.24 and 0.82. Biotic variables had D^2 values between -0.2 (0.11 before adjustment) and 0.61. In all sites, aside from Cartmel Sands, abiotic models had higher D^2 values indicating a superior fit. This trend was further

supported by the AIC scores for each model. Cartmel Sands was the only site in which the biotic model better explained OTU richness, with a D^2 of 0.61 vs a D^2 of 0.25 for the abiotic model. Significant predictors of OTU richness were also found to be different between sites. None of the abiotic variables were found to be significantly in Abbott's Hall, whereas a significant increase in OTU richness was found in the winter at Fingringhoe Wick. Tillingham was different again with a small but significant relationship between OTU richness and salinity. Within the Lancashire sites, a marginally significant negative relationship was found for pH at Cartmel Sands. Both Warton Sands and West Plain had significant changes in OTU richness in the winter, with Warton Sands showing increased OTU richness and West Plain showing decreased richness. For biotic models, few of the predictors were significant, particularly within the Essex sites. In Cartmel Sands, small but significant positive relationships were found for percentage cover by herbs and forbs.

When OTU richness was modeled at the location level (data were pooled by location rather than by site), abiotic variables once again better explained OTU richness than biotic variables in both Essex (abiotic AIC = 1175.2, biotic AIC = 1214.7) and Lancashire (abiotic AIC = 1262.3, biotic AIC = 1293.1). In both locations, abiotic models fit the data better with D^2 0.52 and 0.41 in Lancashire and Essex respectively. In Essex, OTU richness between sites was found to differ significantly with Fingringhoe Wick and Tillingham significantly more OTU rich relative to Abbott's Hall. None of the biotic predictors were found to be significant within Essex. In Lancashire, West Plain was significantly richer in OTUs than Warton Sands and Cartmel Sands and winter was found to have a significant positive effect on OTU richness. Small significant relationships were found for salinity and soil

moisture. Plant richness and soil root biomass were also found to positively influence OTU richness in Lancashire.

Similarly, when all data were pooled, overall, abiotic variables were superior to biotic variables and the fit of both models was similar to the location level (Abiotic; D2 = 0.59, AIC = 2444.4, Biotic; D^2 = 0.41, AIC = 2520.3). An overall site effect was found with Abbott's Hall containing significantly fewer OTUs than all other sites apart from Warton Sands. A marginally significant negative relationship with pH was also observed. Of the biotic variables, only root biomass was significantly related to OTU richness with a small positive relationship.

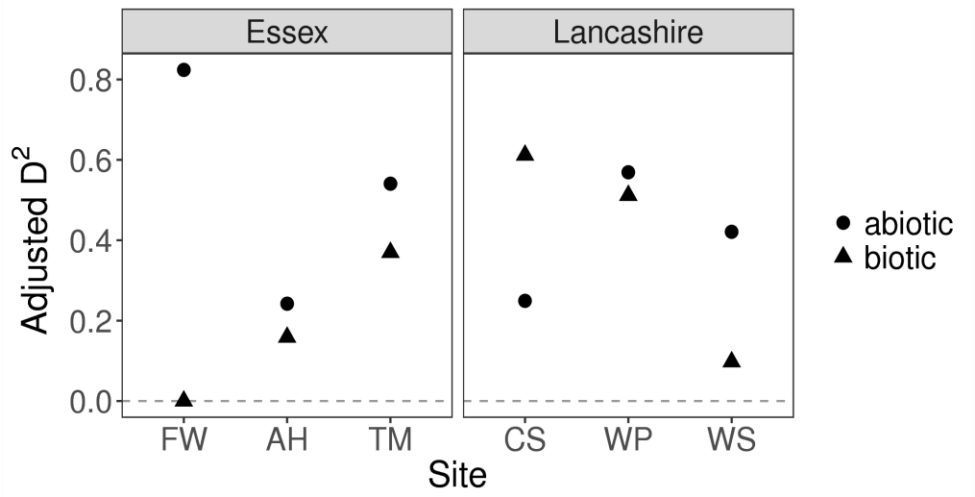

Figure 5-7: D^2 values for GLM fits OTU richness in each site by biotic or abiotic models. In most sites, abiotic models fit the data better, apart from in Cartmel Sands. Note that negative D^2 values are possible due to the process of adjustment to account for differing numbers of variables or observations.

5.4.2. The Relative Roles of the Biotic and Abiotic Environment on Fungal Abundances

At the site level, AIC comparison of biotic and abiotic models showed that, in all sites, the abiotic model better described OTU abundances overall as total AICs

were lower for the abiotic model. However, at the individual OTU level, the relative importance of biotic and abiotic variables was more complex. Few OTUs had a large ΔAIC between biotic and abiotic models and those that did tended to favour the abiotic model (Figure 5-8). Within Essex sites, OTU abundances were better described by abiotic variables with 69.7% (FW), 67.8% (AH) and 71.6% (TM) of OTUs having a lower AIC for the abiotic model. In comparison, OTUs from Lancashire sites were more evenly split between biotic and abiotic models with only 51.2% (CS), 55.1% (WP) and 62.9% (WS) of OTUs having a lower AIC for abiotic variables hinting at a more even role for biotic and abiotic drivers.

In contrast, when examining OTU abundances at the location level (with site included in the abiotic model), most OTUs were better described by the biotic model with 66% (Essex) and 65.6% (Lancashire) of OTU abundances better described by biotic variables. Similarly, to the site level, the total AICs for each model in each site favoured the abiotic model in both Essex and Lancashire.

At the largest scale within our study, abiotic variables were once again found to be better descriptors of OTU abundances. 1,286 of the 1,999 OTUs analysed were best described by the abiotic model. Cumulative AIC for the biotic model over all sites was 4,110,048 compared to 2,958,411 for the abiotic model (excluding site variable) suggesting that the abiotic model was likely the better model overall. Adding site or location variables to each model did not greatly improve either model (Figure 5-9).

Overall, most OTUs the abiotic and biotic factors played a relatively even role in modulating their abundances. However, some OTUs were explained far better by

abiotic rather than by biotic variables, which helped shift the total AICs of each model in favour of abiotic variables.

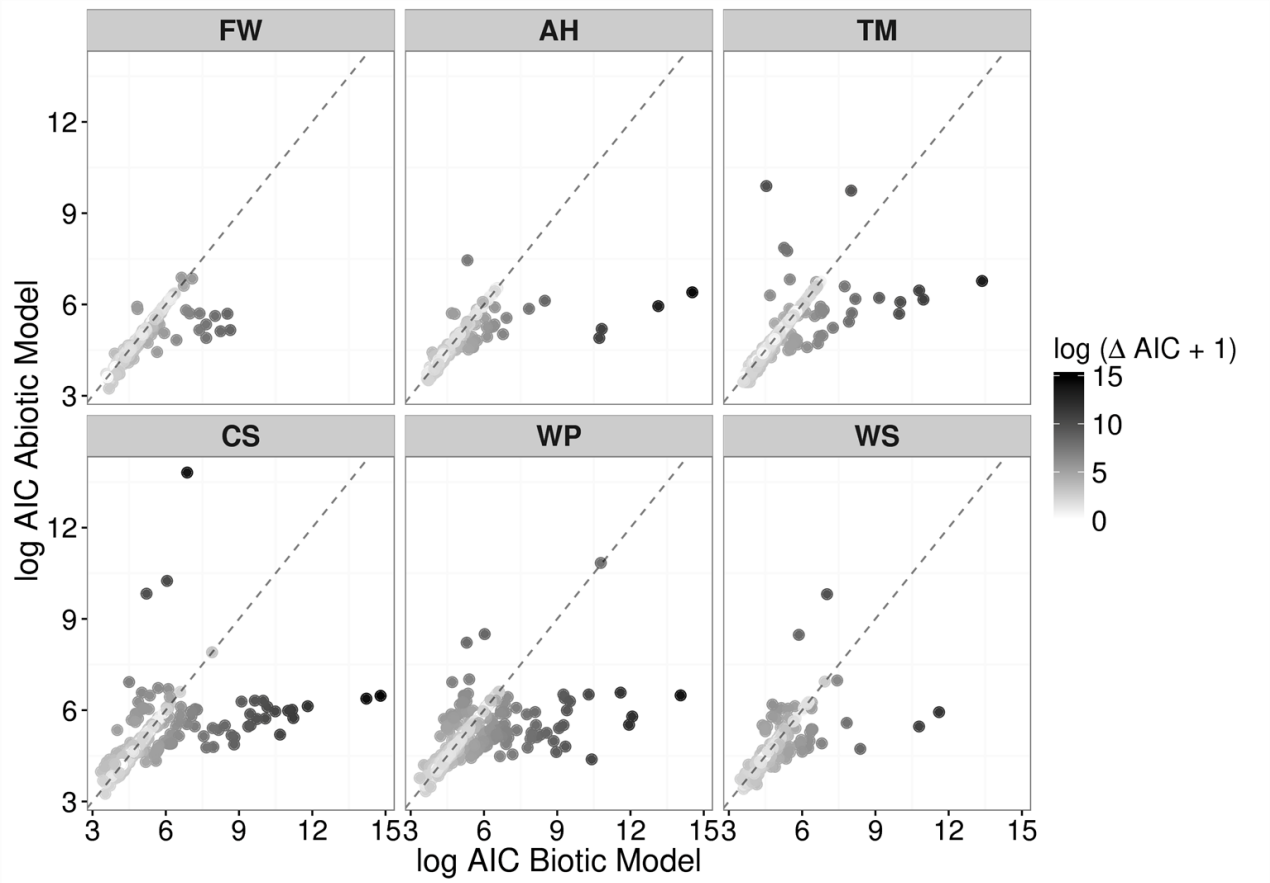

Figure 5-8: AIC scores for biotic and abiotic models for each OTU by site. The dashed line shows a 1:1 relationship (if the two models had equal AIC scores), thus points below this line indicate OTUs better described by the abiotic model and points above the line are OTUs better described by biotic variables. The darker the point, the larger ΔAIC between models, indicating that one model is notably better than the other. Note that the natural log of AIC scores are shown for ease of visualisation.

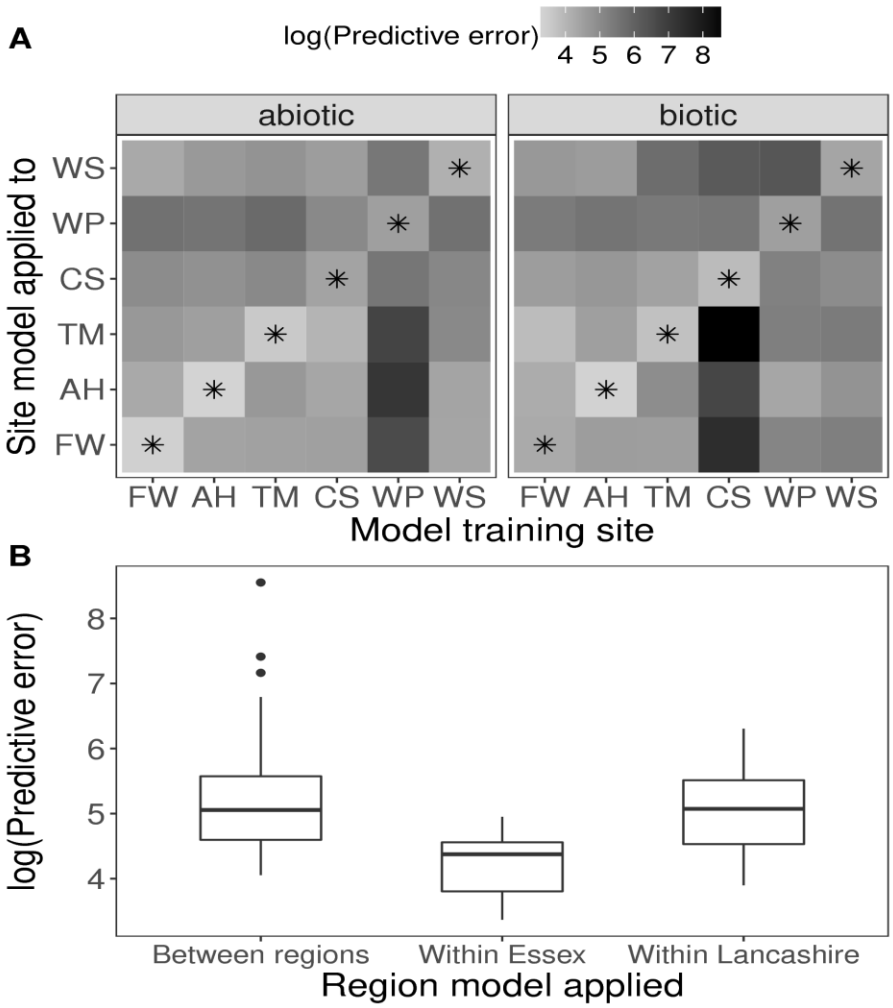

Figure 5-9: Predictive error of OTU richness models when used to predict other sites' OTU richness. (A): The predictive error of an OTU richness model when trained on data from a given site (x-axis) and used to predict OTU richness in another site (y-axis). Asterisks indicate the predictive error of models which were trained and applied on the same site. Lower values indicate a better fit between observed and predicted values (B): Predictive error of models when applied to a site from a different, or the same, region to the site from which they were trained.

