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New wheat root ideotypes for improved resource use efficiency and yield performance in reduced input agriculture

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CONTENTS

1. Abstract

The main objective of this study was to determine the impact of wheat root growth and morphology on the efficiency of nutrient uptake and hence yield and to explore how this interacts with selected environmental and agronomic parameters. The level of genetic variation for root development was assessed in a range of wheat genotypes in controlled environments along with the performance of a sub-set of lines with contrasting root characters under field conditions. Interactions with mycorrhizal fungi were also assessed.

A DNA based assay was developed that shows differences between root phenotypes of UK wheat varieties in field grown plants. The utility of the assay was assessed by comparing DNA results with those from soil washing experiments. Furthermore, it was assessed whether DNA-based assays can distinguish between genotypes that differ in root phenotype. Finally, it was determined whether DNA-based assays are sensitive to changes in root phenotype in response to soil treatments (e.g. nutrient input, soil tillage). This technology is a potential tool for plant breeders and for exploring variation within agronomic trials.

Plant roots interact with a complex microbial community in the soil, including microbes that enter into the root tissue. Prominent among these are the arbuscular mycorrhizal fungi (AMF), which can provide demonstrated benefits for plant growth. Here, an improved tool was developed to describe the diversity of AMF within roots and in the surrounding soil, and tested in a number of different wheat trials, looking at contrasts in treatments and varieties. The approach was to amplify a variable region of the 18S ribosomal RNA gene using AMF-specific primers, and then to pool samples and sequence using the Ion Torrent PGM™ platform. Reads are clustered into sets at approximately the level of species so that relative species abundance can be determined. The method revealed high species diversity of AMF in both roots and soil, and high spatial variation in communities from one plot to another. Future studies will need to be carefully designed to take this variation into account in order to detect the effects of variables such as depth in the soil, agronomic treatments, or crop varieties.

Quantification of cereal root systems during plant development to maturity raises several challenges. Cereal root systems are relatively large compared with more commonly explored model species such as *Arabidopsis thaliana*, and vary with plant genotype. Cereal roots are also highly dynamic during the cereal growth season and in response to environmental factors such as nutrient availability. This research aimed to quantify root phenotype in UK wheat varieties at four growth stages, using one of two methods: i) a flat bed filter paper based system to characterise roots at seedling stage; and ii) a metre-length rhizotube system to characterise roots at stem elongation, anthesis and maturity. Root responses to nitrogen supply were also assessed using the rhizotube system. Significant differences were found between varieties in root size and in rooting depth and root shape. The maximum rooting volume occurred at anthesis, with the majority of the root

expansion being found between 0 cm and 30 cm deep in the rhizotubes. The overall increase in root size with development and depth depended on wheat variety but was significantly affected by the length of the growing season to anthesis. Changes in both root size and shape were found in response to nitrogen treatment, and in addition there was evidence of differences in the type of responses between varieties.

Taken together, the three strands of research have created methods and datasets of value to root researchers, agronomists and plant breeders that will furnish a toolkit useful in development of new varieties and cultivation methods for UK growers.

2. Introduction

Cereals account for 70% of the world's food production and, in the UK, wheat is the single largest crop. To safeguard future food supplies plant breeders are challenged to maintain yield as environmental conditions change, to reduce dependence on limited resources and to reduce CO_2 emissions. These considerations drive agriculture towards reduced input regimes, particularly through the decreased use of NPK fertilisers, limited soil tillage and exploitation of plant varieties suited to reduced inputs. Historically, cereal breeding has focussed on the growth of above ground organs and their impact on yield. In contrast, the development of roots and their contribution to yield is less well understood. Crop plants require a properly established root system that ensures uptake of water and nutrients and offers structural support. Plant architecture genes such as Rht have an influence on yield and harvest index but their effect on partitioning of biomass between roots and shoots are less well understood. In addition to genotype, operations that affect rooting might include position in the rotation, nitrogen application and timing, cultivation type, seed rate, sowing date and plant growth regulator applications (Hoad, 2001; Bayles *et al*., 2002).

The main objective of this study was to determine the impact of wheat root growth and morphology on the efficiency of nutrient uptake and hence yield and to explore how this interacts with selected environmental and agronomic parameters. In order to achieve this prime objective the level of genetic variation for root development in a range of wheat genotypes was assessed in controlled environments, as was the performance of a sub-set of lines with contrasting root characters under field conditions. To enable these assessments to be done, methods to study root systems in both controlled environments and field experiments were identified and developed. A panel of 100 wheat lines was assembled, selected on the basis of information from UK projects on adaptive traits, or that feature prominently within extant experimental populations, or diverse material available from pre-breeding programmes. The root phenotypes for this panel were extensively explored in controlled environments and a subset phenotyped in field experiments.

Interactions with mycorrhizal and other fungi may have implications for wheat yields. The potential of wheat genotypes to form mutualistic associations with mycorrhizal fungi was assessed by investigating the diversity of mycorrhizal and other fungi associated with wheat roots, and associated soil, sampled in field experiments.

2.1. Semi quantitative estimation of wheat roots in soil using DNA

A DNA based assay was proposed that was intended to show differences between root phenotypes of UK wheat varieties in field grown plants.

- Firstly, the utility of the assay was assessed by comparing DNA results with those from soil washing experiments.

- Secondly, it was assessed whether DNA-based assays can distinguish between genotypes that differ in root phenotype.

Root phenotyping is a rapidly developing field (George *et al*., 2014). The simplest approaches rely on digging up the growing plants, taking cores of soil within trial plots, digging observation trenches or introducing observation tubes into the soil column (Zhu *et al.*, 2011). Washing the roots free of soil and quantifying the root length, diameter or surface area is labour intensive and therefore costly, though image analysis may be used for data capture (Bauhus & Messier, 1999). The results obtained by these methods are informative on the proportions of fine to coarse roots and directly relevant to field grown crops but results may not be transferable among different soil types (Kücke *et al*., 1995). Root phenotyping by soil coring among Australian wheat lines showed considerable variation for deep root traits and it was notable that above ground traits were not useful predictors of root traits (Wasson *et al*., 2014). Non-invasive geophysical methods such as ground penetrating radar and electrical resistivity tomography have been successful in measuring large tree roots (Butnor *et al*., 2001, Paglis, 2013). Where fine root structures have dimensions similar to soil grains and pores, these techniques may be confounded (Amato *et al*., 2009). Root electrical capacitance has been shown to correlate to root mass for *H. vulgare* in glasshouse experiments (Dietrich *et al*., 2013). The use of rhizotron laboratory based systems is well established (James *et al*., 1985) and amenable to automation (Lobet & Draye, 2013). Growing media for these experiments can include soil, artificial growing media such as sand or potting composts, gels or hydroponics systems (Zhu *et al*., 2011). Rhizotron methods allow for repeated measurements of the developing plant and allow greater of control environmental variables such as temperature, irrigation and fertilisation. However, as rhizotrons are artificial environments, these techniques are a step removed from field conditions. Watt *et al.* (2013) found that correlations between artificial systems and field assessments were high during the vegetative growth phases but low for the reproductive growth phases. Allied to rhizotrons are X-ray Computed Tomography (CT) systems; developed originally for biomedical uses, CT is capable of visualising detailed structures in media such as soil that are opaque to visible light. The advent of industrial micro CT systems with resolutions of 500 nm or less (Mooney *et al*., 2012), coupled with automated systems for sample presentation and data processing (Mairhofer *et al*., 2012) represents a significant advance in potential for root phenotyping.

Species specific DNA sequences are present in every cell of a plant, including the roots. Quantitative DNA detection methods, coupled with robust extraction techniques are commonplace and have been deployed to address identification and quantification of roots in soil. Real-time PCR has been used to differentiate between species in mixtures of roots washed out of soil (Mommer *et al*., 2008) and used to measure roots and seeds from a mixed population of meadow grasses using DNA extracted directly from soil (Riley *et al*., 2010, Haling *et al*., 2011; Haling *et al*., 2012). Detecting roots by DNA based methods is not straightforward (Mommer *et al*., 2011): soil contains humic acids known to

inhibit PCR by binding $MgCl_2$, thus a DNA extraction method suitable for soil must be selected. As roots comprise a small part of the total soil volume, a high sensitivity final assay will be needed. This suggests high copy number targets such as ribosomal DNA internal transcribed spacer region (rDNA ITS), provided care is taken to design species specific primers. DNA based assays targeting rDNA ITS have been used to demonstrate differing responses to drought among the root phenotypes of twenty Australian wheat varieties (Huang *et al*., 2013).

The aim in this work package was to develop and deploy a novel method for measuring cereal root biomass in soil samples taken from field experiments.

2.2. Characterising the mycorrhizal fungal community associated with wheat roots

The roots of the majority of land plant species are colonised by fungi in the phylum *Glomeromycota*, which form the distinctive intracellular arbuscular mycorrhizal (AM) symbiosis (Smith & Read, 2008). In many cases, this association has been demonstrated to be beneficial to the plants in various ways. The best known of these is the provision of soil phosphate that can be harvested more effectively by the fungal hyphal network in the soil than by the plant roots themselves. Other demonstrated benefits include other nutrients, drought tolerance, and the suppression of pathogens (Smith & Read, 2008). Plant roots are associated with a rich microbial community (Donn *et al*., 2015; Lundberg *et al.,* 2012), and no doubt there are other fungi and bacteria that have significant effects, positive or negative, on plant performance (Berendsen *et al.,* 2012). There is a parallel here with the complex microbiota of the human gut, which has consequences for health that are increasingly recognized (Shreiner *et al.,* 2015).

While the arbuscular mycorrhizal symbiosis is generally considered to be beneficial to plants, it is important to recognise that the effects will depend on the environmental conditions, the host plant, and the type of fungus. *Glomeromycota* is an ancient phylum that encompasses many divergent fungal species (Schüßler *et al*., 2001) and these fungi have unfortunately proved hard to study because they cannot be obtained in pure culture and their genetic systems are very poorly understood (Young, 2015). An important first step is to establish which fungi are present, and over the past two decades a number of molecular tools have been developed for this, and some knowledge about the distribution of AM fungi (AMF) in association with plants around the world. After some early studies of other genes, the first breakthrough was the design of the AMF-specific DNA primer AM1 that could be used in a polymerase chain reaction (PCR) that was robust enough to work reliably on a diversity of field-collected material (Helgason *et al.,* 1998). Together with the universal primer NS31, this amplified part of the small subunit ribosomal RNA (SSU rRNA) gene that was sufficiently variable to reveal the diversity of AMF in the field. The first publication included results from wheat and other crop plants as well as woodland plants, and reported that there was AMF diversity in arable crop roots, but much greater diversity in woodland plants (Helgason *et al.,*

1998). The NS31-AM1 primer system was widely used to assess AMF diversity in plants from the field, either by cloning and Sanger sequencing of the mixed PCR product or by a more rapid but less detailed approach such as TRFLP (Vandenkoornhuyse *et al.,* 2003). As knowledge of AMF increased, new, distantly related lineages were discovered and some of these were not recognised by the AM1 primer. Lee *et al*. (2008) reassessed the situation, taking into account the expanded set of known AMF sequences, and designed new primers AML1 and AML2 that have been extensively used since.

Although the PCR primers just described have proved to be valuable tools, the sequences that they amplify (550-800 bases) are too long for sequencing using high-throughput technologies. In order to realise the huge potential of these technologies, a shorter product is needed (ca. 200 bases), which retains sufficient diversity to distinguish different AMF and can be amplified from all AMF but not from other organisms, and especially not from plants.

The aim here was to develop such a tool and to apply it to investigate the diversity of AMF on wheat roots.

2.3. Dissecting root traits at key development stages

The main challenges for wheat breeding are to stabilise yield as environmental conditions change, to reduce dependence on limited resources (particularly mineral fertilisers), and to reduce environmental impacts. However, limited availability of key resources like nitrogen (N) and water can severely reduce wheat yield. There is an urgent need for alternative crop management strategies to allow food production to keep pace with population growth without exhausting global resources. Deploying wheat genotypes that exhibit efficient use of nitrogen and water is one option for improving resource use efficiency and maximising wheat yields.

Above-ground plant characteristics have been the main focus of studies to identify traits relating to cereal yield. In contrast, the contribution of root traits to resource use efficiency and yield of wheat is less well understood and root traits have rarely been used as selection criteria in breeding for improved resource acquisition. A more directed search for root traits that underpin resource use efficiency is now imperative. As fertiliser- nitrogen is highly soluble and mobile in water, the efficient use of water and nitrogen are likely to be correlated; thus, assessing wheat root traits in relation to both of these resources is likely to be the most productive approach.

Initial growth of wheat roots involves the emergence of seminal roots from the seed, followed by roots emerging from the base of the tillers. During early plant growth, root dry mass increases rapidly and exponentially up to anthesis, after which growth rate declines and root mass even decreases towards plant maturity (Barraclough, 1984; Barraclough & Leigh, 1984; Gregory *et al.,* 1978). The majority of the root dry matter is present in the top 20 cm of the soil profile (Barraclough & Leigh,

1984). Variation in root size and shape are thought to be important determinants of the efficiency of nutrient uptake in many plant species and, for this reason, root phenotype is proposed as a suitable focus for further crop improvement (Den Herder *et al*, 2010). However, root phenotyping of crops and other plant species is challenging, due to the difficulties of characterising plant structures below the soil surface, particularly in field conditions (George *et al*, 2014)). Despite these challenges, data are needed to understand whether wheat genotypic variation in agronomic characteristics such as yield and nitrogen uptake (Barraclough *et al*., 2010) is underpinned by varietal differences in root traits, as these traits could provide a useful basis for future wheat improvement programmes.

This work package aimed to identify root traits that might improve wheat performance in reduced input systems. The primary objective was to characterise the extent to which root traits vary at key development stages using controlled environment phenotyping in a wide range of wheat genotypes. A second objective was to assess the degree of variation in nitrogen acquisition, and to determine any relation with root phenotype.

3. Materials and methods

Soil samples were collected from wheat variety trials over the course of three growing seasons.

Table 3.1: Variety trials sampled for root quantification and characterising the mycorrhizal fungal community associated with wheat roots in the course of this study

Site	Year	Trial design	Purpose	Grid reference	Soil series	Soil texture
Terrington St Clement, Norfolk	2012	Three varieties in two field reps	DNA quantification (Pilot)	TF 496 226	Wisbech	Silt loam
Westfield Farm. Settrington.	2012	Six varieties in one field reps	AMF assessment (Pilot)	SE 813 719		
Nelson Field, Stanaway Farm, Otley, Ipswich, Suffolk	2013	One variety (cv Santiago) in two cultivations	DNA quantification AMF assessment	TM 536 205	Beccles / Hanslope	Fine loam over clay
Nitrogen fungicide interaction, Morley St Botolph, Norfolk*	2013	One variety (cv Santiago) in four treatment combinations	DNA quantification AMF assessment	TM 955 055	Burlingham	Sandy loam:
Stonham	2013	Three varieties in one field reps	AMF assessment	TM 122 612	Beccles	Clay loam
Burkees Field, Eastland Bank, Walpole St Andrew, Norfolk	2014	Eighteen varieties and four breeders' lines in three field reps	DNA quantification	TF 500 184	Blacktoft	Silty Clay loam
Willow Tree Field, Burman Farm, Terrington St Clement, Norfolk	2015		DNA quantification	TF 537 239	Wisbech	Silt loam

* Nitrogen fungicide interaction, Morley St Botolph, Norfolk. Treatment details

Treatment	Fungicide	Nitrogen
1	Untreated	0 KgN.Ha^{-1}
2	Untreated	320 KgN.Ha^{-1}
3	Treated	0 KgN.Ha^{-1}
4	Treated	320 KgN.Ha^{-1}

Fungicide programme: GS30: Cherokee @ 0.75(l/ha), GS32: Ignite @0.5(l/ha), Bravo 500 @1.0 (l/ha), GS39; Ignite @0.75(l/ha), Bravo 500 @1.0(l/ha), GS59: Prosaro @ 0.6 (l/ha) Comet 200 @ 0.4 (l/ha)

3.1. Semi quantitative estimation of wheat roots in soil using DNA

The development of a novel method to measure root biomass within soil samples taken from field experiments is described below.