5.4.3. Context Dependency in the Importance of Season

The importance of season on fungal communities was analysed using likelihood ratio tests on models with and without season. Within each site, the addition of season to abiotic models resulted in significantly better fits in four of the six sites. Adding season to biotic models again resulted in better models for four sites, with Abbott's Hall and West Plain the exceptions.

At the location level, the addition of season to both biotic and abiotic models resulted in significantly better fits for both Essex and Lancashire marshes. Similarly, when all sites were examined at once, inclusion of season resulted in significantly better fits to both models.

5.4.4. Taxonomic Differences in Environmental Responses

Model coefficients estimated from the overall biotic and abiotic models showed that taxonomically consistent responses to the environment only emerged at relatively high taxonomic resolutions, if present at all. At the phylum and class levels few taxonomically consistent responses to most environmental covariates were observed, with the coefficients for most classes spanning (Figure 5-10). This is indicating that OTUs with both positive and negative responses to the same variable. Notable exceptions to this were in the responses of fungal classes to season. Groups such as the Archaeorhizomycetes, Cystobasidiomycetes, Orbiliomycetes and Sacccharomycetes were all enriched in winter compared to summer. Conversely, the Exobasidiomycetes and Taphrinomycetes were among the few classes whose OTUs were consistently less abundant in winter than in summer.

A similar trend was observed in the biotic model coefficients in which few taxonomically consistent responses were found. The Exobasidiomycetes and

Taphrinomycetes again showed similar responses to plant richness, but not to other biotic variables.

At finer taxonomic resolutions, differences in the environmental responses of closely related fungi emerged. For example, OTUs in the class *Glomeromycetes* (Arbuscular Mycorrhizal Fungi; AM fungi) showed mixed responses to season, with an approximately even split between positive and negative responses. Yet, when examined at the family level, the 5 Glomeromycete families showed distinctly different responses to season with the Archaeosporaceae and Claroideoglomeraceae both were becoming less abundant in winter (Figure 5-10). The largest family, the Glomeraceae showed a mixed response whereas the Paraglomeraceae were enriched in the winter.

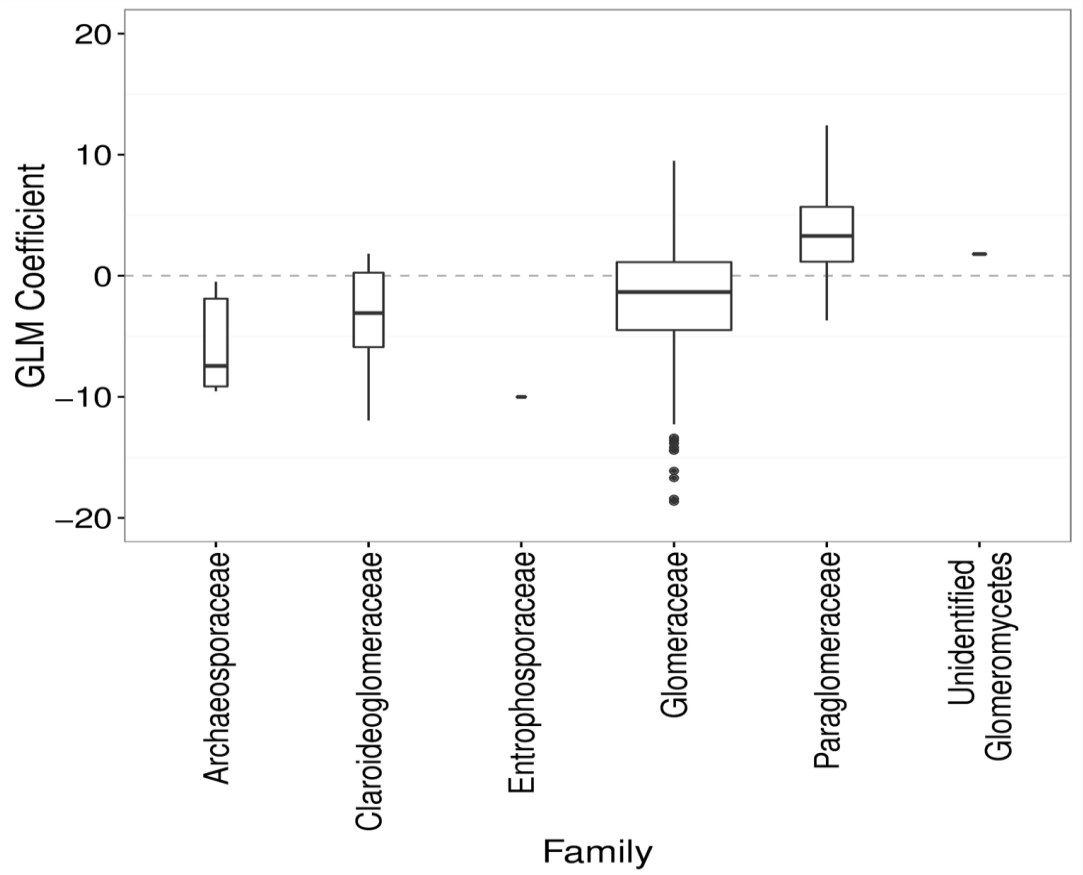

Figure 5-10: Family groups of fungal OTUs show markedly different responses to environmental variables. Coefficient values of 0 indicate little relationship between the given environmental variable and the abundance of OTUs within each family.

5.4.5. Functionally Consistent responses to Environmental Gradients

Finite mixture models were used to group fungal OTUs by their responses to biotic or abiotic variables into 6 archetypes. These archetypes consisted of groups of OTUs with similar responses to the variables and each archetype was characterised by considerably different responses to the variables in each model.

Biotic model archetypes were characterised by fairly strong positive responses to plant species richness and very weak responses to percentage cover by herbs or grass species. Responses to root biomass and percentage cover by forbs or forbs also varied between archetypes.

Abiotic archetypes were similarly variable in terms of the coefficients. In particular, season resulted in archetypes with highly contrasting responses to the winter. Most archetypes had relatively weak responses to soil moisture and intermediate responses to pH and soil salinity.

In terms of membership for each archetype, biotic archetypes were all occupied, with G-1 assigned the fewest OTUs and G-3 the most. Abiotic archetypes G (1-5) were occupied. However, G-6 was not assigned any OTUs, with most OTUs having zero (or very close to zero) probability of clustering within this archetype suggesting that the diversity of responses to abiotic variables was not as great as the responses to biotic variables.

Chi-Squared tests were used to test for association between trophic groups and archetype groups created for biotic and abiotic models. Significant association between trophic groups and archetypes was found for both biotic and abiotic model archetypes (biotic; $\chi 2 = 185.56$, $P < 0.001$, abiotic; $\chi 2 = 192.5$, $P < 0.001$) indicating that environmental responses tended to be consistent within functional groups.

The abiotic model clustered most symbiotrophs into archetype G-2 (Figure 5-11), a group characterised by decreased abundance during the winter compared with the summer, and a relatively strong negative response to pH. Saprotrophs were mostly split between archetypes G-1 and G-2. These archetypes differed mainly in their response to season with G-1 having a positive response to winter and G-2 a negative response. Pathotrophic OTUs were relatively evenly distributed between the 5 populated archetypes, with G-2 and G-5 being the most heavily populated groups. In the biotic model, saprotrophs were mainly distributed between archetypes G-2 and G-3. Archetypes G-2 and G-3 had fairly similar responses to the biotic variables, although G-2 was characterised by stronger responses to plant richness and percentage cover by shrub species. In contrast to the abiotic model, saprotrophs were relatively evenly distributed among the archetypes in the biotic model, and difference between the observed and expected number of OTUs in each group was small indicating that the environmental responses of saprotrophic OTUs were not consistent.

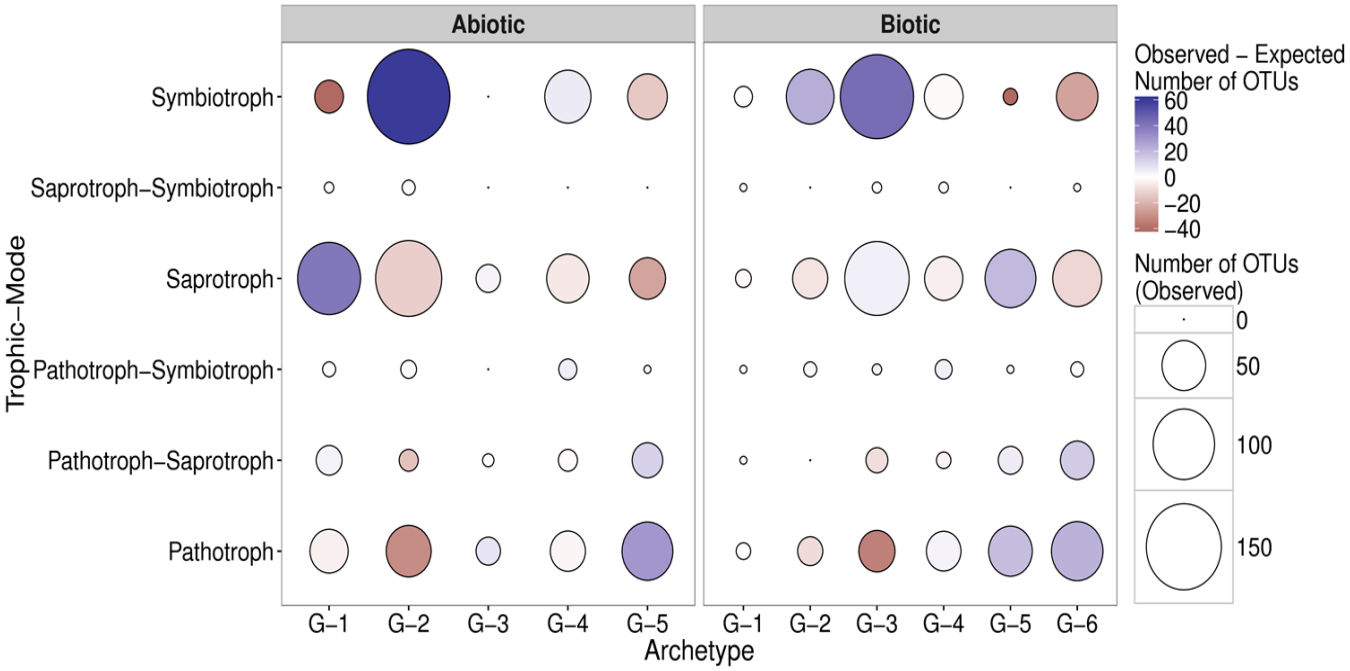

Figure 5-11: The number of OTUs from each trophic group in each archetype group. Groups such as the *symbiotrophs*, *saprotrophs* and *pathotrophs* appeared to aggregate into specific *archetypes*. Circle size is proportional to the number of OTUs observed in each group, and the colour is indicative of the difference between the observed number of OTUs and the expected number, according to the null hypothesis of no association (blue indicates more OTUs than expected, red indicates fewer OTUs than expected).

5.5. Discussion

5.5.1. Drivers of fungal community structure are site dependent

The relative importance of the abiotic and biotic environment to fungal abundance and diversity was found to vary considerably between sites, supporting the hypothesis that drivers of fungal communities are context specific. In most sites and scales, abiotic factors were found to better predict the diversity of fungal communities and the abundance of fungal OTUs.

The importance of the abiotic environment to root-associated fungal communities has been confirmed in many study systems (Dumbrell *et al.*, 2010b; Hazard *et al.*, 2013; Blaalid *et al.*, 2014) and local scale physico-chemical properties frequently emerge as the best predictors of diversity and abundance. In contrast to most previous study systems in which root-associated fungi have been studied, salt marsh sediments are often highly saline, which has been shown to potentially alter fungal diversity (Mohamed & Martiny, 2011). However, there was little evidence to support salinity as a driver of fungal diversity, as it was only found to be a significant predictor of diversity in one site (Tillingham Marsh, Essex) where it had a relatively weak effect. In contrast, season was often found to significantly predict OTU richness in multiple sites, confirming the importance of seasonality to fungal assemblages (Dumbrell *et al.*, 2011; Cotton *et al.*, 2015; Kivlin & Hawkes, 2016). However, the effect of season on fungal diversity also differed between sites, with both positive and negative relationships observed. This can be attributed to the important role of temporal changes in resource availability that were confirmed to affect the intraspecific competition and resource partitioning in AMF communities (May *et al.*, 2007). Thus, carbon might act as the main limiting

resources that regulating the communities of the obligate symbionts like arbuscular mycorrhizal fungi that obtain all their carbon directly from their host plants (Smith & Read, 2008; Helgason & Fitter, 2009).

Indeed, Previous research on root-associated fungal communities has suggested that the biotic environment may be an important determinant of fungal community structure and factors such as plant identity, phylogenetic, functional, and species richness have all been suggested as drivers (Johnson *et al.*, 2004; Burke *et al.*, 2009; Bálint *et al.*, 2015). Here, the biotic environment was found to be inferior at predicting fungal richness and abundances and surprisingly, few biotic drivers were found to have significant relationships with fungal diversity. This result is concordant with other research which has suggested a minimal influence of plant properties on fungal communities (Peay *et al.* 2015; Lekberg & Waller 2016; Erlandson *et al.* 2016).

The ability of abiotic and biotic variables to predict fungal richness and abundances differed greatly between sites, suggesting that drivers of fungal community structure may be site-specific to some extent. Generally, few microbial ecology studies have attempted to study environment-diversity relationships within and between multiple comparable habitats as noted by (Peay *et al.*, 2016). Most studies either take a “macroecological” approach by only studying relationships between sites (eg. Kivlin *et al*. 2011; Tedersoo et al. 2014; Davison *et al*. 2015) or, simply study patterns within one particular habitat (Dumbrell *et al.*, 2010b; Mohamed & Martiny, 2011). This means that it is difficult to determine the generality of environment-diversity relationships across comparable habitats. One of the few studies that have examined microbial communities in parallel sites was by Leff *et al*.

(2015) who found that microbial communities in grasslands showed consistent shifts in response to nutrient addition, despite being initially highly dissimilar. I reconcile the discordance between these results and those of Leff *et al*. (2015) by highlighting two points. Firstly, it is worth noting that the effect sizes observed by Leff *et al.* for fungal communities with respect to nutrient addition are all rather small with R^2 values typically <0.05, suggesting that fungal communities responded consistently but weakly to nutrient addition. Secondly, Leff *et al*. (2015) focussed on soil fungi which are directly exposed to the edaphic environment, whereas the root-associated fungi may be more influenced by the biotic environment, potentially reducing the consistency in the response to environmental variables. Martiny *et al*. (2011) suggested that the drivers of diversity depend on scale, and used the changing slope of a bacterial distance-decay relationship at increasingly large spatial scales to support this. Additionally, the authors note that the relative importance of environmental factors on diversity also changed with scale. These results generally support this finding, and suggest in addition to scale, site should also be considered to affect the relative importance of environmental drivers of diversity and community structure.

5.5.2. Function as a context

Current finite-mixture modeling examined whether the fungal OTUs in the same functional trophic guilds, responded similarly to environmental gradients present in the salt marshes. Grouping the fungal OTUs into archetypes (Dunstan *et al.* 2011) based on their response to both abiotic and biotic environmental variables and, for both abiotic and biotic variables, these archetype groups showed significant association with the functional trophic guilds that the OTUs belonged to. This

suggests that, to some extent, fungal OTUs within the same functional group tend to respond similarly to environmental gradients.

Few other studies have demonstrated functionally conserved responses to the environment in natural systems. However, experimental studies have recorded that different microbial functional groups may respond differently to various habitat manipulations, providing support for our results. Treseder *et al*. (2016) studied the effects of experimental warming in forest litter fungal communities. They found that certain functional groups such as endophytic and ectomycorrhizal fungi increased their abundance under warming, whereas others such as yeasts and pathogenic fungi showed either negative or no response respectively. Xiong *et al*. (2014) also detected that different fungal functional groups changed their abundance in relation to experimental warming, with the endosymbiotic Glomeromycota declining under elevated temperatures but lignin degrading Basidiomycota increasing in abundance. Further evidence for function specific environmental responses was found by Paungfoo-Lonhienne *et al*. (2015) who found that increased nitrogen fertilisation of arable soils resulted in increased abundance of known fungal plant pathogens, with other functional groups such as lignin decomposers declining under nitrogen addition.

The finding that environmental responses may be conserved within functional groups of fungi has significant implications for our understanding of functional redundancy and resilience in microbial communities (Allison & Martiny 2008). Due to their (often) enormous diversity, it is often posited that microbial communities may exhibit functional redundancy (Yin et al. 2000; Bell et al. 2005; Talbot et al. 2014, but see Allison & Martiny 2008; Delgado-Baquerizo et al. 2016), whereby many species

occupy the same functional role. This is typically assumed to confer high functional resilience as, if one species declines, the function is fulfilled by other species present in the community. However, if functionally similar microorganisms respond similarly to the environment, a change in the environment has the potential to affect the abundance of many species which contribute to the same ecosystem function. This may alter processes at the ecosystem level with wide ranging impacts. Hopefully these results pave the way for more empirical research into the consistency of environmental responses within, and between microbial functional groups and how this relates to the functional resilience of microbial communities. As such knowledge is essential in order to better understand how changing ecosystems may impact on ecosystem functions in the face of global change.

5.6. Conclusions

In conclusion, current chapter confirmed that the drivers of root-associated fungal diversity and abundance are largely site dependent, even in highly comparable sites. Models of fungal richness in relation to abiotic or biotic factors explained considerably different amounts of variation in each site, and generalised poorly to other sites. Additionally, the proportion of fungal OTUs whose abundance was best explained by abiotic or biotic variables also changed by site, further emphasising the importance of context to fungal diversity and abundance (Tedersoo *et al.* 2015). Therefore it is possible now to argue against deriving general environment-diversity relationships without empirical validation in multiple sites. Finally, these result show that functionally similar fungi tend to respond similarly to natural environmental gradients, suggesting a possible disconnect between function redundancy and resilience in microbial communities.

Chapter six

6. Temporal and spatial variation of Arbuscular Mycorrhizas in salt marshes, and the role of host plants and other fungi in driving their community composition

6.1. Summary

- Studies have shown abiotic variables in the surrounding soil significantly influence AMF communities. However, AMF obtain all their carbon from their host plant species, which vary in their dependency on AMF. Thus, the degree of benefit provided by AMF varies between plant species. One of the most beneficial roles of AMF is to reduce the risk of infection by pathogenic fungi. However, the mechanism by which this occurs is not clear understood, although studies have shown that the number of pathogenic species reduce with AMF association.
- Applying NGS methods to studies of AMF diversity have greatly enhanced our understanding of the mechanisms that regulate and maintain their distribution at large scales. However, as with any other methods, this is prone to errors, leading to incorrect information on the abundance and diversity of genes due to PCR bias. Thus, different primer sets were used to ascertain if changes in AMF species affect the role of biotic and abiotic factors. Although, the resulting estimates of AMF diversity showed considerable changes in OTU richness within and between sites, no changes in the general response were recorded.
- Other fungi in the rhizosphere may also not have been shown to have significant effects on the abundance of AMF species at this stage. Only *Paraglomeraceae* were shown to have their abundance affected by the

presence of some plant pathogens in the rhizosphere. All the recorded AMF families were shown to be affected by changes in the plant conditions at different spatial scales, and between seasons. The composition of AMF communities in the salt marshes was shown to be related to the condition of their host plants.

6.2. Introduction

Although it has long been recognized that ecological communities are not random collections of species, the mechanisms that shape community assembly are yet to be fully understood (Weiher & Keddy, 2001; Silvertown, 2004). A competitive interaction that is known to limit the long-term coexistence of species with similar fundamental niches was hypothesised to explain these nonrandom species assemblages (Darwin, 1859; MacArthur & Levins, 1967; Webb *et al.*, 2002). This was done by suggesting that the competitive exclusion of closely related species with similar fundamental niches results in communities that are made up of phylogenetically over dispersed, or more distantly related species rather than by chance (Darwin, 1859; Tofts & Silvertown, 2000; Webb *et al.*, 2002; Cavender-Bares *et al.*, 2004). However, due to the difficulties of manipulating large spatial and temporal scales of critical processes in plant and animal communities, this hypothesis remained difficult to be directly tested (Weiher & Keddy, 1995). Recent studies have indicated that both the level of phylogenetic relatedness within a particular community, and the spatial scale of species interactions influence the degree of phylogenetic dispersion (Winston, 1995; Cavender-Bares *et al.*, 2004; Silvertown & McConway, 2006). However, the strength of a phylogenetic signal in

the species composition of communities is often obscured by stochastic processes and dispersal limitations (Tofts & Silvertown, 2000).

This is particularly evident in Arbuscular mycorrhizal fungi(AMF) (phylum Glomeromycota; Schüßler et al., 2001b), which form an important symbiosis with almost 80% of all terrestrial plant species (Smith & Read, 2008). AMF proved to have direct and indirect effects on the diversity and productivity of land-plant communities (van der Heijden *et al.*, 1998) by their central role at the soil–plant interface (Van Der Heijden *et al.*, 2008). This is including their important role in enhancing the pathogenic resistance of their host plant (Vigo *et al.*, 2000; De La Peña *et al.*, 2006). This may be a result of the AMF high taxonomic richness that helped create an intensely competitive area (Wehner *et al.*, 2010), in which other fungi that presumably exploit common resources within the root (Whipps, 2004) become less abundant. On the other hand, interference competition may arise at low carbon availability within intercellular spaces (Graham, 2001), or under decreased area of infection within the root system (Vigo *et al.*, 2000). Both these processes were proved to affect AMF communities' composition.

Although the role niches were confirmed as the primary mechanism regulating the composition and diversity of natural AMF communities in woodland habitats, Dumbrell *et al*., (2010) concluded that these communities still responded to stochastic-neutral processes. AMF communities in woodland habitats showed stronger response to changes in the soil pH compared with their host plants species. However, AMF are obligate biotrophs depending on their green host plants to supply their carbon compounds that are essential for tissue production and survival (Ho & Trappe, 1973). Molecular based studies of AMF communities confirmed an important

role of the host plants in regulating both the composition of AMF (Helgason *et al.*, 2002; Husband *et al.*, 2002; Vandenkoornhuyse *et al.*, 2003) and thier spore production (Bever, 2002). Possible temporal changes in the AMF community evenness and diversity may result from seasonal changing supply of host-plant carbon (Dumbrell *et al.*, 2011). AMF were confirmed to inhabit distinct seasonal niches (Pringle & Bever, 2002), that affect the abundance of their dominant taxa resulting in them to be temporally dependent (Merryweather & Fitter, 1998). AMF species that are known to dominant in the newly germinated seedlings were shown to be almost entirely replaced by previously rare types in the surviving seedlings the following year. The high diversity and huge variation detected across time points, sites and hosts, indicats that the AMF types are ecologically distinct and thus may have the potential to influence recruitment and host composition in tropical forests (Husban*d et al*, 2002). Indeed, significant temporal variation in the colonisation of AMF was observed in the salt marshhabitats. The shift in the diversity of AMF in the high marsh zone was confirmend to be dependent on plant phenological phases. However, in the low marsh plant phenological events are likely to be diluted by stressful conditions like flooding and high salinity (Carvalho *et al.*, 2001).

Therefore, to understand the relative importance of biotic factors, and the presence of other fungi in the rhizosphere to communities of AMF in six UK salt marshes, the current chapter adopted a multiscalar approach, that examined the roles of abiotic factors, competition and spatial structure in determining the distribution of AMF. This approach enabled the examination of the importance o biotic and other fungi to AMF communities, generalised to larger spatial scales.