3.1.1. Trials sites and plant material

Soil samples were collected from wheat variety trials over the course of three growing seasons. A three variety pilot trial was grown with two field replications and sampled in 2012; additional cores were sampled in an adjacent uncultivated area of the site. Two larger variety trials grown and sampled in 2014 and 2015 included 18 varieties and four breeders' lines planted in three field replications. Trial locations are given in Table 3.1. Soil data for each site was taken from the LANDIS Land information system (Landis, 2014).

Soil and root samples were also obtained from two soil treatment trials: STAR Project WW13-002 at Otley and Morley NAC nitrogen dose and fungicide interaction study WW13-9506. The STAR project compared inversion cultivation by ploughing to a depth of approximately 200 mm and shallow non-inversion cultivation using a Sumo Trio working with discs and legs raised to a depth of approximately 100 mm. The Morley trial examined the effect of fungicide and nitrogen treatments (Table 3.1).

The varieties sampled for these larger trials were selected as likely to harbour diverse phenotypes based on information from rhizotron experiments (Karley *et al*., 2012) and broadly representative of the diversity of UK wheat (Appendix 1). Two of the breeders' lines [SHW Xi19 / (Xi19 // SHW-218)>18 and SHW Xi19 / (Xi19 // SHW-218)>19) backcross-derived lines from the cross (Xi19 / (Xi19 // SHW-218)] trace back to different BC1 (back-cross generation 1) plants; plants XS-218>18 and XS218>19, respectively. SHW-218 is CIMMYT synthetic hexaploid wheat with the published pedigree Ceta / *Ae squarrosa* (895) (Gosman *et al*., 2014). The other two breeders' lines were Rht (reduced height) near isogenic lines in a background of cv Mercia available from the Genetic Resources Unit, John Innes Centre, Norwich.

3.1.2. Soil sampling

Soil cores were sampled from 10 x 2 m plots of winter wheat in each experimental trial. The soil cores were sampled when the crops had reached growth stage (GS) 51-65. Ten soil cores measuring 1 m depth x 15 mm diameter were extracted from each plot. The cores were divided into four portions representing 250mm depth intervals in the soil profile. The cores representing each depth interval were bulked into a single sample. In 2012 the bulked cores from one field replicate were sub-sampled to take a portion for estimation of root content by soil washing for comparison with estimates of root biomass using DNA based assays.

3.1.3. DNA Extraction

Soil cores were stored frozen (-18°C) before analysis. Samples were prepared for analysis by drying at 30°C in a re-circulating oven for a minimum of 72 hours. The dried soil was milled to a fine powder using a Humboldt H4199.5F soil mill fitted with a 2mm screen. DNA was extracted from each sample in duplicate using a PowerSoil kit (MO BIO Laboratories, Inc., Carlsbad, USA.) in accordance with the manufacturer's protocols; thus two technical replicates were obtained for each milled sample. This kit has been shown to achieve equivalent DNA yields from soil as commercial soil extraction methods (Haling *et al*., 2011)

Preparation of calibration materials. Wheat (cultivar Xi19) was grown in horticultural sand until it was 'pot-bound'. Root material was washed until free of sand, rapidly frozen in Cardice, freeze dried, milled and stored as frozen lypholised material. DNA was extracted from samples of 100 mg dried root in accordance with Tanksely's method and the extracted DNA re-suspended in 100 µl TBE buffer. Calibration standards were prepared by a series of ten-fold dilutions from this primary reference.

3.1.4. Quantification

Wheat DNA in the soil extracts was quantified by real time PCR using an ABI 7900 with triplicate 6 µl reactions comprising 1.0 µl template from soil extract, 0.5 µl primers with primers and probes at 5 mM, 2.5 µl Thermo Fisher Scientific ABsolute Blue qPCR ROX Mix and 2.0 µl water. Amplification was carried out using 10 minute activation at 95°C followed by 40 cycles of 15s at 95°C then 60s at 60°C, monitoring fluorescence at each cycle.

The primers and probes targeted the wheat internal transcribed spacer region within the 5.8S ribosomal RNA gene (Table 3.1.2). The target sequence was acquired from NCBI Genbank AF438186.1 *Triticum aestivum* (Sharma *et al*., 2002) and the primers and probes were designed using Primer3 (Untergrasser *et al*., 2012).

Table 3.1.2: Primers and probe designed for wheat root quantification

Forward	TritITS2_F	CAACCACCCTCATCGGGAAT
Reverse	TritITS2_R	TCGGATGCACTGCGTTGATA
Internal oligo	TritITS2_Probe	[JOE]GACCGAAGATCGGGCTGCCG[TAM]

Additional data (including seasonality, *Rht*, 1B/1R and *Ppd*) for varieties included in the Terrington St Clements trials were provided by Alison Bentley (*pers. comm*).

3.1.5. Data analysis

All qPCR data were processed using Applied Biosytems SDS 2.2 and the results collated and analysed in Microsoft Excel. Analysis of variance (ANOVA), restricted maximum likelihood (REML) analysis were carried out using Genstat 12.1.0.3338 and R 3.0.1. All statistical analyses were carried out on raw data without prior averaging of technical replicates.

3.2. Characterising the mycorrhizal fungal community associated with wheat roots

3.2.1. Field samples 2012

Wheat trial plots at West Field Farm, Settrington, N Yorkshire were sampled on 20 April 2012 (Table 3.1). Two soil cores were taken from each of six plots, each of a different wheat variety (Oakley, Santiago, Cordiale, Viscount, Solstice, Alchemy). Soil cores were taken to a depth of 30 cm using plastic pipes (40 mm internal diameter). In the laboratory, each core was divided into upper, middle and lower 10 cm lengths, and roots were extracted by washing. Root weights and mycorrhizal colonisation were measured for all samples. The cores from the Alchemy plot were used for the experimental work to devise a new protocol (Section 4.2.1).

3.2.2. Field samples 2013

Soil and root samples were obtained from three trials (Table 3.1): STAR Project WW13-002, Stonham – NTN variety trial WW13-9037, and Morley NAC N dose and fungicide interaction study WW13-9506. The STAR project compared ploughing and shallow cultivation treatments (see paragraph 3.1.1 for details). The Stonham trial explored differences among three wheat varieties: Crusoe, Cordiale, JB Diego (note: trial location TM 122 612 where the soil is described by the Beccles series as a clay loam). The Morley trial examined fungicide and nitrogen treatments (see Table 3.1 for details). In each of the three trials a single 100 cm core was extracted from each of the plots in one field replicate and the cores divided into four equal sections to investigate the mycorrhizal diversity at different depths. The sub sampled portions from each core were processed and described below.

3.2.3. Assessment of mycorrhizal colonisation

Fresh roots were fixed and stained, and the level of colonisation by mycorrhizal structures was determined by microscopy using standard methods (Heinemeyer *et al.,* 2004).

3.2.4. Extraction of DNA from roots or soil

DNA was extracted from roots (fresh or dried, 5 - 50 mg) using the MoBio PowerPlant Pro kit, and from soil (250 mg) using the MoBio PowerSoil kit (MoBio Laboratories, Carlsbad, CA, USA).

3.2.5. Amplification of fungal SSU sequences and sequencing

SSU rRNA sequences were amplified from each DNA preparation using either the AMF-specific primer pair AML1-AML2 (Lee *et al*., 2008) or the universal fungal primer pair AU2-AU4 (Vandenkoornhuyse *et al*., 2003). The resulting products were used as templates for a further amplication step using newly designed universal primers that also incorporated sequences specific for sequencing on the Ion Torrent PGM and, in the case of the forward primer, a 13-base tag sequence that was different for each sample. The forward primers had the structure

CCATCTCATCCCTGCGTGTCTCCGACTCAG-[13 base tag]- GCAGTTAAAAAGCTCGTAGTTGA

and the universal reverse primer was

CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGAT - GCCCCCAACTATCCCTATT

where the 5' part of each primer is required for the sequencing and the 3' part recognises conserved sequences in the SSU rRNA gene. Tagged samples were pooled and sequenced on an Ion Torrent PGM 318 chip using standard protocols for a 200 bp run. In the first run (2012 field samples), 16 samples were pooled, while 32 samples were pooled in each of the second and third runs (2013 samples).

3.2.6. Analysis of fungal diversity

Sequence read data from the 2012 trial were submitted to MG-RAST (http://metagenomics.anl.gov/), which clustered and identified the sequences. As the quality of the annotation was severely limited by the reference databases available to MG-RAST, the prevalent sequence clusters were re-annotated by submitting representative sequences to MaarjAM (http://maarjam.botany.ut.ee/), a specialised database for AMF SSU sequences. Clusters that were not identified as AMF were classified by BLAST search against the NCBI nr nucleotide database. Sequence reads from 2013 were clustered using QIIME (www.qiime.org) , and then the clusters were identified within QIIME using MaarjAM for AMF and SILVA (http://www.arb-silva.de/) for the remaining clusters.

3.3. Dissecting root traits at key development stages

3.3.1. Experimental design

Wheat seed of all varieties tested here was supplied by KWS and NIAB. Seeds were pre-germinated on filter paper for two days prior to seedling transfer to the experiment. In total, four experiments were conducted between 2011 and 2013 (Table 3.3.1).

Table 3.3.1. Summary of experimental design

	Number of Varieties	Experimental design	Nitrogen treatment	Assessment timings
Seedling test	24	Randomised Block	No nutrient supply	7 days post germination
Glasshouse 2011	100	Randomised Block	Standard Nitrogen supply	Maturity
Glasshouse 2012	25	Randomised split block	Standard Nitrogen supply	Stem elongation, Anthesis, Maturity
Glasshouse 2013	15	Randomised split block	Standard and Reduced Nitrogen supply	Stem elongation, Anthesis, Maturity

Nutrient treatments

Macronutrients	Stock concentration	Reservoir concentration	Dripper feed concentration (1/64 dilution by Dosatron)	Dripper rate (µmol per min)
NH_4NO_3	2.56 M			
Standard treatment		0.128 M	2.0 mM	16.6
Reduced treatment		0.016 M	0.25 mM	2.075
KNO_3	2.56 M			
Standard treatment		0.128 M	2.0 mM	16.6
Reduced treatment		0.096 M	1.5 mM	12.45
$MgSO_4.7H_2O$	0.96 M	0.048 M	0.75 mM	6.23
K_2SO_4	0.128 M	0.064 M	1.0 mM	8.33
$KH_2PO_4.H_2O$	0.393 M	0.020 M	0.31 mM	2.58
K_2HPO_4	0.033 M	0.0017 M	0.026 mM	0.22
$CaCl_2.2H_2O$	2.688 M	N/A	42 mM	349

3.3.2. Seedling tests

For analysis of roots at seedling stage, seeds were grown in the system described in Adu *et al*, (2014). Two seedlings were sown per A4 growth chamber, with 3 replicate scans per variety. Root images were captured seven days after seed imbibition, and used to analyse root structure using SmartRoot (Lobet *et al*, 2011).

3.3.3. Glasshouse rhizotube experiments

For analysis of root phenotype at stem elongation, anthesis and in mature plants, pre-germinated seedlings were transferred (in pairs) to small cores or rhizotubes containing a grit-sand-gravel mix (Karley *et al*, 2011). Seedlings were vernalised in cores or rhizotubes at 4-12^{o}C for approximately 8 weeks, prior to transfer to a greenhouse cubicle, where the daytime temperature was gradually increased to 18^{o}C (with the exception of year 1 - 2011). Nutrients and water were delivered to each rhizotube via an automated drip feed system, except for $CaCl_2$ which was supplied weekly (see Karley *et al*., 2011). In year 3 (2013), nitrogen (N) supply in half of the tubes was reduced to one-third of the standard N supply described in Table 3.3.1. All other nutrients were maintained at the same level as the standard N treatment. All experiments were performed as fully replicated randomised blocked designs. The growth and development of each plant was recorded twice per week. Plants were harvested individually at the specific growth stage of interest (stem elongation, anthesis or maturity), following the procedure described in Karley *et al.* (2011), with shoots divided into four fractions (stem, leaf, grain and chaff) and roots divided into twelve sections according to depth within the metre-length rhizotube. The fresh and dry mass of each plant fraction and root section was recorded. Leaf, stem and grain fractions were milled to a fine powder and quantified sub-samples (*c.* 5 mg) were analysed for carbon and nitrogen content using an Exeter Analytical CE440 Elemental Analyzer (EAI, Coventry, UK). All data were double entered and checked for integrity.

3.3.4. Data analysis

Statistics were performed using Genstat and R. For statistical analysis of each dataset, a standard protocol script was developed in R. Principal components analysis was applied either to dry mass data collected for the 12 root-depth sections, or to dry mass data for all shoot fractions and root sections. These analyses were used to select varieties for further experimental work in each successive year of the project. All parameters within each dataset were normalised where possible using the Box-Cox function in R, prior to analysis of variation of the transformed and untransformed parameters using linear mixed models. Models tested included fixed effects (e.g. variety, growth stage, nutrient supply, year and plant position) with random factors including blocks / sub blocks where appropriate.

4. Results

4.1. Semi quantitative estimation of wheat roots in soil using DNA

4.1.1. Primer specificity

The primers were tested for specificity among common agricultural species. Amplicons were obtained for wheat and barley DNA but there was no reaction for maize, oilseed rape or faba beans. The primers were also tested against black grass (*Alopecurus myosuroides*) and found to have no reaction. Cores taken from an uncultivated area within our 2012 trial site gave no positive response when extracted and assayed using our method. The result for wheat and barley is not ideal; however, as this method would be applied in well managed agricultural trials where only one cereal is present this does not represent a practical problem. The ability to exclude weed roots from the total measured root density represents an advance over conventional root washing methods.

4.1.2. Comparison with soil washing experiments

Soil cores were sampled from three varieties in two field replicates from the pilot trial at Terrington St Clements (2012). Cores from this site were sampled and root content determined by our DNA based assay, expressed as µg dry roots / g air dried soil. Cores from field replicate 1 were sub-sampled and root content determined by soil washing, expressed as average root length density (cm/cm^3). Correlations between the root values obtained by the DNA based assay and by soil washing for each variety were high (0.88-0.99) (Table 4.1.1).

Table 4.1.1: Comparison between soil washing and DNA based assay results for three varieties sampled in replicate 1 from the Terrington St Clements 2012 trial. Correlations were calculated between root length per cm^3 soil and root weight per g soil.