Using quantified AMF communities from 219 plant root samples of 32 species, various biotic factors and fungi were measured within the root zone. I hypothesized that these factors might influence the niche-based mechanism that was proved to regulate the abundance of AMF.

Specifically, I hypothesized that the impact of the spatial and seasonal patterns of plants conditions would likely change the composition of the rhizosphere fungal communities. Previous studies on fungal diversity have revealed a suite of different modulating factors of fungal community composition (Dumbrell *et al.,* 2010; Kivlin *et al.,* 2011; Kivlin *et al.,* 2014; Pellissier *et al.,* 2014); a disconnect between some of these factors may be due to differences in study scale or temporal variability. Secondly, I investigated whether this pattern is related to the response of different AMF families to the presence of other fungi in their surrounding area. Research on other microbial groups suggests that responses to various environmental gradients may be taxonomically conserved (Youngblut *et al.,* 2013) such that, closely related organisms respond more similarly than distantly related ones.

6.3. Materials and methods

Sampling design and site description with sediment chemistry analysis and AMF determination were fully explained in the methodology Chapter (Chapter 2). The current chapter includes data for all sediment cores that have AMF association recorded within each of the root samples. The fungal ITS region was amplified using fungus-specific primers (ITS1F; Gardes & Bruns, 1993) and universal primer (ITS2; White *et al*., 1990). For the sake of accuracy and robustness any 18S data were not included in analysis between AMF and other fungi. Most of the surveyed

studies had utilized the primer pair NS31–AM1 covering a *c.* 500-bp central fragment of the SSU rRNA gene. The primer pair is known to amplify most taxa of Glomeromycota, but to exclude the basal families Archaeosporaceae and Paraglomaceae (Daniell *et al.*, 2001).

6.3.1. Data analysis

AMF data were obtained from the general fungal data in the Chapter Five. The R language was then used to calculate species richness, based on the number of operational taxonomic units (OTU). A multivariate generalised linear modelling approach with the Mvabund package to examine which biotic factors better predicted the abundance of OTUs within each site (Wang *et al.*, 2012). Data were first split by site and OTUs, which were removed when occurring fewer than 5 times. In this model, an offset of log (n sequences in the sample) was included to account for random variability in the number of sequences generated for each sample. This approach assigns a covariate (log (n sequences)) and assigns it a value of 1, inferring that OTU abundances are proportional to the number of sequences in each sample. Biotic models could be written as:

OTUs ~ Number plant species + Root Biomass + % cover of forbs + % cover of grasses + % cover by herbs + % cover by forbs

And

OTUs ~ Endophyte Other Plant Pathogen + Plant Pathogen + Plant Pathogen other Wood Saprotroph + Undefined Root Endophyte + Undefined Saprotroph + Wood Saprotroph

The negative binomial error distribution with a log link function used as sequence data is often too over-dispersed for other count distributions such as the

Poisson. To test which model best described OTU abundances, An analysis of deviance was performed using a Likelihood ratio test as implemented in the anova.manyglm() function. 999 montecarlo permutations were used to assess significance.

6.4. Result

An initial ~327 AMF OTUs were obtained from the general fungal sequences, based on the ITS in the previous chapter (Chapter Five). Only less than 1% of the OTUs were not identified, while all the rest were identified up to the family level. The majority of recorded OTUs belong to the Glomeromycotan family *Glomeraceae* (85 %), followed by *Paraglomeraceae* (~13%), and *Claroideoglomeraceae, Entrophosporaceae, Archaeosporaceae* that all form > (2%) of the entire population. The number of AMF obtained with different sets of primers in this study was different, and no AMF were recorded in Tillingham, Essex using the 18S primer set. Although no AMF was amplified from the root samples of Tillingham salt marsh when the 18S primer set was used, the highest number of AMF OTUs were recorded in the salt marshes of Essex compared with Lancashire when the 18S primer set was used (ANOVA; $F_{1, 16}$=5.044, P=0.03). In comparison, samples from west Plain were significantly higher when samples amplified, using the ITS primer set that also amplified AMF in the salt marsh of Tillingham, along with other fungal species. The regression analysis of the seasonal variation in the richness of the AMF OTUs at these sites was not significant (ANOVA; $F_{1, 13}$=2.94, P=0.11). Although the number of OTUs increased during summer in both Carmel Sands (CS) and West Plain (WP), however, Essex salt marshes and Warton Sands (WS) from Lancashire showed the opposite. Seasonal variation arose at the site level regardless to the primers. AMF showed different responses in sites like Abbottshall where high colonisation was recorded during winter when the ITS primer was used. Consequently, the 18S primer showed no recorded AMF in the same site during winter (Table 2-1).

Table 6-1: The recorded number of different AMF OTUs for both primers 18S and ITS per season, location and different sites. Each OUT was assigned to their AMF family and represented as following; F1=*Archaeosporaceae*, F2=*Claroideoglomeraceae*, F3=*Entrophosporaceae*, F4=*Glomeraceae*, F5=*Paraglomeraceae*, F6=*Acaulosporaceae,* F7=*Diversisporcea* and F8=Other.Glomeromycetes.

Primer	Season	Location	Site	F1	F2	F3	F4	F5	F6	F7	F8
ITS	**Summer**	**Essex**	Abbotthall	4	951	1	103777	1017	0	0	88
			Fingringhoe	2	0	0	16120	135	0	0	1
			Tillingham	0	1632	0	45293	7370	0	0	1258
		Lancashire	Cartmel Sands	1	132	0	76289	7483	0	0	62
			West Plain	2769	403	160	137714	40959	0	0	840
			Warton Sands	1962	1191	1	105921	55906	0	0	2038
	Winter	**Essex**	Abbotthall	251	84	0	197400	1631	0	0	3
			Fingringhoe	0	0	0	2796	0	0	0	0
			Tillingham	0	0	0	106740	317	0	0	1
		Lancashire	Cartmel Sands	0	0	0	49278	429	0	0	0
			West Plain	2	977	8	65924	6743	0	0	1378
			Warton Sands	6132	301	0	38930	22191	0	0	79
18S	**Summer**	**Essex**	Abbotthall	646	0	0	492510	553	0	1	0
			Fingringhoe	2	0	0	648420	26	0	6	0
			Tillingham	0	0	0	0	0	0	0	0
		Lancashire	Cartmel Sands	5733	0	0	6047495	2240	57	1014	0
			West Plain	1	0	0	79478	24226	0	2	0
			Warton Sands	2	0	0	637129	3684	47	3917	0
	Winter	**Essex**	Abbotthall	0	0	0	0	0	0	0	0
			Fingringhoe	117	0	0	320295	1037	5	526	0
			Tillingham	0	0	0	0	0	0	0	0
		Lancashire	Cartmel Sands	0	0	0	104437	2103	0	19	0
			West Plain	4	0	0	36746	735	0	5	0
			Warton Sands	1210	0	0	575078	8179	2192	15191	0

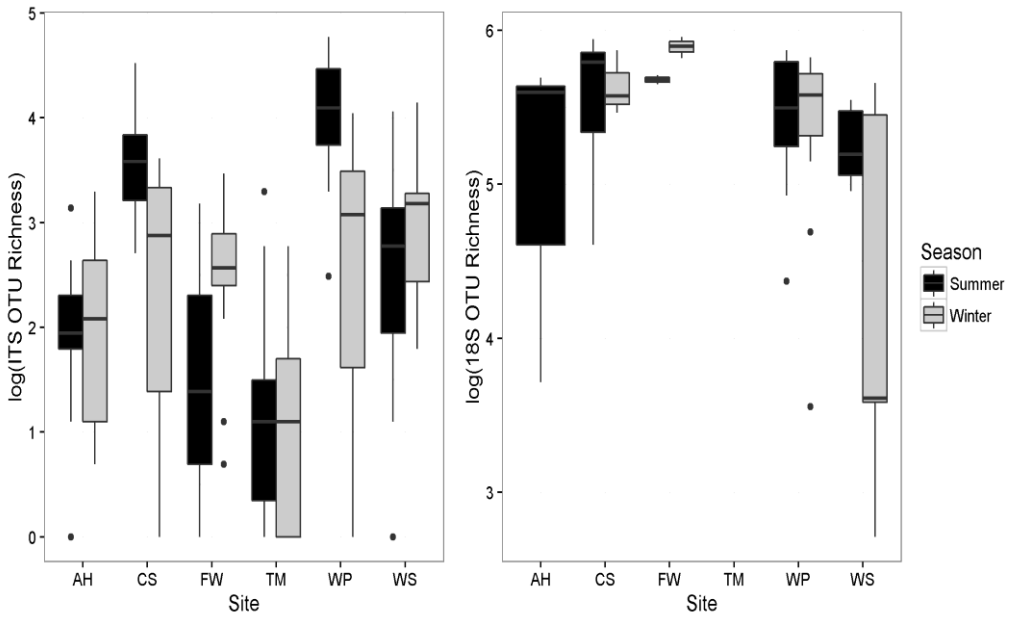

Figure 6-1: Regression analysis of the log OTUs richness, showing variation between sites in summer and winter when different primers are used. Each box represents the OTUs richness and season is identified with different colours. Points outside these boxes are outlier samples.

Different salt marshes at these two locations were inhibited by different number of plant species that significantly varied at site level (ANOVA; $F_{5, 203}$=280.91, *P*=0.001), however, no significant differences between seasons were observed (ANOVA; $F_{1, 203}$=2.95, *P*=0.08). Generally, salt marshes of Lancashire recorded higher number of plants species compared with Essex (Figure 6-2). Indeed, the highest plants richness was recorded at the salt marsh of West Plain during summer. Between the two seasons, there was a higher number of plants species during summer compared with winter of the same year, with the exception of Fingringhoe Wick, Essex.

Unlike species richness, the root biomass of these plant species significantly differed in different seasons (ANOVA; $F_{1, 206}$=37.7, *P*=0.001). However, the seasonal effect within each site was different even within the same location. Essex salt marshes had lower root biomass compared with Lancashire. The lowest root biomass was recorded in the salt marsh of Tillingham, Essex, and the highest root biomass among other sites from both locations was recorded in the salt marsh of West Plane, Lancashire.

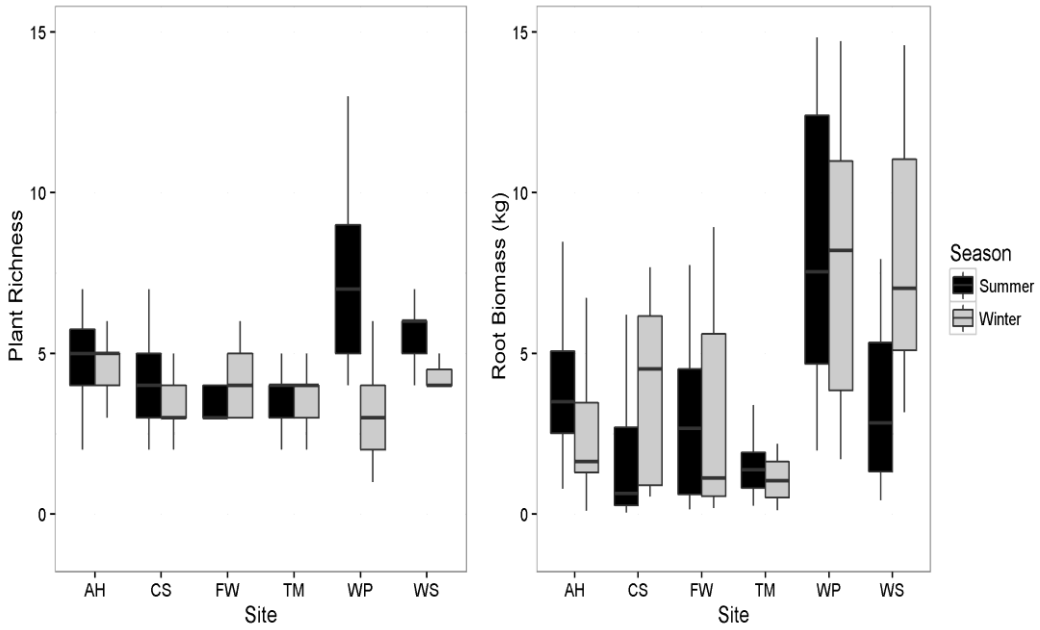

Figure 6-2: Regression analysis of plant species richness and root biomass at the site level, from both Lancashire and Essex during summer and winter. Sites are labelled as follows: AH (Abbottshall), TM (Tillingham), FW (Fingringhoe) for sites at Essex and CS (Cartmel Sands), WS (Warton Sands), WP (West Plane) for Lancashire salt marshes. Outliers outside the range -30:30 were removed (post calculation of quartiles) for ease of visualisation.