			Variety		
		Depth (mm)	Alchemy	Oakley	Viscount
Soil washing	Root length density	0-250	3.2	3.0	3.2
	(cm/cm^3)	250-500	2.1	1.7	1.8
		500-750	1.4	0.9	1.1
		750-1000	0.6	0.6	1.0
DNA based assay	Biomass of roots in dry soil	0-250	41.2	9.1	44.2
	(µg mg /g)	250-500	8.4	6.1	13.2
		500-750	5.3	1.2	6.1
		750-1000	0.2	0.2	0.7
Correlation			0.92	0.98	0.99

ANOVA for the root washing data (one replicate) showed the differences in root content by depth were highly significant ($p < 0.001$), but that differences among varieties were not significant. Similarly, ANOVA for the DNA assay data showed the differences in root content by depth were highly significant ($p<0.001$), but that differences among varieties were not significant. However, the F value for difference among varieties had a low probability ($p = 0.051$) despite data coming from a low powered pilot experiment with only three varieties in two field reps. This suggested that a more powerful experiment might allow discrimination among varieties hence a series of larger trials were planned.

4.1.3. Results from variety experiments

For soil cores sampled from two full scale trials in 2014 and 2015, roots were quantified within the soil profiles at levels between 0.00 and 4400 µg/ gm soil (dry matter roots in air dried soil). The data were highly variable with large differences among technical replicates in a small number of cases. Differences among technical replicates may be explained by the relatively small samples taken for each assay (0.25g) and the presence of a small number of visible, but not necessarily evenly distributed, large root fibres within the milled soil. Initial analytical results were inspected and the result was removed for those technical replicates where the result was greater than 500 µg/gm soil and the two technical replicates for that sample differed by a factor of ten or more. Results for four technical replicates were removed in this way. Once these outliers were removed roots were quantified within the soil profiles at levels between 0.00 and 760 µg/ gm soil (dry matter roots in air dried soil) with a mean of 31.2 µg/ gm soil. The mean values for the 2014 and 2015 trials were 29.5 and 33.0 µg/ gm soil respectively; this difference was not significant. The mean across two years for all varieties by depth are shown in Table 4.1.2.

In general, the highest levels of root biomass were measured in the upper parts of the soil profile, the lowest levels were found at depth. Taking individual plot data for each level in the cores from these trials, the root biomass was measured and varied between 0.7-721 µg /g (0-250 mm), 0.9 - 394 µg /g (250-500 mm), 0.0 - 119 µg /g (500-750 mm) 0.0 - 42.3 µg /g (750-1000 mm). The mean for all varieties by depth separated by year are shown in Appendix 2.

Over 50% of the measured biomass was in the upper 500 mm of the soil profile in all but two of the plots sampled in each field experiment. The proportion of root biomass in the upper part of the profile averaged 79% in 2014 and 88% in 2015 and this difference between years was significant ($p < 0.01$) showing that while the mean values for root biomass within the profile does not differ significantly between years, the distribution of root biomass within the soil profile does differ significantly. This distribution of biomass within the profiles for each trial plot was consistent with reported results obtained by soil washing (White et al, 2015).

Table 4.1.2: Table of means for µg /g roots in dry soil for varieties by depth. The standard error of means was 10.5, 5.5 and 21,1 for variety, depth and variety by depth means respectively, least significant difference was 21.2, 9.0 and 38.5 for variety, depth and variety by depth means respectively.

Variety	0-250	250-500	500-750	750-1000	Mean
Alchemy	94.3	27.7	22.7	7.3	38.0
Avalon	114.0	66.9	32.2	7.6	55.2
Beaver	36.8	12.2	3.6	2.6	13.8
SHW Xi19 / (Xi19 // SHW-218) >18	82.1	33.0	11.3	1.7	32.0
SHW Xi19 / (Xi19 // SHW-218) >19	149.2	67.6	32.5	19.4	67.2
Buster	44.2	17.4	16.4	2.1	20.0
Cadenza	51.6	29.3	28.5	17.4	31.7
Cappelle_Deprez	68.1	22.4	11.3	1.3	25.8
Glasgow	29.4	14.4	2.5	1.8	12.0
Hereward	30.2	13.3	4.3	2.2	12.5
Mercia	32.0	11.9	5.7	2.1	12.9
Mercia_Rht8	60.9	20.1	4.6	1.4	21.8
Mercia_Rht_D1b	49.7	27.1	6.1	2.8	21.4
Norman	109.3	45.2	18.5	9.5	45.6
Oakley	47.5	21.3	7.6	7.6	21.0
Paragon	109.2	51.3	23	4.6	47.0
Rialto	35.5	25.5	6.5	0.9	17.1
Robigus	39.5	12.1	5.4	1.7	14.7
Savannah	82.9	42.8	14.2	2.4	35.6
Soissons	38.3	33.4	21.6	1.4	23.7
Spark	94.9	45.7	11.0	7.1	39.7
Xi19	212.8	87.4	6.5	7.2	78.5
Mean	73.3	33.1	13.5	5.1	31.2

4.1.4. Distinguishing between genotypes that differ in root phenotype

An analysis of variance (ANOVA) was carried out for the measured root biomass and this showed the differences by depth, the differences among varieties and the variation among varieties by depth were all highly significant ($p < 0.001$). The standard error of differences of means of varieties, depths and variety by depth were 10.5, 5.5 and 21.1 respectively (df = 953). Comparing the least significant differences calculated (20.6, 8.8, 41.3 for varieties, depths and variety by depth respectively) with the mean values are given in Table 4.1.2, it is suggested this assay is of value in describing varietal

differences among root biomass phenotypes. Inspection of the analysis of variance tables shows the method is vulnerable to random variation, with high variance terms observed for the field replication and for each analytical batch run for qPCR.

The varieties under test varied in their seasonal growth habit, their status at the semi dwarfing Rht loci, the photoperiod response Ppd loci and the rye translocation (1B/1R).

Highly significant differences ($p < 0.001$) were found for the root phenotype among varieties with different seasonal growth habits: spring types had the greater average root content within the soil profile, followed by alternative and winter types. However, the data set was unbalanced, with only one spring type and one alternative type among a set of mainly winter types so the results need to be treated with caution.

When the two years' trials were considered, the variation at Rht loci did not account for variation in the root biomass phenotype, though the F ratio had a low probability ($p=0.073$). Drilling down within the data, when considering the 2014 trial in isolation, variation at the Rht loci did explain significant variation ($p < 0.001$) in the root biomass phenotype. In 2014, wheat varieties harbouring wild type alleles and Rht2 had greater average root content within the soil profile than those harbouring Rht1 and Rht8; this trend was not observed in the 2015 data. No significant variation in the root biomass phenotype was accounted for by the presence or absence of the rye translocation or variation at the Ppd loci.

4.1.5. Changes in root phenotype in response to soil treatments

The result from the nitrogen and fungicide interaction study at Morley in 2013 showed that soil sampled from wheat plots in the high nitrogen treatments (Table 4.1.3) generally had a higher root content as did soil sampled from fungicide treated plots. As previously observed, the upper levels sampled from the cores had higher root content when compared with cores taken from lower in the soil profile. ANOVA and REML showed that the differences observed for depth and nitrogen treatment were highly significant ($p<0.001$) while the differences observed for fungicide treatment were not significant (Table 4.1.3).

The results from the STAR tillage experiment at Otley in 2013 were not conclusive; soil sampled from plots in the 'shallow tillage' treatment had higher root content than those from the 'plough treatment' but these differences were not significant (Table 4.1.3).

Table 4.1.3: Table of means for µg /g roots in dry soil for soil treatments by depth for the cultivar Santiago in one replicate

	STAR Cultivation Trial		**Nitrogen / Fungicide Interaction Trial**			
	Shallow tillage	Plough Tillage	Standard N / Standard Fungicide	Standard N / Zero Fungicide	Zero N / Standard Fungicide	Zero N / Zero Fungicide
Depth (mm)						
0-250	63.4	53.3	56.9	42.1	18.3	21.2
250-500	59.0	32.9	29.3	25.0	36.4	9.9
500-750	35.5	17.0	28.9	15.0	17.1	8.5
750-1000	0.9	0.5	26.4	13.4	8.7	6.1

4.2. The mycorrhizal fungal community associated with wheat roots

4.2.1. Design of the new protocol

A new protocol was designed to amplify part of the SSU rRNA gene of AMF from plant roots and to sequence the resulting amplicon pools using the Ion Torrent PGM high-throughput sequencer. From an alignment of sequences of this gene from the widest known range of AMF and some representatives of other organisms, an informative region of suitable length was identified. Unfortunately, the flanking sequences were so conserved that it was not possible to design primers that would specifically target AMF, although good universal primers could be found. Accordingly, a two-stage amplification was designed, in which AML1-AML2 were first used to amplify an 800-base sequence specifically from AMF, and then a small number of cycles were used to obtain the 130-base informative sequence using universal primers that also incorporated sample-specific tags and the standard flanking sequences for Ion Torrent sequencing. Blast search revealed that these primers should amplify not only sequences from AMF but from many other fungi, as well as other eukaryotes including plants. This potentially allows them to be used with other flanking primer sets, such as AU2-AU4, which have been used as universal primers for all fungi.

To provide material for method development, wheat roots were sampled by soil coring from six field trial plots at Settrington, N Yorkshire (i.e. six varieties). Roots were recovered at all depths in all cores, and microscopy indicated good colonisation with AMF (mean values of root length colonised were 26.1% for 0-10 cm depth, 43.7% for 10-20 cm, 21.7% for 20-30 cm). Cordiale had the lowest root mass in the 20-30 cm sample and these roots had significantly lower AMF colonisation. Other differences among the wheat varieties were not significant,

Fungal ribosomal RNA gene sequences were successfully amplified from the washed roots using both AM-specific and general fungal PCR primers. The two cores from the plot of the Alchemy variety were chosen as examples to test the protocol for sequencing. DNA was successfully extracted from roots from the upper, middle and lower 10-cm sections of the cores, both when fresh roots were used immediately and when the roots were first dried in an oven at 60 °C (which would be more convenient). Amplification with the AMF-specific primers was successful for all of these samples. The amplified products were sequenced with the Ion Torrent PGM™, yielding several hundred thousand high-quality reads per sample. These were grouped and identified using the MG-RAST pipeline. A consistent set of fungal species was identified in each sample, with the proportions varying between samples. Thus, it has been successfully demonstrated that this new methodology can provide details of the fungal community (both of AM and of other endophytic fungi) within wheat roots from the field. In addition, the general fungal (FUN) primers were used, successfully, to amplify sequences from the four middle-section samples (arbitrary selection). All sequences amplified by the AMF primers AML1-AML2 were identified as from *Glomeromycota*. Those amplified with AU2-

AU4 were all fungal, and included a significant but variable proportion of AMF sequences (Figure 4.2.1).

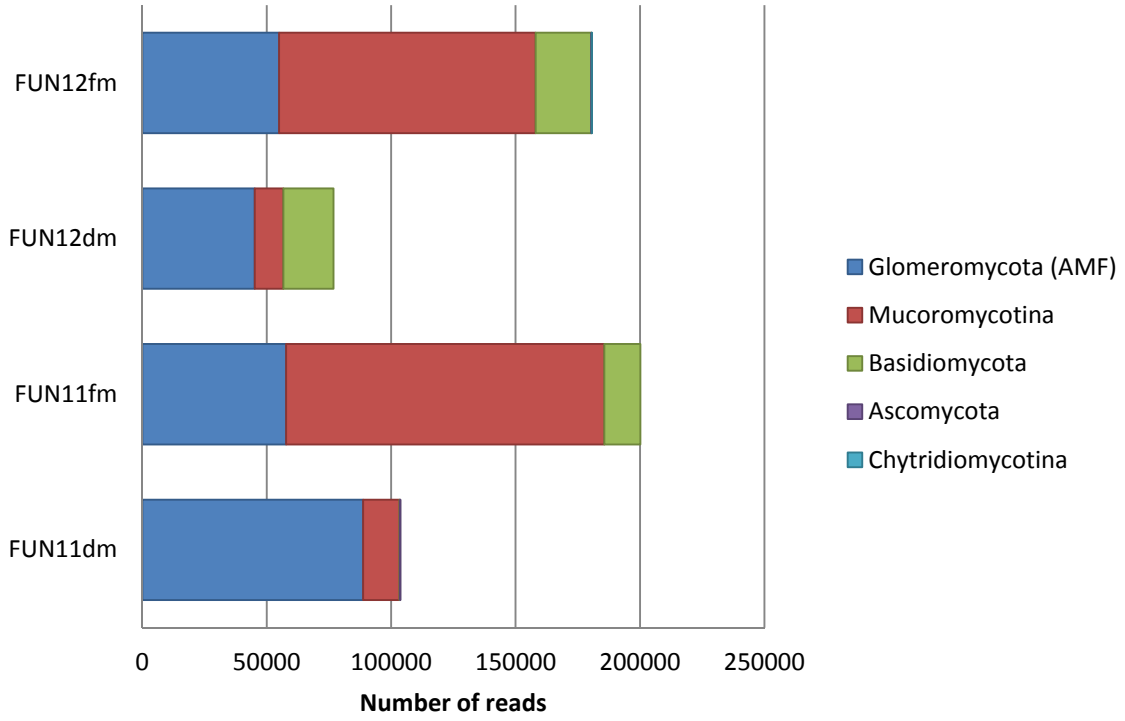

Figure 4.2.1. Taxonomic distribution of reads amplified with universal fungal primers AU2 and AU4 from four wheat root samples for Alchemy from Settrington, 2012. Sample identifiers can be decoded as follows: FUN = general fungal primers; 11 or 12 = core number; f or d = fresh or dried; m = middle section of core.

The most abundant non-AMF sequences were from Mucormycotina, especially *Mortierella*, but Basidiomycetes and Ascomycetes were also represented. When the relative abundance of AMF sequences amplified by the AU2-AU4 primers was compared to that obtained using AML1-AML2, there was generally a very good match (Figure 4.2.2a-d; sample identifiers can be decoded as follows: FUN = general fungal primers; AMF = AMF-specific primers; 11 or 12 = core number; f or d = fresh or dried; m = middle section of core), which is encouraging because it suggests that the primers are not introducing significant bias in the estimation of relative abundance.