6.4.1. The Relative Roles of the host plant and other fungi in the soil on AMF species abundance

At the site level, AIC based comparison of host plants and other fungi models suggested there was little difference in the relative influence of these variables to most OTUs. Few OTUs had large **Δ**AIC between host plants and other fungi models, and those that did, tended to favour the other fungi model (Figure 6-3). Within Essex sites, OTU abundance was better described by host plant variables with 68.3% (FW), 67.4% (AH) and 71.6% (TM) of OTUs having a lower AIC for the host plant model. In comparison, OTUs from Lancashire sites were more evenly split between host plant and other fungi models in both (WP) and (CS), whereas, (WS) OTU abundance had a lower AIC for host plant variables.

The likelihood ratio tests on models with and without season showed that, within each site, the addition of season to other fungi models resulted in significantly better fits in five of the six sites (Table *6-2*). Adding season to host plants models again resulted in better models for four sites, with Fingringhoe Wick and West Plain as the exceptions. At the location level, the addition of season to both plant condition and other fungi models resulted in significantly better fits for Essex only. Finally, when all sites were examined at once, inclusion of season resulted in significantly better fits to host plants models.

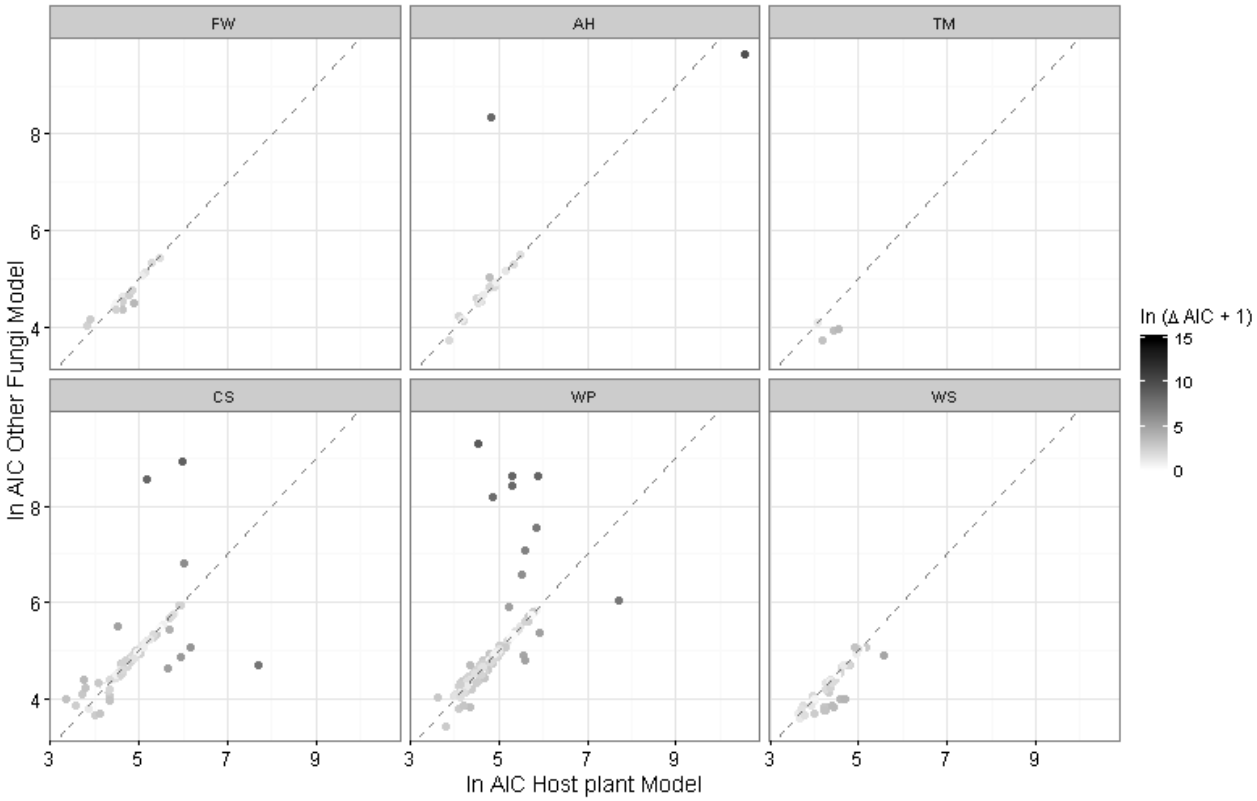

Figure 6-3: AIC scores for biotic and abiotic models for each AMFs OTU within each site. The dashed line shows a 1:1 relationship (if the two models had equal AIC scores) and darker points are those with a larger ***Δ***AIC between models. Note that the natural log of AIC scores is shown for ease of visualisation.

Table 6-2: Results of the likelihood ratio tests between the GLM models with and without season. *P* values in bold indicate a result in which inclusion of season significantly improved the model. Significant values are labelled as follows (0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1).

Site/Location	Other fungi Model with season - Other fungi model without season				Host plant model with season- Host plant model without season			
	Residual degrees of freedom	Degrees of freedom	Test statistic	P value	Residual degrees of freedom	Degrees of freedom	Test statistic	P value
AH	14 13	1	48.52	0.002***	15 14	1	95.58	1
FW	9 9	0	0	0.02**	10 9	1	34.35	0.019**
TM	2 1	1	74.58	0.001***	3 2	1	49.34	0.001***
CS	25 24	1	1	0.002***	25 24	1	963.7	0.001***
WP	29 28	1	1913	0.16	29 28	1	7834	0.103
WS	13 14	1	712.4	0.001***	14 13	1	147.8	0.008***
Essex	37 36	1	1937979	0.001***	40 39	1	224.7	0.001***
Lancashire	80 79	1	89856	0.27	82 81	1	66904	0.76
Overall	127 122	1	121421	0.22	128 127	1	1648	0.001***

6.4.2. Spatial patterning of host plants, and other fungi in the soil on the structure of AMF communities

Model coefficients estimated from the biotic model including (plants species richness, root biomass as well as the other fungi living in the rhizosphere) suggested considerable variation in responses at different sites (Figure 6-4). Generally, both plant species richness and root biomass had significant effects on the abundance of AMF VTXs (Table 6-2). However, in the saltmarsh of Tillingham in Essex, the abundance (number of individuals) of AMF was reduced in response to the presence

of plant pathogens. This indicated that niche based biotic factors had a low effect at the site level. However, at larger scale (Location level) root biomass had a greater effect on the abundance of the AMF in Essex compared with Lancashire. Both plant richness and root biomass resulted in fluctuating OTUS number of each AMF VTX in Lancashire (Figure 6-8). Indeed, the abundance of the AMF VTXs was reduced as a result of the increased number of the Endophyte plant pathogens. Generally, the effect of the other fungi in the rhizosphere on the abundance of AMF in these salt marshes was low in Essex compared with Lancashire.

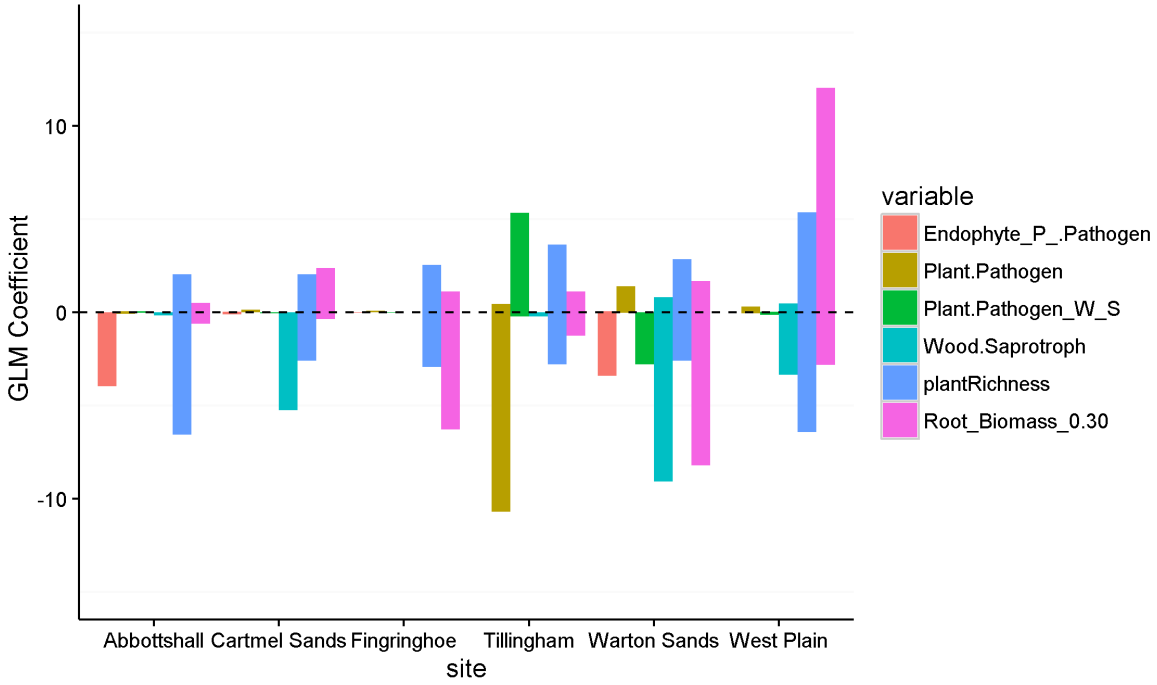

Figure 6-4: Different guilds of fungal OTUs show markedly different responses to environmental variables at site level. Coefficient values of 0 indicate little relationship between the given environmental variable and the abundance of OTUs within each guild.

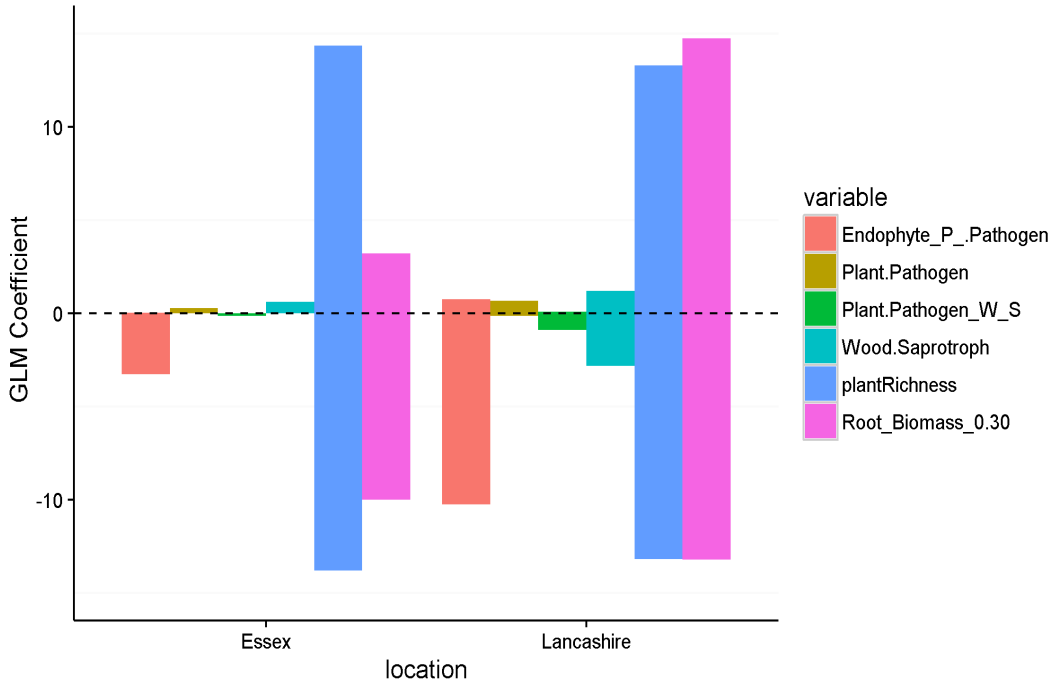

Figure 6-5: Different guilds of fungal OTUs show markedly different responses to environmental variables at large spatial level. Coefficient values of 0 indicate little relationship between the given environmental variable and the abundance of OTUs within each guild.