The main exception (sample 11DM) involves two very similar *Glomus* sequences (VTX65 and VTX342) and may reflect an accident of cluster formation in MG-RAST that led to VTX65 being amalgamated into VTX342 in the analysis of the FUN11DM sample

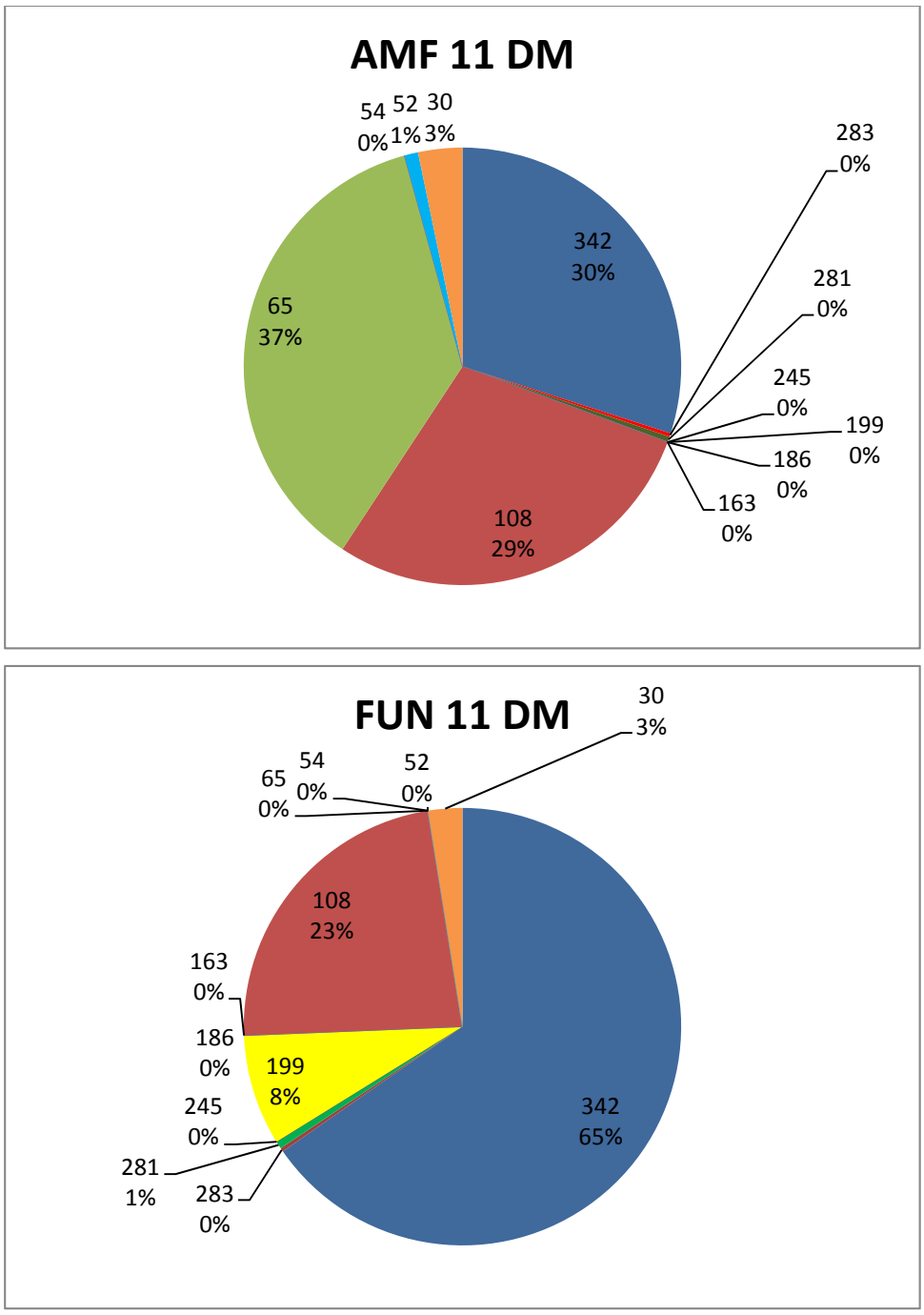

Figure 4.2.2a. Relative abundance of AMF taxa amplified with AMF-specific primers (top) or general fungal primers (bottom) from the same root DNA samples. The virtual taxon (VTX) numbers in the MaarjAM database are shown, together with the percentage of the sequence reads. The VTX numbers correspond to the following taxa: 30 (orange) *Acaulospora* Acau2 AF074346; 52 (light blue) *Scutellospora* MO-S2 AJ496115; 54 (purple) *Diversispora* sp. AJ315524; 65 (olive) *Glomus caledonium* Y17635; 108 (dark red) *Glomus* Whitfield type 7 AY330278; 163 (blue) *Glomus* MO-G25 AM849298; 186 *Glomus* Ligrone07-sp AM412533; 199 (yellow) *Glomus* MO-G7 AM849311; 245 *Archaeospora trappei* AJ006800; 281(green) *Paraglomus laccatum* AM295493; 283 (red) *Ambispora fennica* AM268193; 342 (dark blue) *Glomus VeGlo18* FN429114.

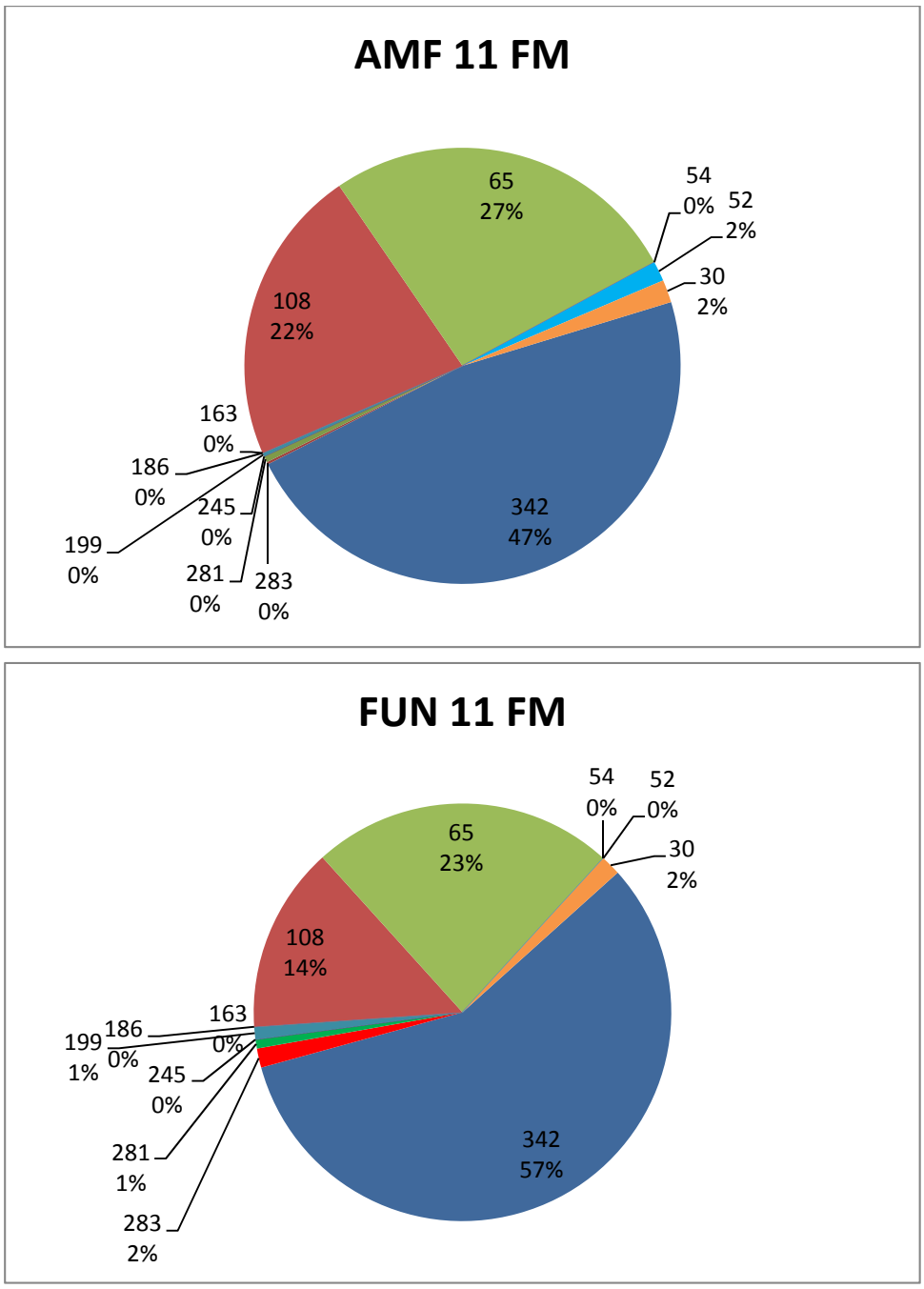

Figure 4.2.2b. Relative abundance of AMF taxa amplified with AMF-specific primers (top) or general fungal primers (bottom) from the same root DNA samples. The virtual taxon (VTX) numbers in the MaarjAM database are shown, together with the percentage of the sequence reads. The VTX numbers correspond to the following taxa: 30 (orange) *Acaulospora* Acau2 AF074346; 52 (light blue) *Scutellospora* MO-S2 AJ496115; 54 (purple) *Diversispora* sp. AJ315524; 65 (olive) *Glomus caledonium* Y17635; 108 (dark red) *Glomus* Whitfield type 7 AY330278; 163 (blue) *Glomus* MO-G25 AM849298; 186 *Glomus* Ligrone07-sp AM412533; 199 (yellow) *Glomus* MO-G7 AM849311; 245 *Archaeospora trappei* AJ006800; 281(green) *Paraglomus laccatum* AM295493; 283 (red) *Ambispora fennica* AM268193; 342 (dark blue) *Glomus VeGlo18* FN429114.

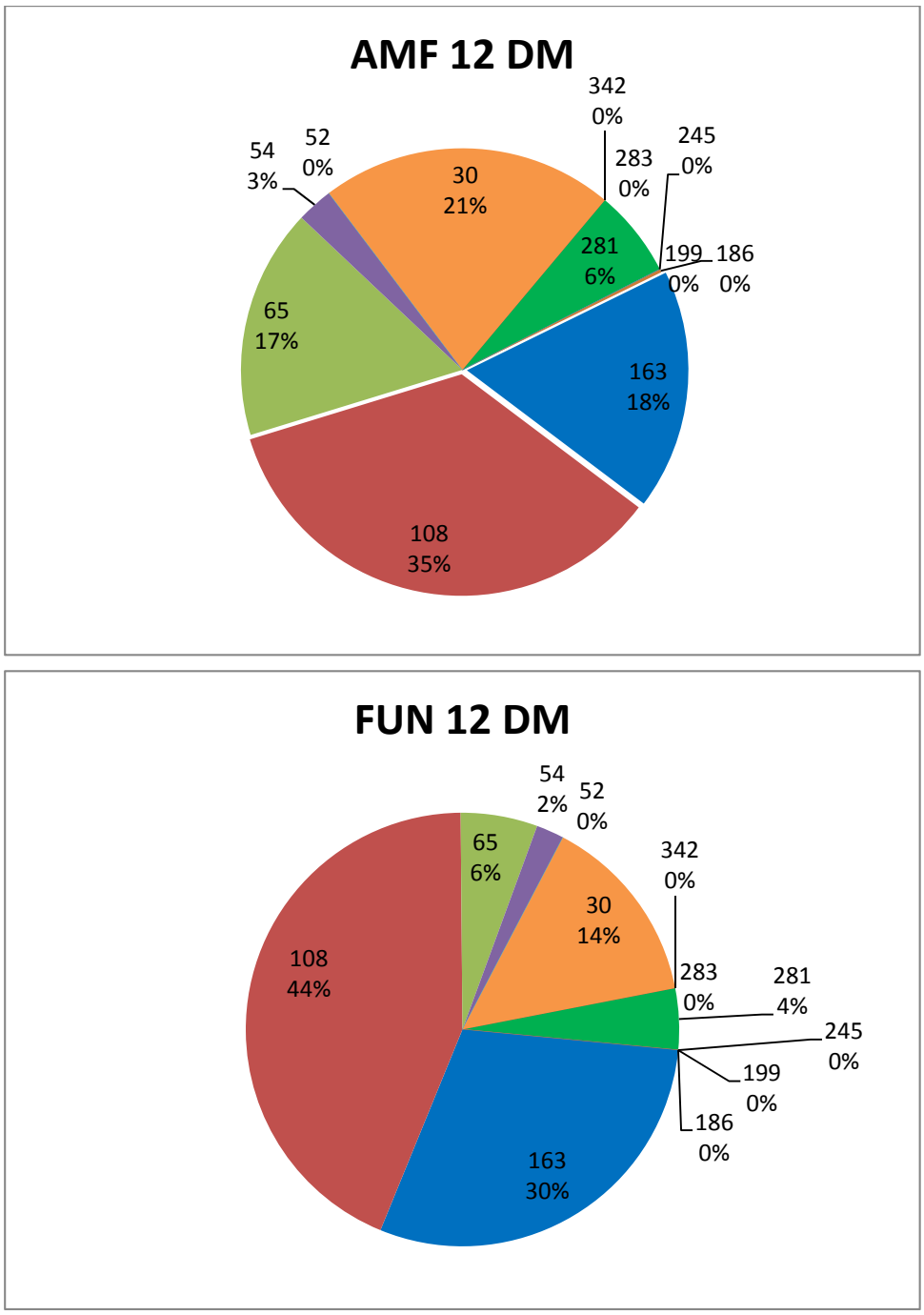

Figure 4.2.2c. Relative abundance of AMF taxa amplified with AMF-specific primers (top) or general fungal primers (bottom) from the same root DNA samples. The virtual taxon (VTX) numbers in the MaarjAM database are shown, together with the percentage of the sequence reads. The VTX numbers correspond to the following taxa: 30 (orange) *Acaulospora* Acau2 AF074346; 52 (light blue) *Scutellospora* MO-S2 AJ496115; 54 (purple) *Diversispora* sp. AJ315524; 65 (olive) *Glomus caledonium* Y17635; 108 (dark red) *Glomus* Whitfield type 7 AY330278; 163 (blue) *Glomus* MO-G25 AM849298; 186 *Glomus* Ligrone07-sp AM412533; 199 (yellow) *Glomus* MO-G7 AM849311; 245 *Archaeospora trappei* AJ006800; 281(green) *Paraglomus laccatum* AM295493; 283 (red) *Ambispora fennica* AM268193; 342 (dark blue) *Glomus VeGlo18* FN429114.