6.4.3. Seasonal patterning of host plants species and other soil fungi on the structure of AMF communities

The seasonal shift in the relative abundance of different fungi within the rhizosphere resulted in significant change of the relative abundance of the AMF (Table 6-2). For example, the relative abundance of AMF in Warton Sands, Lancashire was reduced during winter compared with summer. This shift causes the relative abundance of the Endophytes to increase as a result of low AMF during winter. On the other hand, the enhanced the OTU number of AMF VTXs in the salt marsh of Abbottshall, Essex during winter had a negative effect on the abundance of plant pathogens. Regardless of the season, reduced abundance of the AMF enhanced the abundance of other fungi within the rhizosphere.

Model coefficients estimated from the biotic model including (plant species richness, root biomass as well as other fungi living in the rhizosphere) suggested considerable variation in responses at different seasons. Both root biomass and plant richness had greater effects during winter compared with summer. During summer, the abundance of AMF reduced as a result of the enhanced wood Saprotrophs, compared with winter (Figure 6-6). Similar effects were recorded for the pathogenic wood Saprotroph.

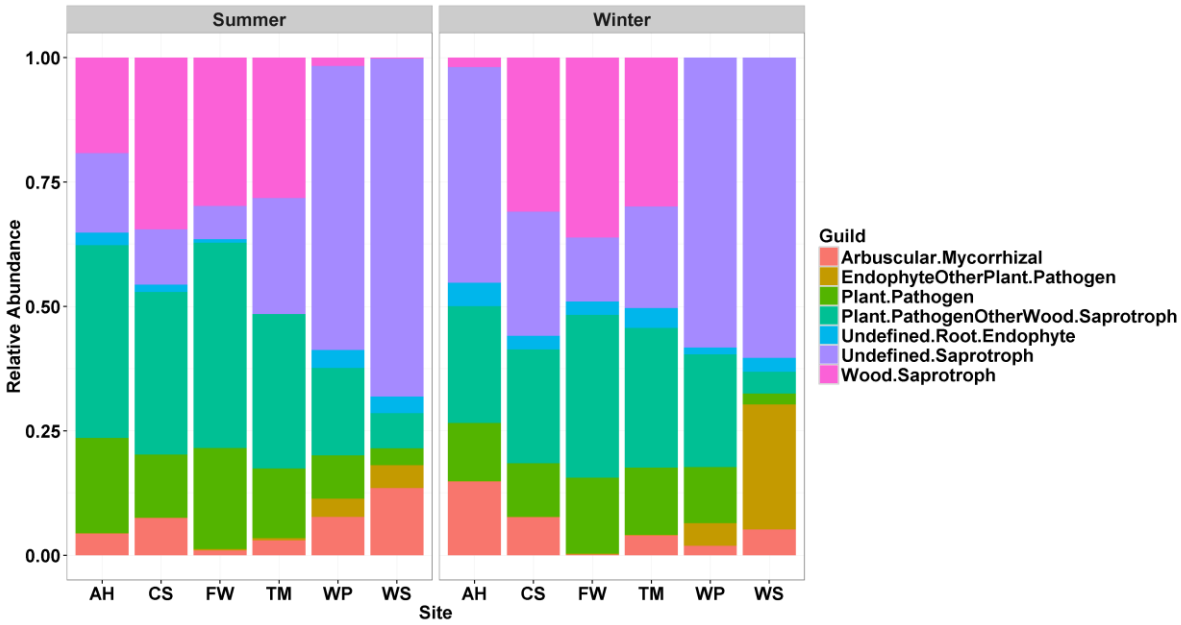

Figure 6-6: The relative abundance of different trophic groups compared with AMF in each site at summer and winter. Note that the relative abundance is scaled to total 1 after removing "undefined" fungi.

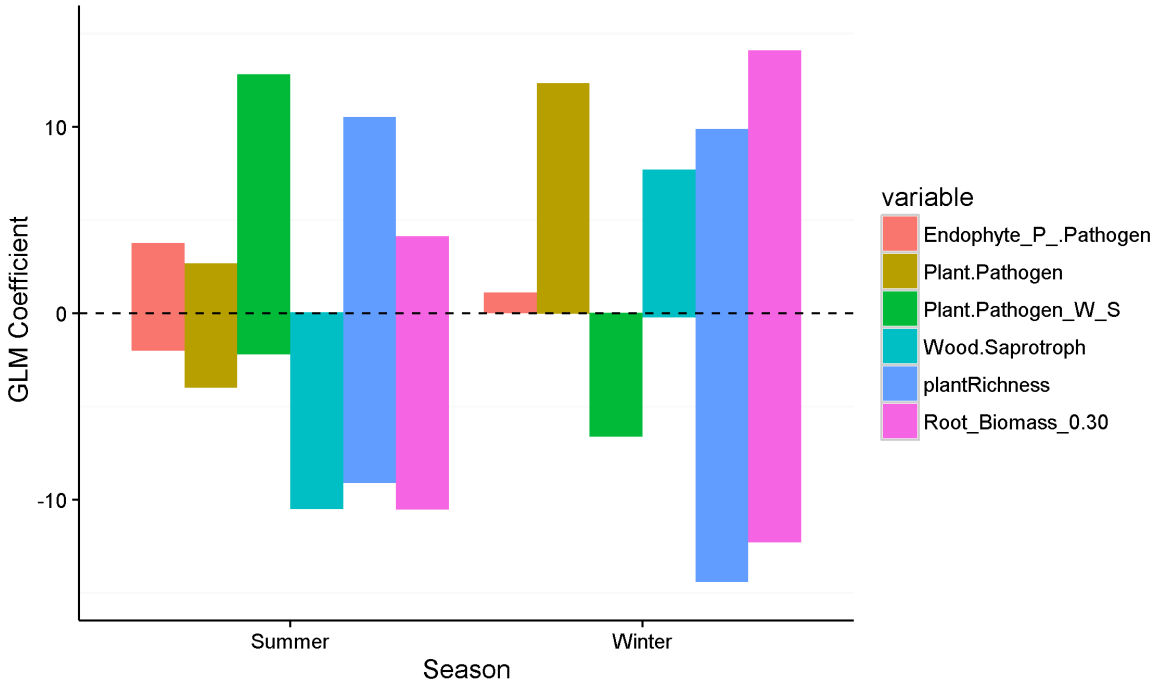

Figure 6-7: Different guilds of fungal OTUs show markedly different responses to temporal variation in the measured environmental variables. Coefficient values of 0 indicate little relationship between the given environmental variable and the abundance of OTUs within each guild.

6.4.4. AMF response to changes in host plants and other fungi based their phylogeny

The result in Chapter Five showed that, at larger scales, plant type had a low effect on the OUT abundance of AMF VTXs from different families compared with both other fungi, root biomass and plant species richness in these salt marshes. At finer taxonomic resolutions, differences in the environmental responses of closely related fungi emerged. For example, OTUs in the *class Glomeromycetes* (AMF) showed mixed responses to season, with an approximately even split between positive and negative responses. Yet, when examined at the family level, the 5 *Glomeromycete* families showed distinctly different responses to season with the *Archaeosporaceae* and *Claroideoglomeraceae* both becoming less abundant in winter (Chapter Five). The largest family, the *Glomeraceae* showed a mixed response whereas the *Paraglomeraceae* were enriched in the winter. Here the GLM models showed that the OUT abundance of most VTXs was not significantly affected by the presence of other fungi in the rhizosphere, with the exception of the AMF family *Paraglomaceae,* which was significantly derived by this factor (ANOVA; $F_{217, 211}$=5582, *P*=0.009). The presence of both endophyte plant pathogens and wood saprotrophs has reduced the abundance of most species compared to other plant pathogens (Figure 6-8). Different AMF families showed different response to the seasonal pattern of their host plants species (Figure 6-9). When analysing the role of host plants, both species richness and root biomass to explained significant variation (ANOVA; $F_{214, 207}$=17.08, *P*=0.05). These factors affected the abundance of AMF families *Archaeosporaceae*, *Claroideoglomeraceae* and *Entrophosporaceae*. Plants species richness also resulted in enhanced abundance of AMF family *Glomeraceae.*

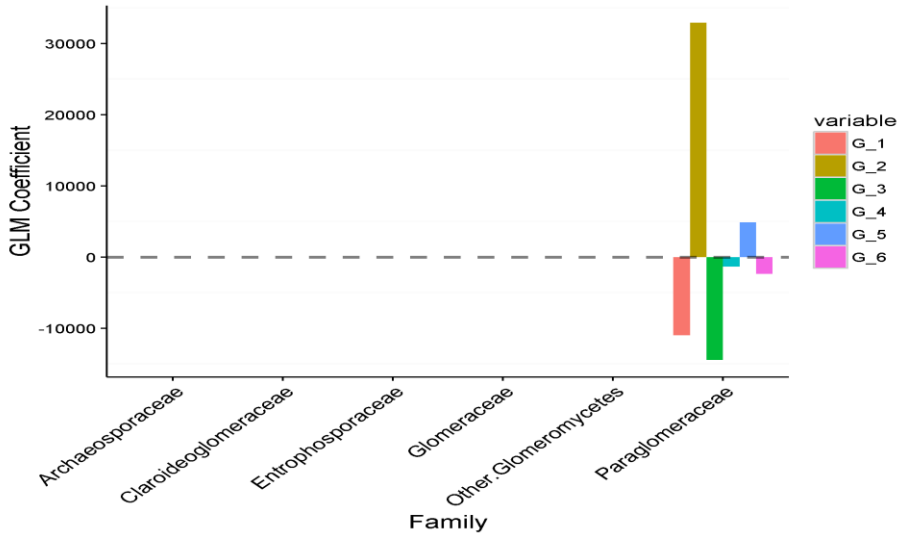

Figure 6-8: Different families of AMF OTUs show markedly different responses to the presence of different fungal guilds. Coefficient values of 0 indicate little relationship between the given guilds and the abundance of OTUs within each AMF family. These six groups of soil fungi represent endophyte plant Pathogen (G_1), plant pathogen (G_2), plant pathogen wood saprotroph (G_3), undefined root endophyte (G_4), undefined saprotroph (G_5) and wood saprotroph (G_6).

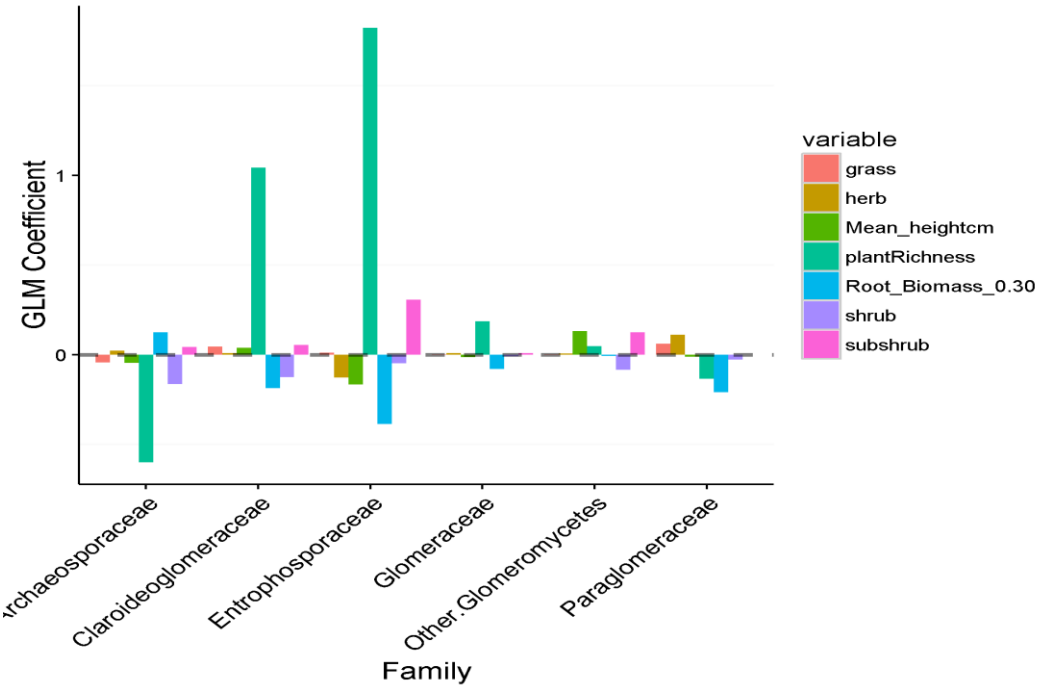

Figure 6-9: Different families of AMF OTUs show markedly different responses to the environmental variables. Coefficient values of 0 indicate little relationship between the given guilds and the abundance of OTUs within each AMF family.