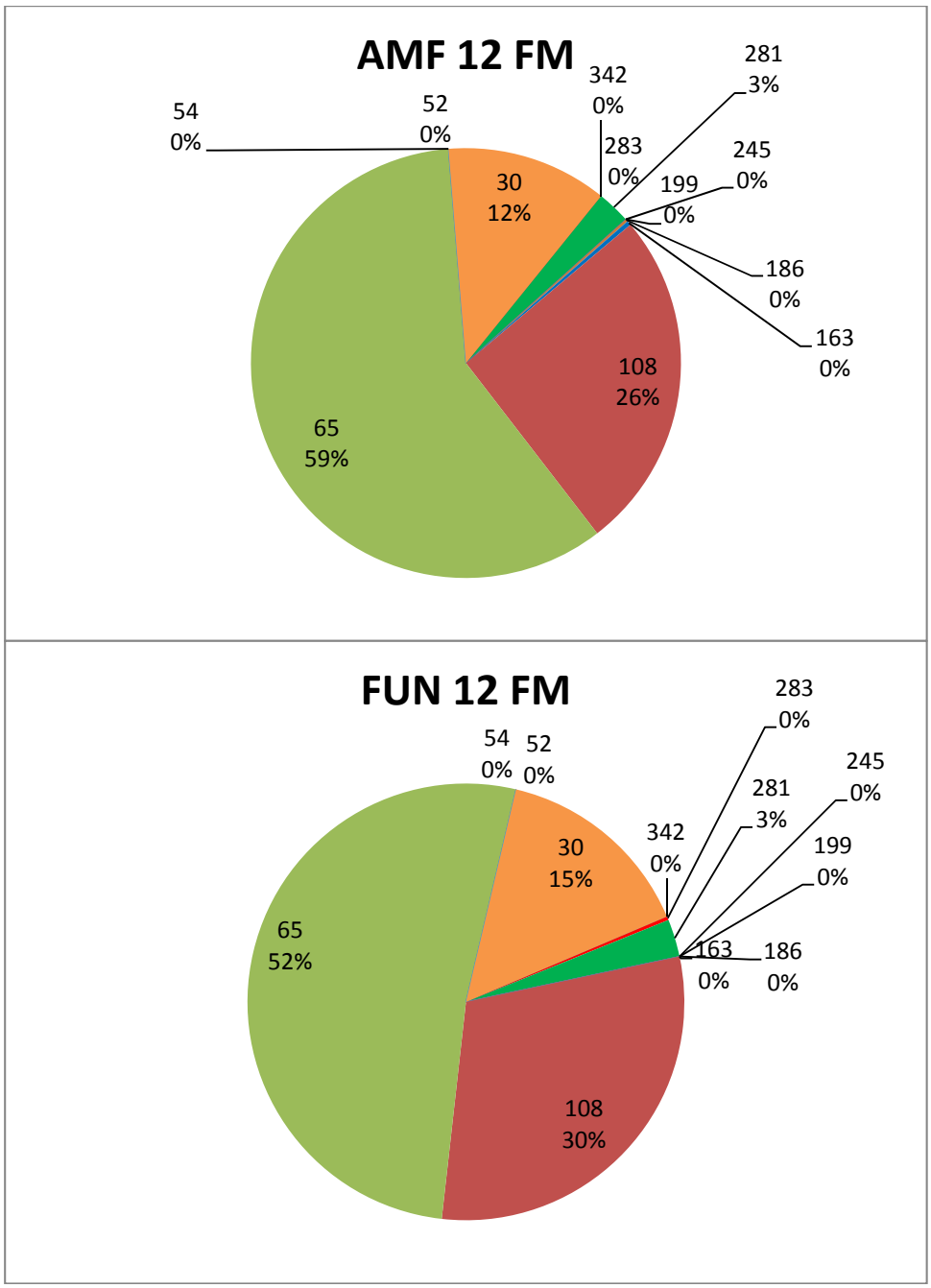

Figure 4.2.2d. Relative abundance of AMF taxa amplified with AMF-specific primers (top) or general fungal primers (bottom) from the same root DNA samples. The virtual taxon (VTX) numbers in the MaarjAM database are shown, together with the percentage of the sequence reads. The VTX numbers correspond to the following taxa: 30 (orange) *Acaulospora* Acau2 AF074346; 52 (light blue) *Scutellospora* MO-S2 AJ496115; 54 (purple) *Diversispora* sp. AJ315524; 65 (olive) *Glomus caledonium* Y17635; 108 (dark red) *Glomus* Whitfield type 7 AY330278; 163 (blue) *Glomus* MO-G25 AM849298; 186 *Glomus* Ligrone07-sp AM412533; 199 (yellow) *Glomus* MO-G7 AM849311; 245 *Archaeospora trappei* AJ006800; 281(green) *Paraglomus laccatum* AM295493; 283 (red) *Ambispora fennica* AM268193; 342 (dark blue) *Glomus VeGlo18* FN429114.

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4.2.2. Effects of cultivation and depth

Both root and soil samples were analysed from the STAR project, which compared ploughing and shallow cultivation. Two replicate plots of each treatment were used, with samples from four depths. The general fungal primers AU2-AU4 were used for all amplifications, which were successful for all samples except for soil from the deepest sections (75-100cm), where amplification was patchy so these samples were not taken further. There was considerable variation in the relative abundance of different fungal taxa (Figure 4.2.3), including the proportion that were *Glomeromycota*.

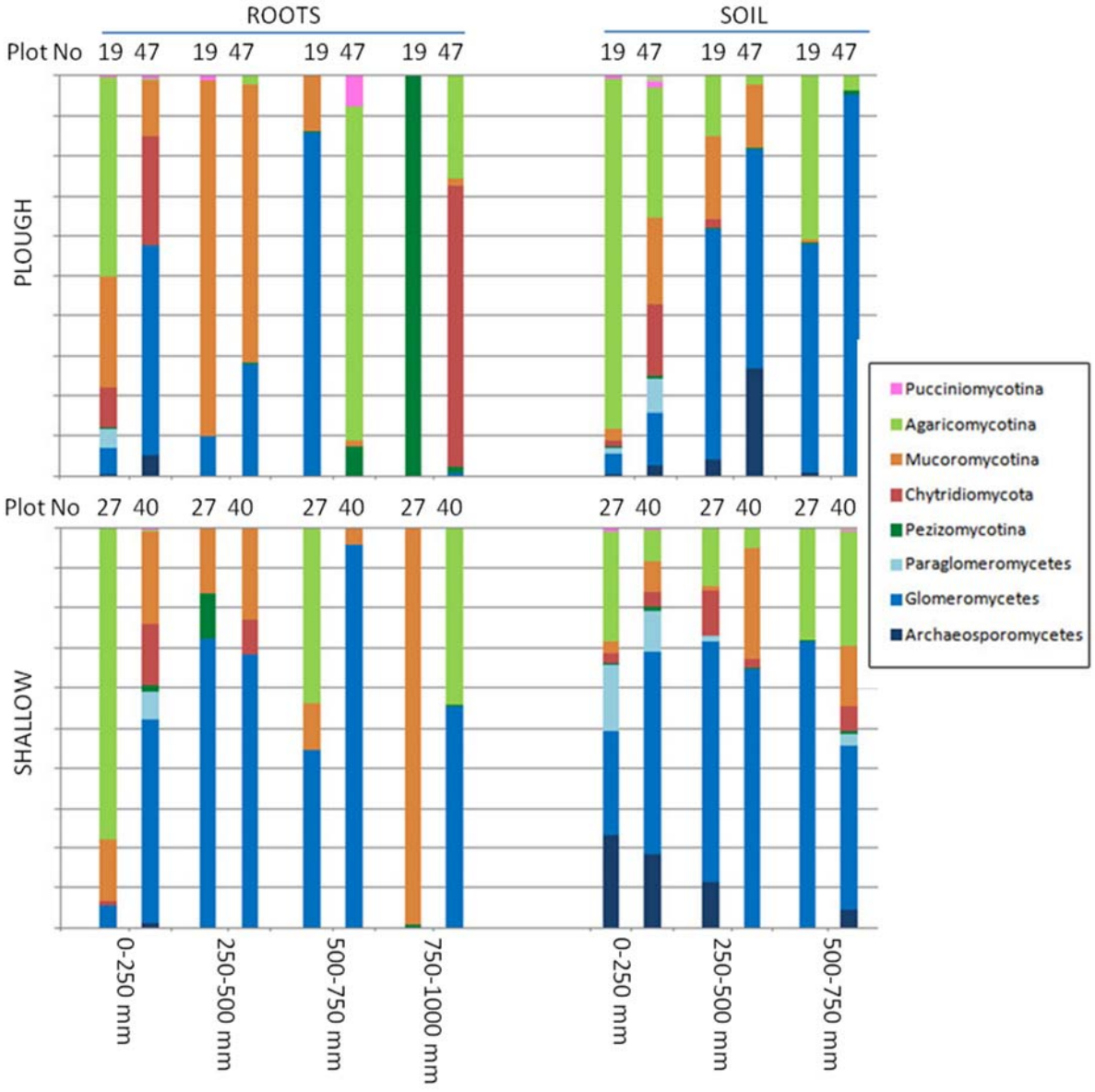

Figure 4.2.3. Depth profile of fungal diversity associated with cultivar Santiago in the 2013 STAR project from replicate plots 19 and 47 (ploughed), 27 and 40 (shallow cutivation). AMF fungi (*Glomeromycota*) comprise the three orders Paraglomeromycetes, Glomeromycetes and Archaeosporomycetes shown in blue.

A high diversity of AMF was detected in most samples (Figure 4.2.4).

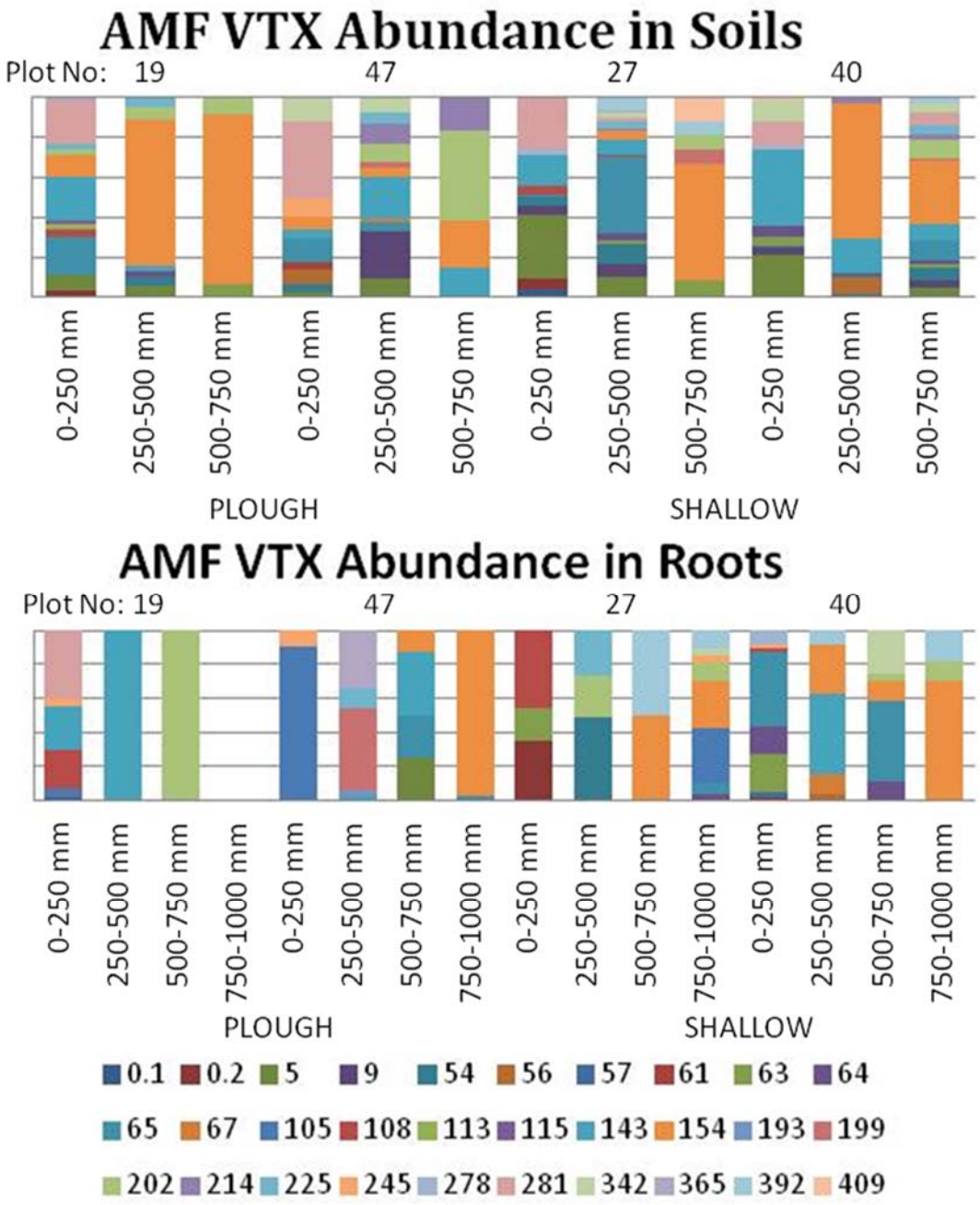

Figure 4.2.4. AMF taxa identified in the 2013 STAR project from plots 19 and 47 (ploughed), 27 and 40 (shallow cutivation). SSU sequences of AMF fungi were classified into virtual taxa (VTX) using the MaarjAM database.

The diversity was significantly lower in roots than in the surrounding soil, and lower in ploughed plots than in those with shallow cultivation (Figure 4.2.5).

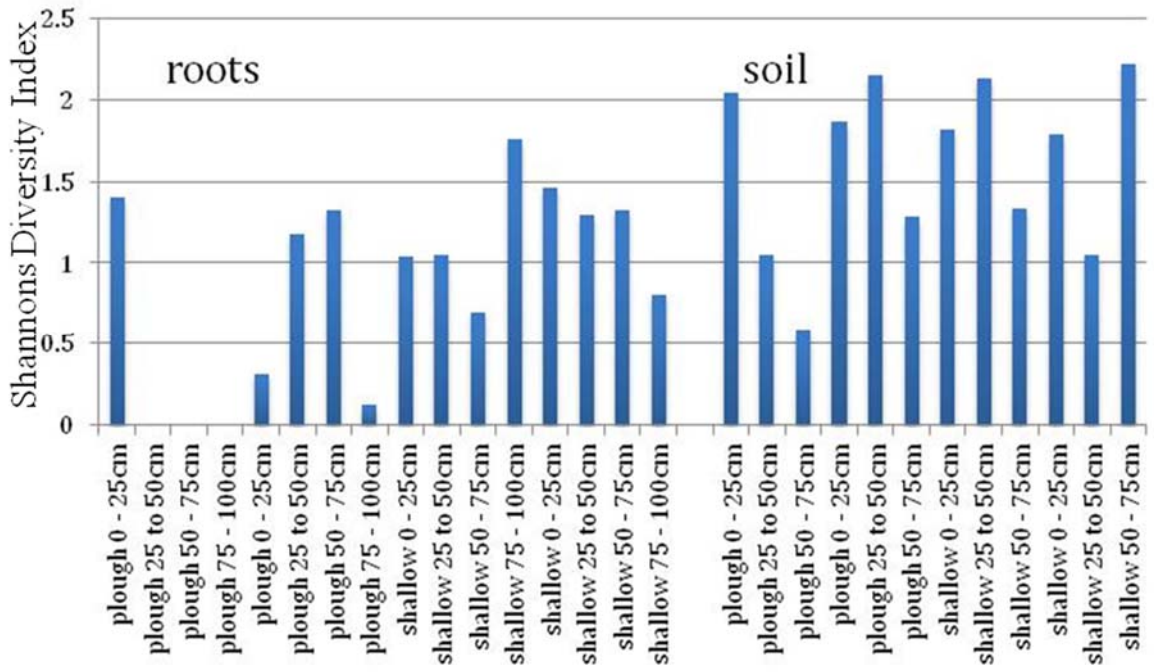

Figure 4.2.5. The diversity of AMF (expressed as Shannon Diversity Index shown on y-axis) in the 2013 STAR project, based on the taxa identified in Figure 4.2.4.

4.2.3. Effects of fungicide and nitrogen

Soil samples from the upper layer (0-25 cm) of the Morley NAC 2013 study were analysed using the AU2-AU4 primers. Replicated plots had fungicide treatment or not, nitrogen fertiliser or not (see Table 3.1.1 for treatment details and timings). The soil cores were taken at GS 51-65. The AMF communities differed greatly among plots, but there were no clear trends relating to the treatments (Figure 4.2.6).

4.2.4. Comparison of wheat varieties

The 2013 Stonham trial compared the AMF species diversity associated with three wheat varieties: Crusoe, Cordiale, JB Diego. Fungal sequences were amplified from roots and soil from two plots of each variety, see materials and methods sections 3.2.2 and 3.2.4 for details. There were large differences in the communities among plots, and differences in the species diversity of the AMF (Figure 4.2.7). There were no clear effects of wheat variety, but it appeared that the first replicates (lower plot numbers, lighter colours in Figure 4.2.7) had higher diversity, both in soil and roots.

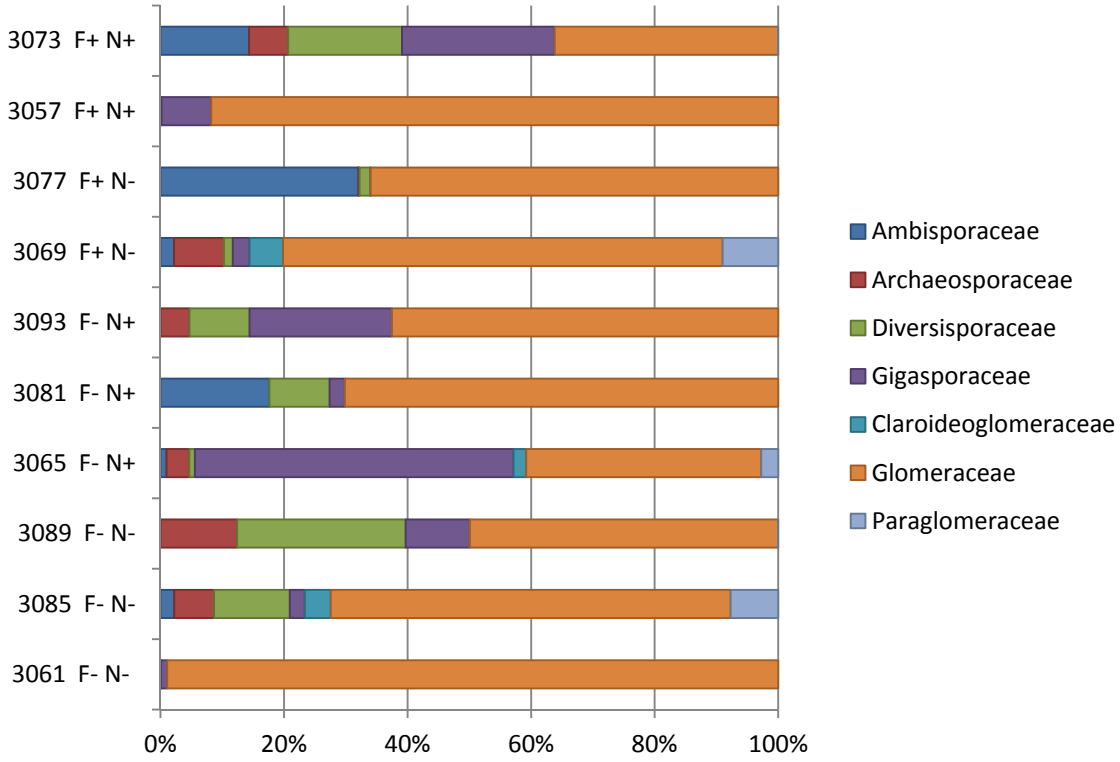

Figure 4.2.6. The diversity of AMF families in soils from the Morley NAC study 2013. F-: no fungicide (3 replicate plots, numbers 3061 etc.); F+: with fungicide (2 replicate plots); N-: no added nitrogen; N+: 320 kg.ha^{-1} added nitrogen. Note that variation among replicates is as great as between treatments.

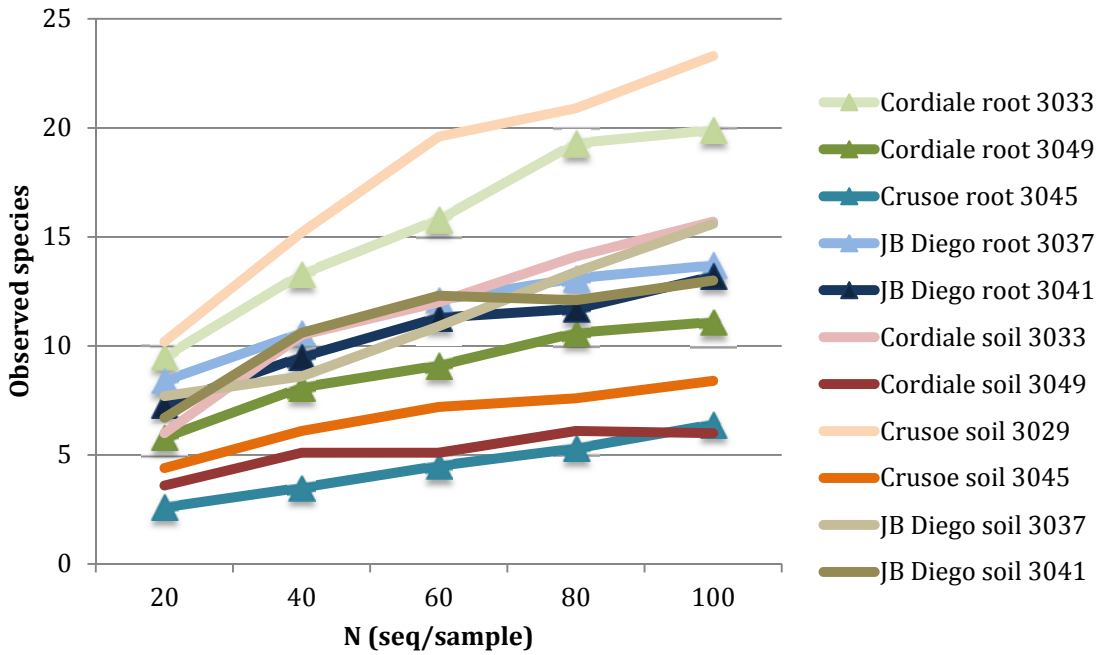

Figure 4.2.7. AMF species diversity in the Stonham NTN variety trial 2013. Differences in alpha diversity are illustrated using the multiple rarefactions method of QIIME.

4.3. Dissecting root traits at key development stages

4.3.1. Seedling roots

Significant differences in early seedling root traits were found between varieties grown on filter paper (Table 4.3.1).

Table 4.3.1. Seedling assay – mean trait parameter values

Variety	Individual root length		Total Root length (cm)	Longest root (cm)	Number of seminal roots
	Mean (cm)	Standard deviation			
Alchemy	6.52	2.53	27.69	9.23	4.33
Avalon	6.68	2.29	21.54	8.85	3.20
Beaver	6.36	2.91	23.84	9.34	3.83
Buster	4.63	2.46	21.29	7.49	4.33
Cadenza	6.42	3.4	32.12	10.27	5.00
Cappelle Deprez	5.63	3.46	24.98	8.63	4.33
Glasgow	6.72	2.63	25.59	8.97	3.60
Glenson	9.9	2.02	29.71	12.13	3.00
Hereward	9.71	1.48	29.14	11.34	3.00
Mercia	7.74	2.08	25.81	9.71	3.40
Mercia-Rht-D1b	8.36	1.90	25.08	10.49	3.00
Mercia-Rht8	6.03	1.38	17.68	7.27	2.67
Norman	6.09	2.46	22.31	8.68	3.83
Oakley	6.98	1.98	20.94	8.97	3.00
Paragon	8.03	2.22	22.5	9.9	2.83
Rialto	6.65	1.61	20.64	8.21	3.17
Robigus	6.67	2.37	22.9	9.04	3.50
Savannah	6.91	2.75	23.82	9.68	3.50
SHW-173	5.26	2.10	15.78	7.47	3.00
Soissons	6.85	3.45	28.31	10.25	4.17
Spark	6.78	2.49	23.21	9.61	3.67
Xi19	6.00	2.88	22.81	8.65	3.75
XS-218>18-1	6.93	2.77	23.95	9.76	3.50
XS-218>19-5	5.72	2.41	20.72	8.63	4.00

Seedling Root Analysis - Effect of variety	F	P
Individual root length (T)	1.8762	0.02142
Mean Individual root length	1.703	0.033
Standard deviation of individual root length	1.6044	0.05241
Longest root	1.106	0.3601
Number of seminal roots	3.5243	P<0.001
Total Root length(T)	1.5424	0.06859

Twenty-four varieties were tested and, a summary of the average parameter values for each variety can be found in Table 4.3.1. Variety Glenson had the longest average individual root length (9.9cm) with Buster producing the shortest average individual root length (4.6cm). Mercia-Rht8 produced the smallest number of seminal roots (mean of 2.7 per seedling) compared with Cadenza that produced approximately double the number of seminal roots (mean of 5 per seedling). Overall the combined root length and number of seminal roots resulted in Cadenza producing the highest total root length (at 32cm per seedling) versus SHW-173. No varieties that appeared in the top 30% of the mean length of seminal roots produced appeared in the top 30% of varieties ranked by the number of seminal roots that were produced, suggesting a trade-off between number of seminal roots and rate of root elongation at this early stage of growth (Figure 4.3.1).

There was also evidence that some varieties produced roots of more consistent length, whereas other varieties produced a range of root lengths; this was indicated by the fact that the standard deviation of the variation in root length within individual plants was significantly different for variety and this did not correlate with the average root length of the plant (r^2 across all varieties 0.15). For example Rialto had a mean root length of 6.6cm with a standard deviation of 1.6cm compared with Soissons with a mean root length of 6.8 cm but with a standard deviation of root length of 3.4 suggesting a much greater variation in root behaviours in the latter variety.

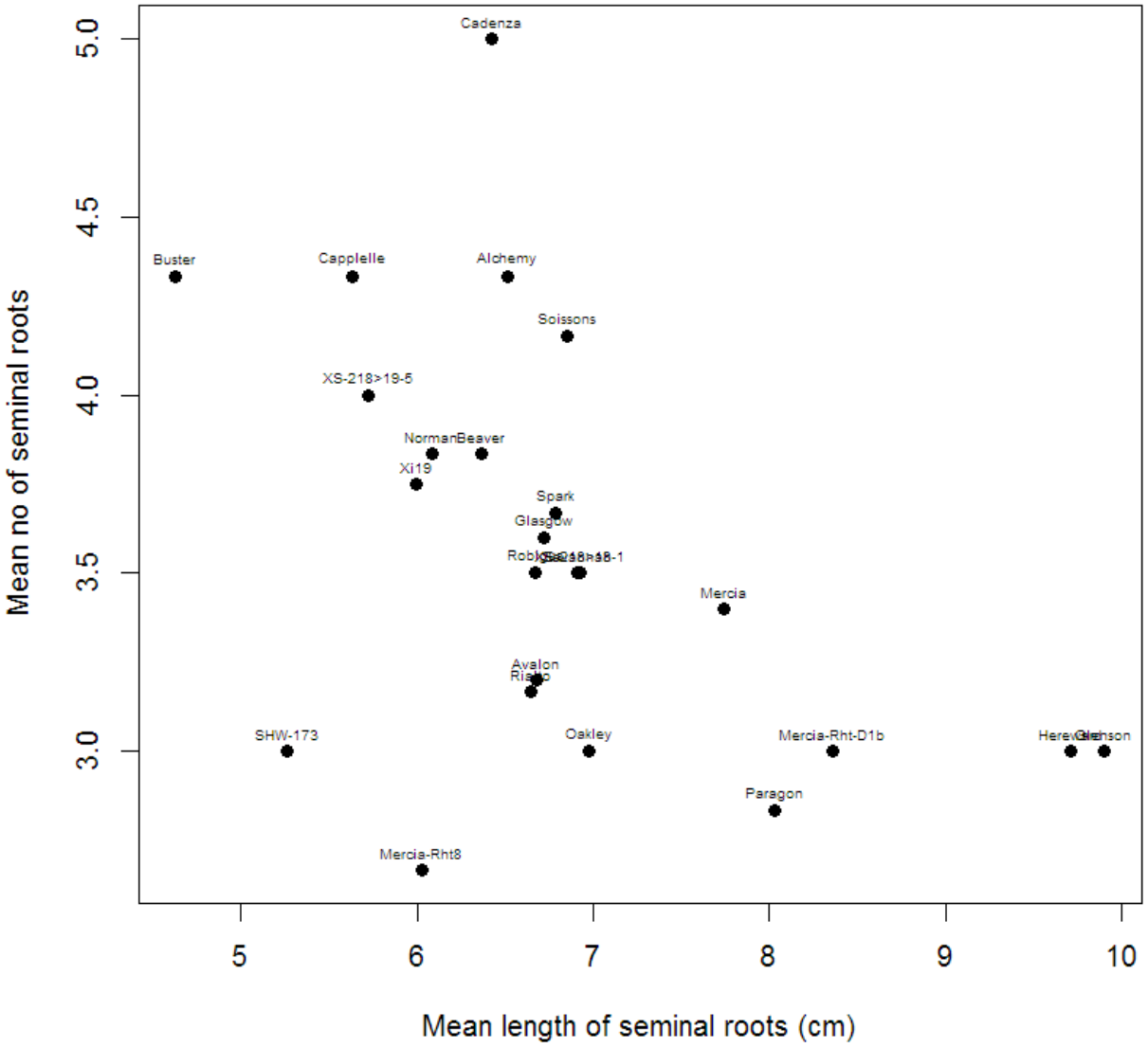

Figure 4.3.1: Wheat root phenotypes at 7 days post germination. Root phenotypes of seedlings grown on filter paper in a scanner bank as detailed in Adu *et al.* (2014). Variety means are shown for average length of seminal roots per individual plant and the number of seminal roots per plant.

4.3.2. Glasshouse grown plants

Differences in plant growth and development due to variety, growth stage or treatment were also found at later growth stages. This is illustrated by the principal component analysis of all 12 root sections for all rhizotube experiments shown in Figure 4.3.2.

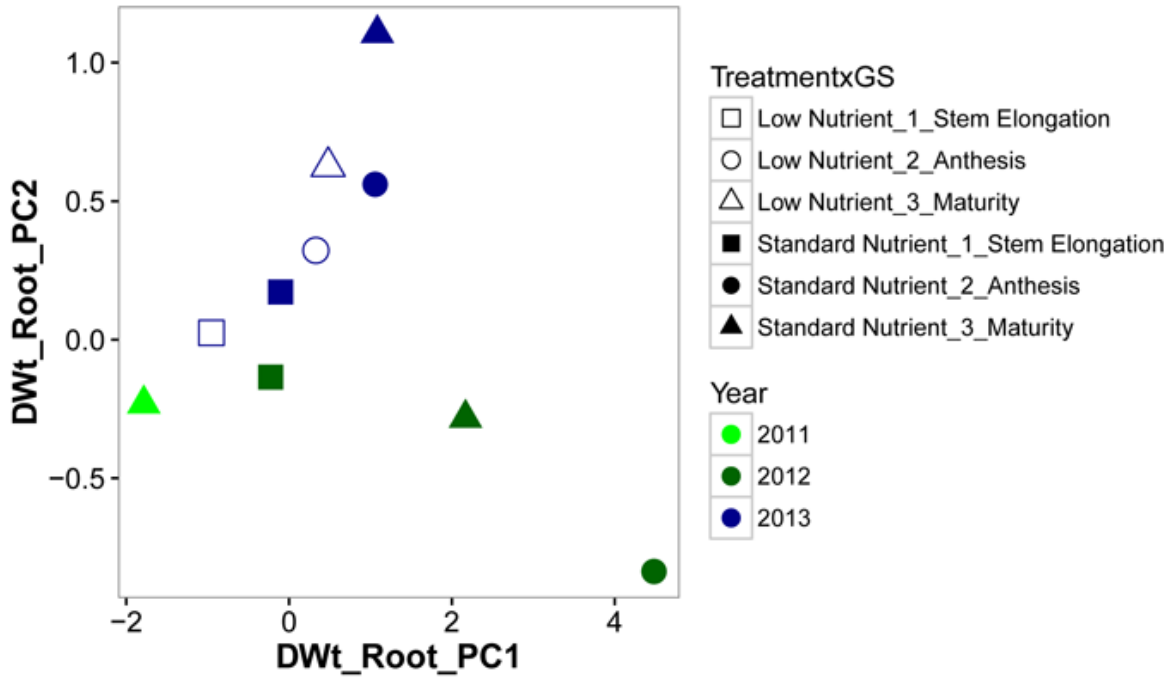

Figure 4.3.2: Principal component analysis of greenhouse grown plants. Principal component analysis of the dry root mass found in each rhizotube section, for all experiments. Only the varieties that were common to each experiment (i.e. 15 Varieties) were included. PC1 and PC2 data points are averaged for all varieties for each harvest growth stage by treatment for each experiment. Colour coding = Experiments: Light green = 2011 (maturity only), Dark green = 2012, Blue = 2013. Shape coding = Harvest Growth Stage, square = Stem elongation, circle = Anthesis, triangle = Maturity. Fill/Unfilled = Nutrient Treatment: Filled = Standard Nutrient treatment, Unfilled = Low Nitrogen Nutrient treatment.