6.5. Discussion

Using different sets of primers to amplify different regions of fungal rRNA for better detection of the whole group of AMF, these salt marshes proved to be dominated by the AMF *Glomeraceae* and *Paraglomeraceae* along with other AMF families like *Entrophosporaceae, Claroideoglommeraceae* and *Archaeospraceae.* Although the frequent detection of *Glomus* species in roots was suggested to be related to the use of primer pairs (e.g., AM1-NS31) that amplify mainly *Glomus*-related sequences (Dumbrell *et al.*, 2010a), obtaining similar results with different primers may highlight the important role of propagation mechanisms (mycelial fragments and mycorrhizal root fragments) that enable them to be more resilient and widespread, compared to other AMF that require spore germination (Helgason & Fitter, 2009). Using the NGS technique enabled large scale sampling and deep sequencing, revealing higher AMF diversity compared to other studies that showed these habitats to accommodate low diversity of AMF due to the low plants diversity (Hildebrandt *et al.*, 2001). However, under the conditions of the salt marshes, the role of other AMF families like *Claroideoglommeraceae* or *Archaeospraceae* might not be as important as the *Glomeraceae* or *Paraglomeraceae.*

6.5.1. Effect of changed conditions of AMF host plant and other fungi on AMF diversity and community structure at different scales and seasons

The evaluation of plant species presents in two different sites in the marsh as given in the fourth chapter neither showed that, the occurrence of AMF colonization did not depend on position within the salt marsh nor, consequently, on the tidal

flooding regimes which create different levels of anoxia around the roots, nor on salt gradients at large scale. This suggests that AMF distribution does not coincide with zonation pattern of vegetation. The absence of significant effects of abiotic variables at large spatial scale seems indicative that AMF can tolerate flooding and salinity levels in the UK's salt marshes. The communities of AMF were confirmed to be determined by the role of different biotic factors at both site and location levels on these salt marshes. Although soil pH, phosphorus and C/N ratio were considered to have a deterministic effect on the composition of AMF communities, AMF β-diversity was shown to be related to changes in host plant composition as well as soil variables (Dumbrell *et al.*, 2010a). The AIC from different models showed changed conditions of plants over scale and seasons to have greater impact on the diversity of AMF families compared with the effect of other fungi in the rhizosphere. At larger scales, the observed high plant species richness in Lancashire had a negative impact on the abundance of the OTUs of the predominant families like *Glomeraceae* or *Paraglomeraceae.* High plant richness allows more AMF to form, resulting in increased below-ground biomass. AMF community composition deferred significantly between soil types, due to co-occurrence within an otherwise fairly homogeneous area, potentially confounding factors such as host plant (Bever *et al.*, 1996).

The effect of plant variables on the abundance of AMF in these salt marshes was even greater at larger scales (at location level) as they represent the effect of different soil type (Ford *et al.*, 2016). In the current chapter, both lower percentages of forbs compared to grass species and higher root biomass in Lancashire had positive effects on the abundance of rare AMF families' like *Claroideoglomeraceae* and *Entrophosporaceae.* AMF showed a stronger and significant relationship

between phenology of prairie grasses and mycorrhizal responsiveness compared with forbs due to their dependency pattern that was confirmed to be different among plant life history and taxonomic groups (e.g., grass, forb, legume, annual, perennial), and phonological guilds (Wilson & Hartnett, 1998). Previous studies have shown that AMF diversity can be maintained through differentiation among host plant species and spatial separation (Rosendahl & Stukenbrock, 2004). The observed divergence of AMF communities between sand and clay in the current chapter suggests that a regional variety of soil types may be important for maintaining high AMF diversity (Lekberg *et al.*, 2007). However, greater root biomass increases the C availability within the soil, allowing for more AMF to increase the root area, and improve soil stability. Similar variation in the AMF niche breadth was recognised by many studies (Oehl *et al.*, 2003; Lekberg *et al.*, 2007), that showed a group like *Gl. intraradices ribotype* (Gl. intra rt1) to be widely distributed due to their ability to tolerate a broad range of soil conditions (Lekberg *et al.*, 2007). On the other hand, the distribution of *Gl. mosseae* was shown to be restricted to clay soil, and this may be a result of some degree of specialization or restriction (Johnson *et al.*, 1992; Landis *et al.*, 2004; Lekberg *et al.*, 2007). Likewise, the sandy salt marshes of Lancashire were likely helped by increasing the value of AMF that are known to play a key role in soil aggregate formation and stabilization (Leifheit *et al.*, 2014). These fungi defer in their ability to promote plant growth; thus specific fungi may be essential for the establishment of some plant species, especially under such stressful conditions. This is also true at the salt marshes of this present study. Current results showed the composition of AMF in these different salt marshes, following the distinct vegetation

that is dominated by grass species in the salt marshes of Lancashire compared to the forb at Essex (Chapter four; Figure 4-1).

Indeed, grass species like *Festuca.rubra* are known for their high root biomass, that enhances the availability of root colonizers such as the AMF, due to available habitats. Thus, compared to Essex, higher diversity of AMF was recorded in the Lancashire salt marshes, which accommodated other AMF, including those from the family *Paraglomeraceae*. Other fungal species were also attracted by the high root density in the Lancashire salt marshes, similar to the way in which the AMF from family *Paraglomeraceae* are attracted to roots. These fungal species were also likely to have competed with the AMF *Paraglomeraceae* over the same source of C, resulting in a significantly changed composition of their community. Unlike the AMF *Paraglomeraceae, Glomeraceae* seems to colonise a wider range of plant species at these habitats, and this is likely the reason that made them dominant at both locations. Moreover, it seems that the AMF family *Glomeraceae* were not affected by the presence of other fungal species due to their ability to associate with plant species that are growing under stressed conditions, where other fungal species are less likely to survive. Thus, no significant effect of other fungal presence in the rhizosphere was recorded on the abundance of the AMF *Glomeraceae.* It has been suggested that AMF colonization might affect rhizosphere interactions and particularly pathogen-infection development by changing root systems morphologically, as well as in the meristematic and nuclear activities of root cells (Atkinson *et al.*, 1994). This might also be the result of enhanced branching by AMF colonization, resulting in a relatively larger proportion of higher order roots in the root system (Atkinson *et al.*, 1994).

The significant effect of spatial scale at the site level, within soil types, indicates that spatial structure may indeed be a good surrogate for dispersal limitation, as suggested by others (Chase J.M & Chase, 2005). The variance partitioning procedure allowed evaluating the independent contributions of spatial structure and biotitic factors to observed AMF distributions. These analyses indicated that both biotic factors and geographical variables explained substantial portions of the variability in AMF community composition, but biotic factors had a slightly stronger influence (Table 6-2). No evidence showed that AMF communities were structured by neutral processes alone. However, some of the sites in the current study may harbour competitively inferior species simply because the best competitors have not yet arrived, or because the competitive inferiors are rescued from competitive exclusion by immigration from communities where they are good competitors, which in this case might mean the pioneer zone. Similarly, Cottenie (2005) conducted a meta-analysis on 158 community-level studies using the same variance partitioning approach as I took here, and discovered that a majority of communities were structured by biotic factors while very few were structured by neutral processes alone.

6.5.2. Temporal pattern of host plants and other fungi on the AMF community structure

The evaluation of the AMF response to the temporal pattern of both other fungi and biotic factors indicated a significant role of plant phenology compared to the presence of other fungi in their surroundings. The highest levels of colonization corresponded to the period of the highest plant growth and the flowering period in both species, summer and winter, respectively, in agreement with the results of Van

Duin *et al*. (1989). Similar seasonal patterns have been observed in the study of AMF in woodlands (Dumbrell *et al.*, 2011). These differences may be related either to the different behaviour of each AMF species, even in similar ecosystems (Klironomos *et al.*, 1993), or to the influence of different environmental conditions. The pattern of the seasonal variation in the condition of AMF host plants resulted in lower abundance of the AMF OUT. With the exception of *Paraglomeraceae,* all other AMF families in these salt marshes had their abundance decreased during winter. This might confirm the significant effect of changed phenology of the AMF host plants that is likely to affect the C supply. In their study on AMF Dumbrell *et al*., (2011) concluded that similar pattern reflected the winter–spring transition (winter samples) and main vegetation period (summer). The impact of seasonal variation in the plant richness, percentage cover of grass, and forbs on the abundance of AMF varies based on their family. Nevertheless, seasonal changes of the root biomass have negatively affected most of the recorded families, which confirms the relationship between AMF communities' composition and plant phenology. Although, singular dependence may appear unlikely, given that many fungal species have been shown to associate with the roots of individuals of this plant species (Bever *et al.*, 1996), there is a great possibility that plant performance is highly dependent on an abundance of this fungal species at different growth levels. Thus, the increased level of organic carbon during these growth levels may enhance AMF with less competitive ability, which are less likely to be predominant. Similarly, in their study Husband *et al.* (2002) showed a strong repeating pattern, whereby the dominant mycorrhizal types are replaced by previously rare types in the surviving seedlings. A shift in the AMF diversity has also been confirmed in a study of saltmarsh grass

(*Spartina patens*) colonized by AMF, which decreased from 26.6% during vegetative growth to 11.5% during dormancy (Burke *et al.*, 2003). AMF are obligate symbionts, obtaining all their C from their host plants, thus, any change in the C supply will be reflected on their community structure.

6.6. Conclusions

The results of this chapter emphasize that the role of the host plant rather than the presence of other fungi in the rhizosphere is responsible for AMF distribution. Like the plant species, AMF may have developed adaptive strategies to tolerate this stressful environment that enable some families like the *Glomeraceae* to become predominate species due to their ability of associating with a wider range of plants species. Additionally, this chapter demonstrates an important scale dependency, which needs to be considered in future research focusing on interactions between organisms with different dispersal abilities; annual plants and soil fungi like those in Lancashire are an excellent example of this disparity. The results of this study raise questions that are important to our understanding of the role of mycorrhizas in the ecology of salt marshes.

Chapter seven

7. General discussion

7.1. Thesis objectives

Although most ecosystem services are heavily dependent upon microorganisms (Delgado-Baquerizo *et al.,* 2016; van der Heijden *et al.,* 2008), the way in which the structure of these microbial communities respond to biotic and abiotic variables is yet to be fully determined (Bossio *et al*, 1998). Soil harbours a huge variety of microorganisms, many of which are still unidentified, yet most of the identified species are probably contributors to globally important ecosystem functions (Delgado-Baquerizo *et al.,* 2016). This includes rhizosphere fungi that play a role in soil stabilisation (Rillig *et al.,* 2003), carbon cycling (Rillig *et al.,* 2001; Treseder *et al.,* 2000), nutrient cycling (Read & Perez-Moreno, 2003), plant pathogenicity (Dean *et al.,* 2012) and plant symbiosis (van der Heijden *et al.,* 1998).

Recently developed NGS has significantly improved our understanding of how the structure of fungal communities respond to various environmental factors (Dumbrell *et al.,* 2010, Cotton *et al.,* 2015; Dumbrell *et al.,* 2011; Peay *et al.,* 2010; Rudgers *et al.,* 2014, Kivlin & Hawkes, 2016). Whilst most studies are starting to address such questions at larger spatial scales (Tedersoo *et al.,* 2014; Kivlin *et al.,* 2011; Davison *et al.,* 2015), multi-scalar approaches are comparatively rare, especially in a habitats like salt marshes, where obligate aerobes like the AMF are thought of as rare, or of no significance due to the anoxic conditions (Khan, 1974; Miller & Bever, 1999).

Such knowledge is critical to understand the generality or context specificity of ecological patterns (Noda, 2004). Indeed, this can help in determining the potential threats and consequences delivered under future climate or habitat change (Walther

et al., 2002). Thus, the main objective of this thesis was to improve our understanding of the ongoing changes in AMF diversity over time and space that may alter the functioning quality of the salt marsh ecosystem.