The points shown are the averages of the varieties that were common to all experiments and the figure shows the shift in PC1 (typically represented some elements of total root size) and PC2 (typically represents elements of root depth balance i.e. root shape) in response to experiment year, growth stage within each experiment and treatment in the 2013 experiment.

The length of the growing season for each of the experiments varied with 2012 having a longer growing season than 2013, with 2011 having the shortest growing season. This resulted in a higher overall value of PC1 in 2012 for the root PCA (Figure 4.3.2) even at maturity, compared with 2013 and the shortest season being experienced by the plants in 2011. This was partly due to external climactic conditions, glasshouse temperature and light control, and the sowing dates in each year. Despite this in a comparison of experimental years 2012 and 2013 (standard N only treatment), significant effects of year, variety, and growth stage on root traits were demonstrated. This is illustrated by the variation in DWt_Root_PC_1 shown in Figure 4.3.2.2.

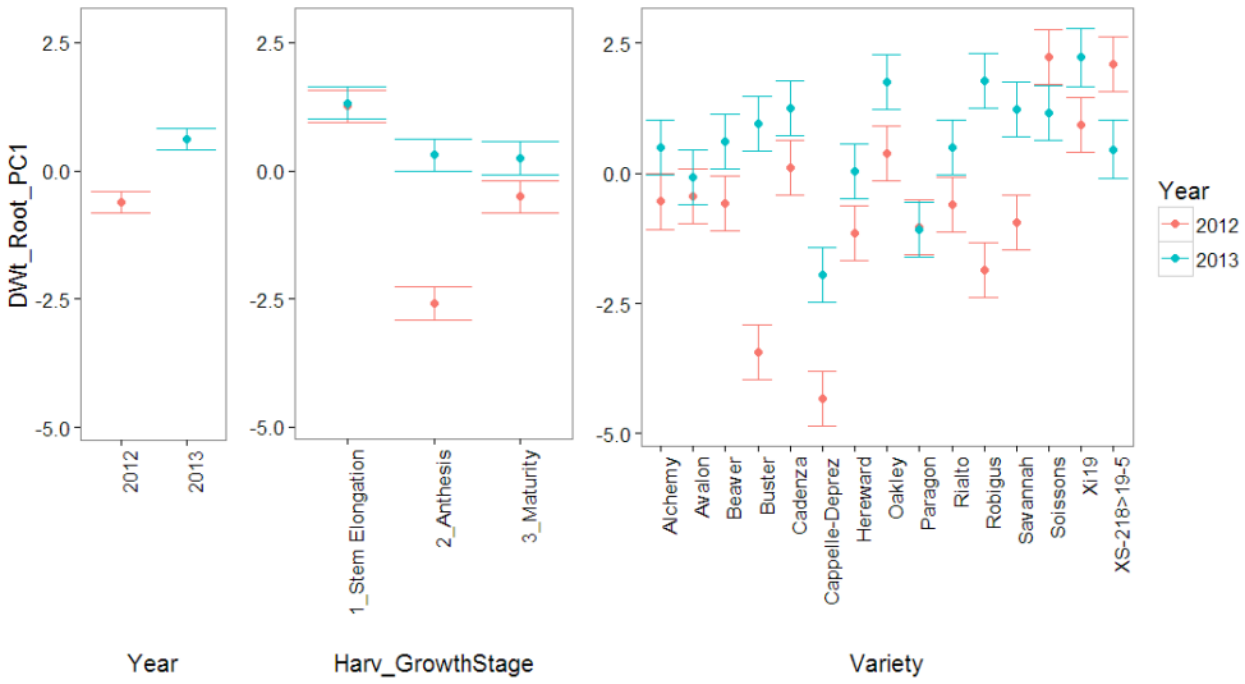

Figure 4.3.2.2 Effect of Year, Year : Harvest growth stage and Year: Variety on DWt_Root_PC1. REML analysis – Year (Y, p<0.001, F= 45.6), Harvest growth stage (HGS p<0.001, F=37.0), Variety (V p<0.001, F=12.27), Y:HGS (P<0.001, F= 12.50), Y:V (p < 0.001, F= 4.83), HGS:V (p<0.001, F=2.16), Y:HGS,V (p<0.001, F=1.60)

4.3.3. Evaluation of the effect of variety and harvest growth stage on 25 wheat varieties

Many different parameters were measured during the growth of the plants, therefore not all the effects on individual parameters are given in detail. Figure 4.3.3 gives an overview of the significant interactions found for a sample of the parameters measured.

Figure 4.3.3: Statistical analysis of plant growth and development parameters for 2012 experiment REML analysis for each plant growth and development parameter for the 2012 experiment, showing responses to greenhouse position, harvest growth stage, and variety. The colour coding indicates where significant effects were observed (i.e. Dark green p<0.001, mid green p<0.01, light green p<=0.05.

Root and shoot biomass varied significantly with both variety (p<0.001, p<0.001) and growth stage (p<0.001, p< 0.001) but there was limited evidence of an interaction between variety and harvest growth stage (p=0.07, p=0.271). Figures 4.3.4 and 4.3.5 summarise the plant mass fraction data obtained. Shoot mass was highest at maturity, whereas root mass was highest at anthesis. The highest average shoot biomass was produced by Hereward, Rialto, Cappelle-Deprez and Beaver (all producing approximately 11.5 g shoot biomass per plant). The lowest overall shoot biomass was produced by SHW-173 at 2.27g per plant). The highest average root biomass was produced by variety Cappelle-Deprez at 1.52 g root biomass per plant in contrast to the lowest root mass produced by variety SHW-173 at 0.167g per plant, followed closely by Glenson at 0.31g per plant. The overall height of the bars represents that total biomass, whereas the size of each single coloured section represents the contribution of that section to the overall biomass.

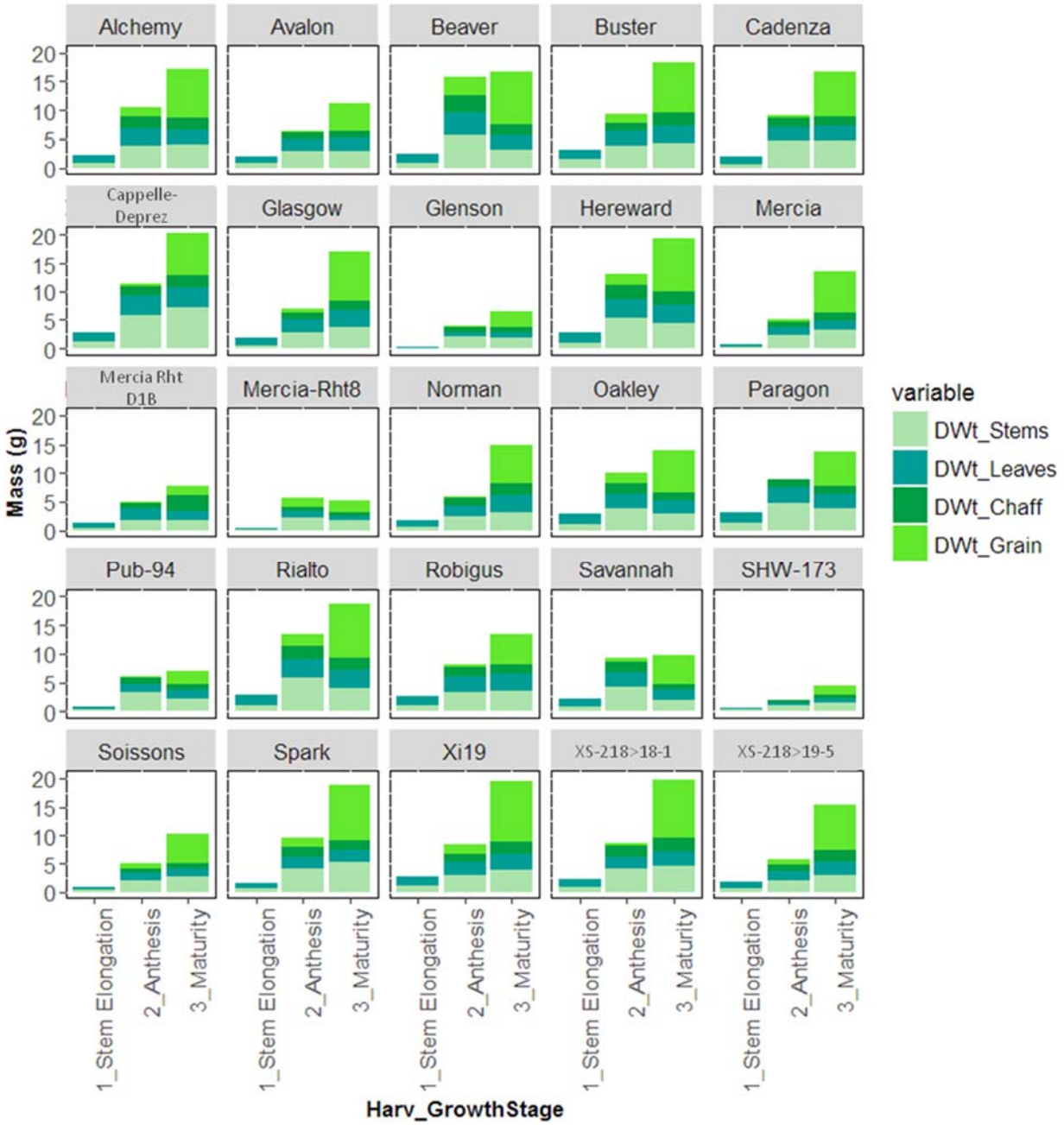

Figure 4.3.4: Shoot mass accumulation by wheat varieties in 2012 experiment. Shoot dry mass partitioning in 25 wheat varieties grown in rhizotubes with standard nutrient feed at the three different stages of growth when the plants were destructively harvested. Total shoot biomass is represented by the height of the bars. Individual colour sections represent the proportion of total shoot biomass contributed by each of the 4 shoot sections. Grain includes immature seeds.

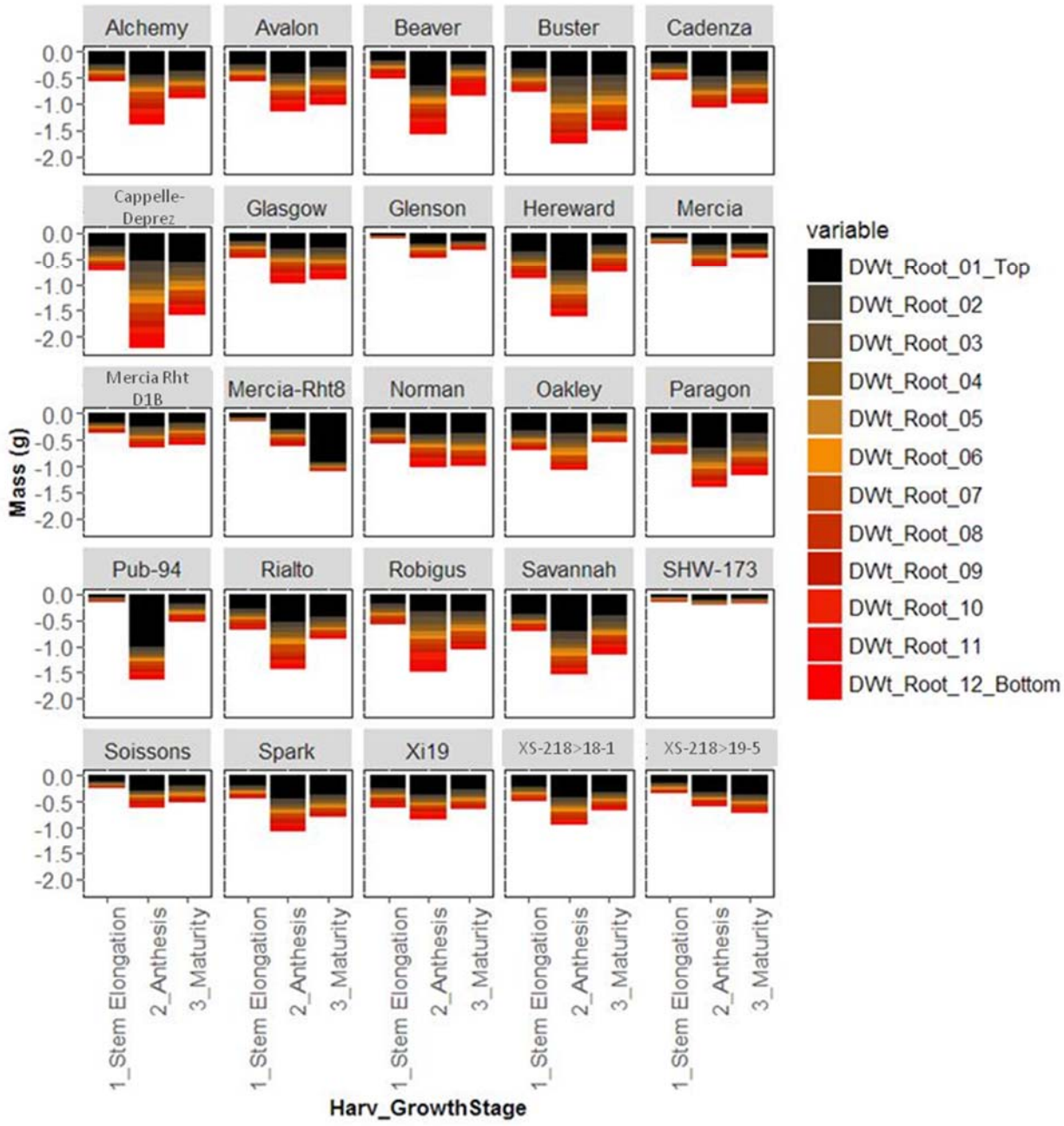

Figure 4.3.5: Root mass accumulation by wheat varieties in 2012 experiment. Root dry mass partitioning in 25 wheat varieties grown in rhizotubes with standard nutrient feed at the three different stages of growth when the plants were destructively harvested. Total root biomass is represented by the height of the bars. Individual colour sections represent the proportion of total root biomass contributed by each of the 12 root sections.

Root systems can also vary by shape as well as size. To assess potential differencing in roots between varieties, both differences between the individual depth fractions, and the principal components of the 12 root fractions were assessed. All root sections showed significant differences between varieties and growth stage. Only Root fraction 3 (10cm-15cm depth) showed evidence of a variety x growth stage interaction ($p=0.004$). However, analysis by principal component analysis revealed variety effects for PC1 ($p<0.001$), PC2 ($p=0.009$), PC6 ($p=0.011$) PC11 ($p=0.020$). Harvest growth stage significantly affected PC1 ($p<0.001$), PC3 ($p = 0.013$, associated with bulking out of

the root from approximately 10-30cm in depth usually at anthesis). Only PC1 showed a significant interaction (p=0.005).