7.2. Summary of thesis and main findings

- Chapter 3 examined the role of local biotic and abiotic factors that are likely to influence the distribution of AMF across the physiochemical gradient of each salt marsh. Results of this chapter indicated significant influences of both soil salinity and pH on the abundance of AMF species. Unlike their host plants, the species of AMF did not necessarily follow the zonation pattern of high salinity and moisture, and predominant species were obtained from different zones. Although landscape-scale distribution patterns of soil microorganisms were rarely investigated, studies like those conducted by Green *et al*. (2004) suggested similar effect of the abiotic variables at the local scale. The resultant AMF communities were dominated by few species with the ability to resist the ambient conditions in these habitats. This resulted in ab increase in AMF species seaward, as a result of lower below-ground competition that in turn resulted in low plants species richness. In conclusion, this chapter shows that although the composition of both AMF and plants at local scales is a result of changed physicochemical properties of their surrounding soil.
- Chapter 4 of this thesis examined the spatial and seasonal effect on the role of these biotic and abiotic variables, using a multiscaler sampling design, that was repeated over summer and winter. AMF communities were significantly affected by the spatial variation of abiotic factors like pH and nitrate levels. At larger scales, however, the role of the biotic factors was superior. Percentage

cover of different plant types at each location (grass, forb) and root biomass had significant effects on the composition of these communities. The resultant AMF communities in Lancashire had a higher number of AMF species compared to those in Essex. In Lancashire, the vegetation cover was dominated by annual species that are known to harbour more AMF during their seedling growth phase. These grass species like *Festuca rubra* in are known for their dense roots that increase AMF habitat as well as the available C in the rhizosphere. This also emphasises the important role of the plants' temporal pattern on the available carbon that was also confirmed to influence the composition of AMF communities in other habitats (Dumbrell *et al.*, 2010a). Finally, the results of this chapter indicated that the composition of AMF communities at large spatial sales is most likely to reflect the niche of their host plants. Higher plant diversity allows the AMF predominant species to be reduced as a result of C availability that is known to enhance other low competitive species.

- The analysis based ITS amplicons of general fungi in the fifth chapter allowed me to evaluate whether the response of AMF communities' composition to spatial and temporal patterns of these biotic and abiotic variables were phylogenetically dependent. Functionally similar fungi responded similarly to the environmental gradients, suggesting a possible disconnect between functional redundancy and resilience in microbial communities. I also investigated whether the responses of fungal communities to these factors were taxon or functional group specific. Analysing the abundance of these fungal communities suggested an important role of the spatiotemporal

patterns of these abiotic and biotic factors in explaining the relative abundance of different fungal communities within the rhizosphere. A considerable variation in environmental responses was observed both within and between fungal taxa. Finite mixture models were used to group fungal OTUs by their environmental responses into *G* archetype species. Setting *G* equal to the number of trophic groups (6) revealed archetype species with considerably different environmental responses. Both biotic and abiotic model archetypes were characterised by fairly strong positive responses to plant species richness and very weak responses to percentage cover by forb or grass species. The abiotic model clustered most symbiotrophs into archetype G-2, a group characterised by decreased abundance during the winter compared with the summer, and a relatively strong negative response to pH. In the biotic model, saprotrophs were mainly distributed between archetypes G-2 and G-3 that had fairly similar responses to the biotic variables. Significant influences on the fungal communities can result from spatiotemporal patterns in biotic and abiotic factors. However, the way in which these fungi respond to these patterns, is phylogenetically dependent.

- Chapter 6 examined whether the role of temporal and spatial patterns of plant species determining the composition of AMF communities is phylogenetically dependent. This was done using multiscaler GLM models that also evaluated the effect of these factors on the relationship between different AMF families and the presence of other fungal species including plant pathogens in the rhizosphere. The results indicated a significant role of seasonal and spatial patterns in determining the composition of different AMF families. Although

the response to this pattern was different, dominant AMF families like the *Paraglomeracea* and *Glomeraceae* were among families that were most affected. Different AMF families had differed in their response to the presence of other fungi in the rhizosphere. The abundance of the AMF *Paraglomeracea* was significantly affected by the presence of other plant pathogens, which may indicate an important role of the C based-niche in different habitats. Finally, the conclusion of this chapter demonstrates an important scale dependency which needs to be considered in future research focusing on interactions between organisms with different dispersal abilities; annual plants and soil fungi like those in Lancashire are an excellent example of this disparity. The relationship between the AMF community composition and the presence of other fungi in the rhizosphere was a result of plant-niche processes that indicated no significant influence on AMF families.

7.3. Role of environmental factors in determining the composition of fungal communities in salt marshes differ from that of plants

Plants diversity in salt marshes follow the stressful conditions of high salinity and soil anoxia that increase seaward causing few species to grow in the lowest point, known as the pioneer zone, where such physical stress reaches its maximum level (Ford *et al.*, 2016). Being obligate aerobes (Khan, 1993; Miller & Sharitz, 2000) with extraradical mycela that are in physica contact to the soil, most wetland plants especially those growing in the pioneer zone were thought of as non-mycorrhizal (Khan, 1974; Anderson *et al.*, 1984), and AMF were thought to have little significance under these conditions (Bohrer *et al.*, 2004). However, a positive correlation was observed between the abundance of different AMF species and the

increased stress in salt marsh habitats (Chapter Three). Although current results indicated that both soil salinity and pH have a determinative effect on the composition of AMF communities in Lancashire saltmarshes, the low phosphate and nitrate in Cartmel Sands have created a stressful condition that enhanced the demands of AMF association. The low number of plant species in the pioneer zones reduces below-ground competitions and thus the AMF diversity as a result of reduced habitats. Such phenomena are known to promote unevenness in AMF communities' due to suppression of any late colonising species.

In these habitats, the recorded AMF species favoured the natural soil pH. The abundance of AMF species was negatively correlated with low soil pH in both West Plan and Cartmel Sands. Both sites were shown to have a significantly different range in soil pH compared with Warton Sands that was narrower and almost natural. Soil pH is also widely known to reduce up to 50% of soil carbon solubility (Clar*k et al*, 2005), and this normally results in decreased rates of decomposition, as well as the solubility of phosphate compounds that heavily influence the ratio of organic carbon and other abiotic factors (Chapman and Reiss, 1999). Moreover West Plain soil was rich with P and N factors, that were shown to influence the AMF community composition in other habitats (Johnson *et al.*, 1991; Dodd *et al.*, 2000; Egerton-Warburton & Allen, 2000; Carvalho *et al.*, 2001; Dumbrell *et al.*, 2011). Indeed, N and P are limited elements in the soil, and were shown to have negative effects on the abundance of AMF in several studies (Fitter *et al.*, 2000; Gamper *et al.*, 2004). In the low-P (high N:P) soils, plant dependency on AMF for P acquisition increases (Hetrick *et al.*, 1990), but not in the high-P soils elsewhere (Bever *et al.*, 2001) (Anderson et al. 1994, Schultz et al. 2001). On the other hand, in the soil that is N

limited, and relatively P rich, N fertilization decreases AMF abundance and species richness (Johnson *et al.*, 2003). However, knowledge about the mechanism of these factors and how other biotic and abiotic factors interact in natural environments is still largely unresolved.

7.4. Spatiotemporal partitioning of fungal communities in salt marshes, role of biotic and abiotic factors

Although AMF species have low specificity to their host plants species in these salt marshes, species-specific interactions do not always occur, and AMF could be more specific to plant functional groups than to individual plant species (Öpik *et al.*, 2009). Moreover, the structure of both AMF and their host community may be influenced by differential effects between individual AMF–host partners causing similar variations to what were recorded at different zones. Plant diversity at the location scale showed a significant effect in the biotic variables, resulting in distinct AMF compositions in these salt marshes (Chapter Four). Lancashire vegetation is formed of grass species like *Festuca rubra,* that is known to form high root biomass which increases the habitat availability of root colonisers like AMF. Stronger relationships always resulted under conditions of high root biomass between plants and these obligate symbionts in Lancashire. This means that more AMFs can gain an axis to the available carbon from the plants root. Similar effects were also observed for other fungi sharing the same resources as the AMF. The composition of AMF communities may be strongly influenced by the host species, through differential effects on hyphal growth and/or sporulation (Bever et al. 1996; Daniels Hetrick and Bloom 1986; Eom et al. 2000; Johnson et al. 1992; Sanders and Fitter 1992). In return, AMF host plant community structure may be strongly

influenced by the specific composition of the associated AMF and the effectiveness of each of the fungal species in promoting growth of each host (Grime et al. 1987; Hartnett et al. 1994; Streitwolf-Engel et al. 1997; van der Heijden et al. 1998a, 1998b). Thus, the increased number of AMF *Glomus* in the roots of pioneer plants may be a result of the water-stress at this zone rather than salinity, which seems to have lower effect.

7.5. Taxonomic and functional responses to environmental spatiotemporal patterning of rhizosphere fungal communities

Considerable variations in environmental responses, both within and between fungal taxa were observed in relation to the spatiotemporal pattern of these biotic and abiotic factors. For example, at the class level, few taxonomically consistent responses to most environmental covariates were observed, with the coefficients for most classes spanning around positive and negative effect (Chapter Five and Six). Notable exceptions to this were in the responses of fungal classes to season. Groups such as the *Archaeorhizomycetes*, *Cystobasidiomycetes*, *Orbiliomycetes* and *Sacccharomycetes* were all enriched in winter compared to summer. Conversely, the *Exobasidiomycetes* and *Taphrinomycetes* were among the few classes whose OTUs were consistently less abundant in winter as compared to the summer. At finer taxonomic resolutions, differences in the environmental responses of closely related fungi emerged. For example, OTUs in the *class Glomeromycetes* showed mixed responses to season, with an approximately even split between positive and negative responses. Yet, when examined at the family level, the 5 *Glomeromycete* families showed distinctly different responses to season, with the *Archaeosporaceae* and *Claroideoglomeraceae* both becoming less abundant in

winter (Chapter Five). The largest family, the *Glomeraceae* showed a mixed response, whereas the *Paraglomeraceae* were enriched in the winter. Unlike their response to the environmental factors, different fungi had nearly similar responses to the spatiotemporal patterns of biotic factors at different sites. Plant species richness positively influenced the species abundance of different fungal communities in these habits. Responses to root biomass and percentage cover by grass or forb varied between archetypes.

7.6. Future work

Overall, current result have developed our understanding of fungal ecology in an underexplored habitat and highlighted mechanisms by which fungal communities may improve plants' tolerance to a physiologically stressful environment. These results also highlighted specific AMF groups which appear to thrive in particularly saline areas of the salt marsh, regardless of zonation. These fungi may confer tolerance against saline conditions to plants, allowing them to grow in more extreme, but less competitive areas. Furthermore, current results have shown that for both AMF and other root associated fungi, the drivers of community structure and diversity are highly context specific. The balance between abiotic and biotic drivers changed between site, scale, and season. This infers that general patterns of diversity may not exist in salt marsh, and other, fungal communities. I also showed that AMF families respond in different ways to the presence of other fungal functional groups, such as plant pathogens. This suggests that the mechanisms by which AMF suppress plant pathogenic fungi may differ between taxonomic groups.

Though these results have hinted at a possible role for specific AMF groups in improving plant's tolerance to stressful or unfamiliar environmental conditions, it is not possible to be sure of this without an experimental approach. By subjecting plants to stressful environmental conditions, it would be possible to measure the changes in the associated fungal community. However, to understand whether the fungal communities associated with plants in stressful conditions confer tolerance, plants from less stressful environments could be transplanted into new conditions. This would subject the plant to both the stressful conditions and the potentially beneficial AMF groups. If these AMF groups associate with the plant, and the plant is

able to survive outside of it's realised niche, then it is likely the new AMF symbiosis has had greatly extend the environmental niche of host plant species.

Although different AMF species can have considerable influence on different plants species under different environmental stresses, however, their response to changes in their local environments is phylogenetically dependent. This alone can explain how an understanding of the AMF diversity is important to predict their role in improving ecosystem functionality. Such knowledge is crucial to help conserve ecosystem functioning, and protect biodiversity from ongoing environmental change. Indeed, having control over biotic factors may allow determining the effect of other abiotic factors like the soil pH, that significantly affects the natural communities at different scales. This will also allow the determination of plant types and their root architecture effect on carbon and the availability rhizosphere microbes in the habitat. Greater attention should also be paid to the phylogenetic variation in responding to these biotic and abiotic factors. Finally, sequential sampling procedures over different seasons would be valuable in determining the annual turnover of these communities if they occur. Such findings can be used as a model for other ecological studies to evaluate possible changes in the diversity of the microbial communities.

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