Significant variety effects were also found for Stem %N (p=0.003, highest Savanah 2.16%, lowest spark 0.31%), Stem total N(p<0.001, highest Cappelle-Desprez 0.06 g per plant, lowest SHW-173 0.009 g per plant), Leaf %N (p=0.048 highest Pub-94 2.52, lowest X218>18-1 0.52%) and Leaf total N (p<0.001 highest Buster 0.065 g per plant, lowest X218>18-1 0.012 g per plant), Grain % N (p = 0.003, highest SHW-172 2.78%, lowest Savannah 1.54%), Grain total N (p<0.001, highest Spark 0.22 g per plant lowest Mercia-Rht-D1b 0.03 g per plant).

4.3.4. Evaluation of the effect of Nitrogen treatments on 15 varieties at 3 different growth stages

Experimental year 2013 was designed to extract differences in plant behaviour to reduced nitrogen supply. Significant effects of variety, growth stage and nutrient interactions were found for root behaviour (Figure 4.3.6).

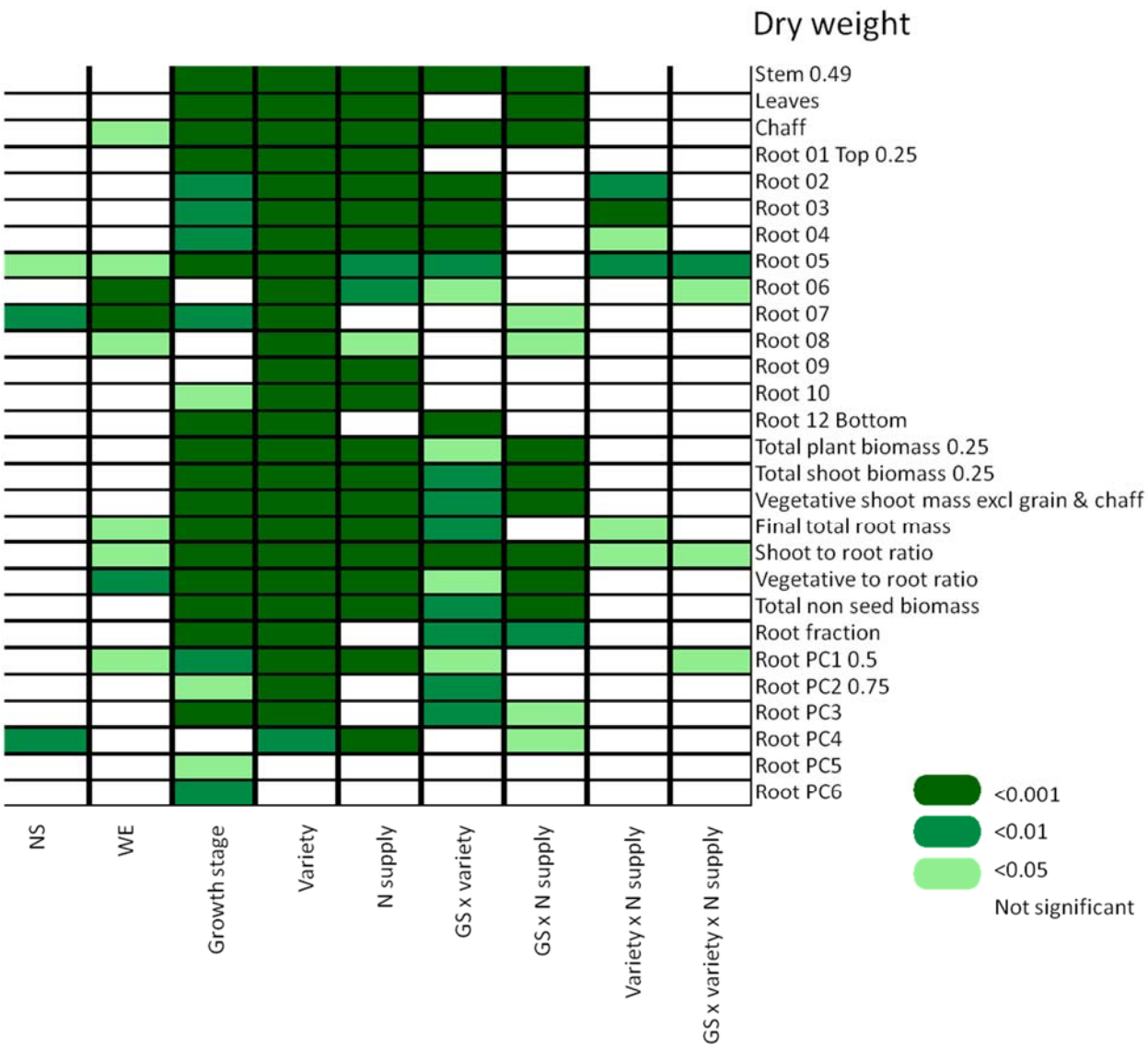

Figure 4.3.6: Statistical analysis of plant growth and development parameters for 2013 experiment. REML analysis for each plant growth and development parameter for the 2013 experiment in response to greenhouse position, harvest growth stage, variety, and nitrogen supply. The colour coding indicates where significant effects were observed (i.e. Dark green p<0.001, mid green p<0.01, light green p<=0.05.

Changes in plant biomass are shown in Figures 4.3.7 and 4.3.8.

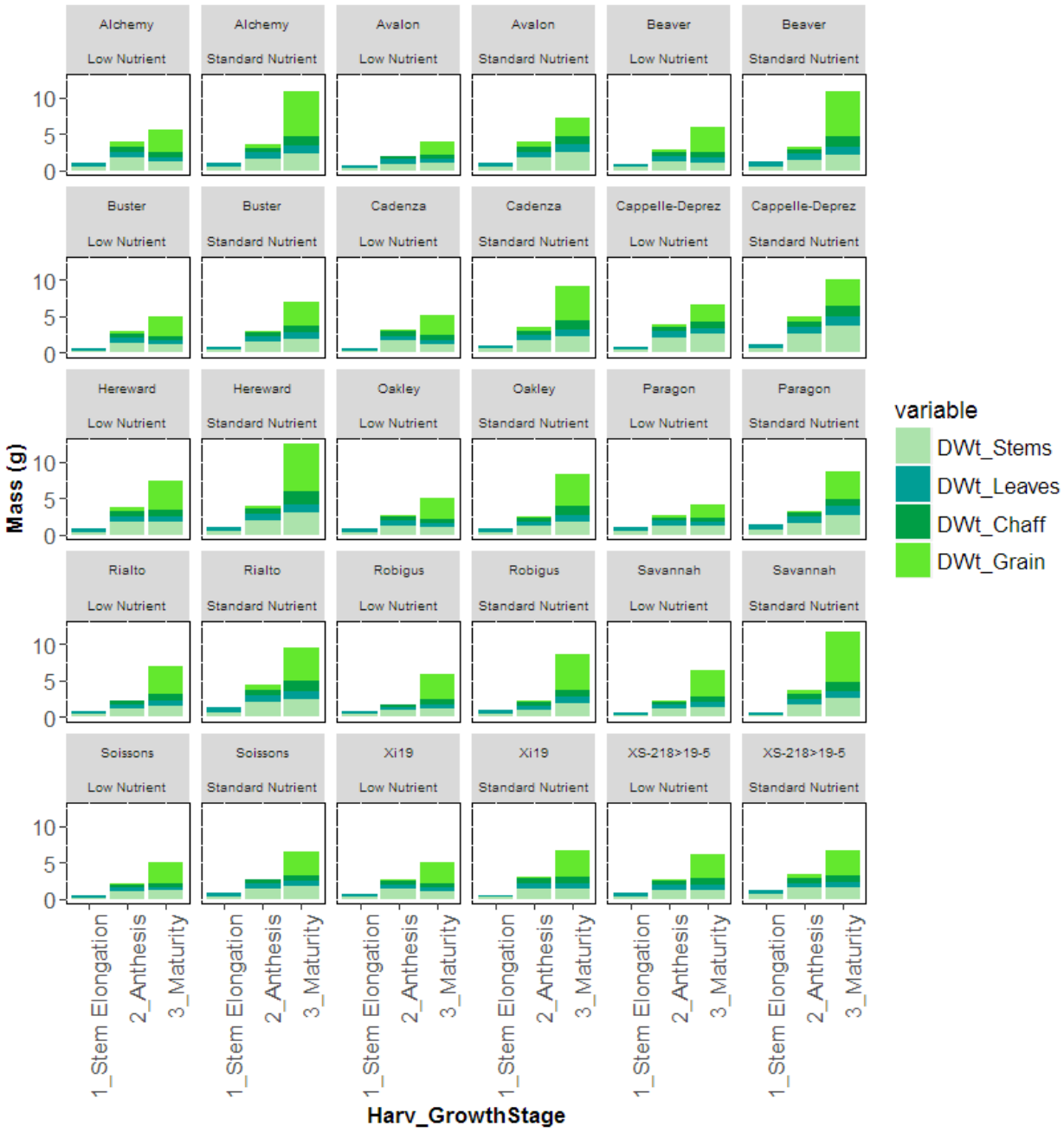

Figure 4.3.7 Shoot mass accumulation by wheat varieties in 2013 experiment. Shoot dry mass partitioning in 15 wheat varieties grown in rhizotubes with standard nitrogen or reduced nitrogen nutrient feed at the three different stages of growth when the plants were destructively harvested. Total shoot biomass is represented by the height of the bars. Individual colour sections represent the proportion of total shoot biomass contributed by each of the 4 shoot sections. Grain includes immature seeds.

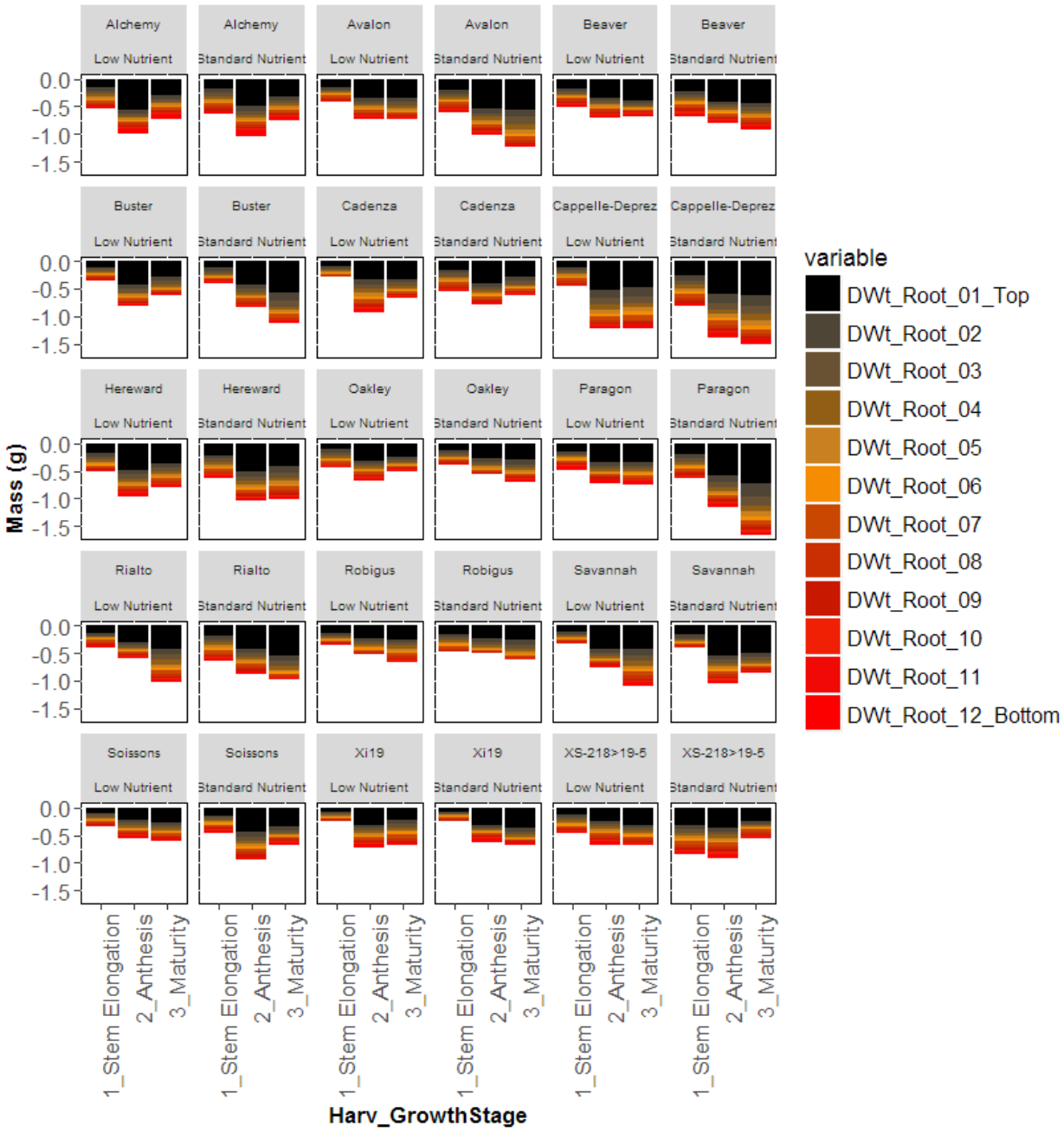

Figure 4.3.8: Root mass accumulation by wheat varieties in 2013 experiment. Root dry mass partitioning in 15 wheat varieties grown in rhizotubes with standard nitrogen or low nitrogen nutrient feed at the three different stages of growth when the plants were destructively harvested. Total root biomass is represented by the height of the bars. Individual colour sections represent the proportion of total root biomass contributed by each of the 12 root sections.

Reducing the supply reduced the overall shoot biomass produced from 4.4g to 3.04g per plant ($p<0.001$) and the root biomass from 0.80g to 0.65g per plant ($p<0.001$). There were no significant interactions between treatment and varieties for shoot biomass (or its constitutive parts) on standard vs low nitrogen supply ($p = 0.42$) i.e. if the plants produced high shoot biomass on standard nitrogen then they produced high shoot biomass on low nitrogen. However, there was a significant interaction for root biomass between varieties and treatment ($p<0.018$). While overall the root biomass was smaller in the low nutrient treatments, varieties Alchemy, Cadenza, Oakley, Savannah and Xi19

were able to maintain a similar root biomass in the low nitrogen treatment to that produced in standard nitrogen treatments. Avalon, Paragon and Cappelle-Desprez suffered particularly in terms of root biomass in response to the low nitrogen treatment (See Figure 4.3.8). The differences in root behaviour were also explored further using principal components analysis and examining the individual section behaviour. Significant interactions between variety and nitrogen supply were found for PC1 as an interaction with harvest growth stage and for root sections 2, 3, 4, 5 and with sections 5 and 6 as an interaction with harvest growth stage). There were also significant differences between treatment and variety for H1-PC3 ($p=0.034$). This is likely to be related to changes in growth of the root system around the middle 10-30cm depth since PC3 is usually associated with this area of the root system. Figure 4.3.9 shows the variation in root mass found between the varieties for the first two root principal components. The interaction between variety, treatment and growth stage was also significant for shoot to root ratio ($p<0.045$).

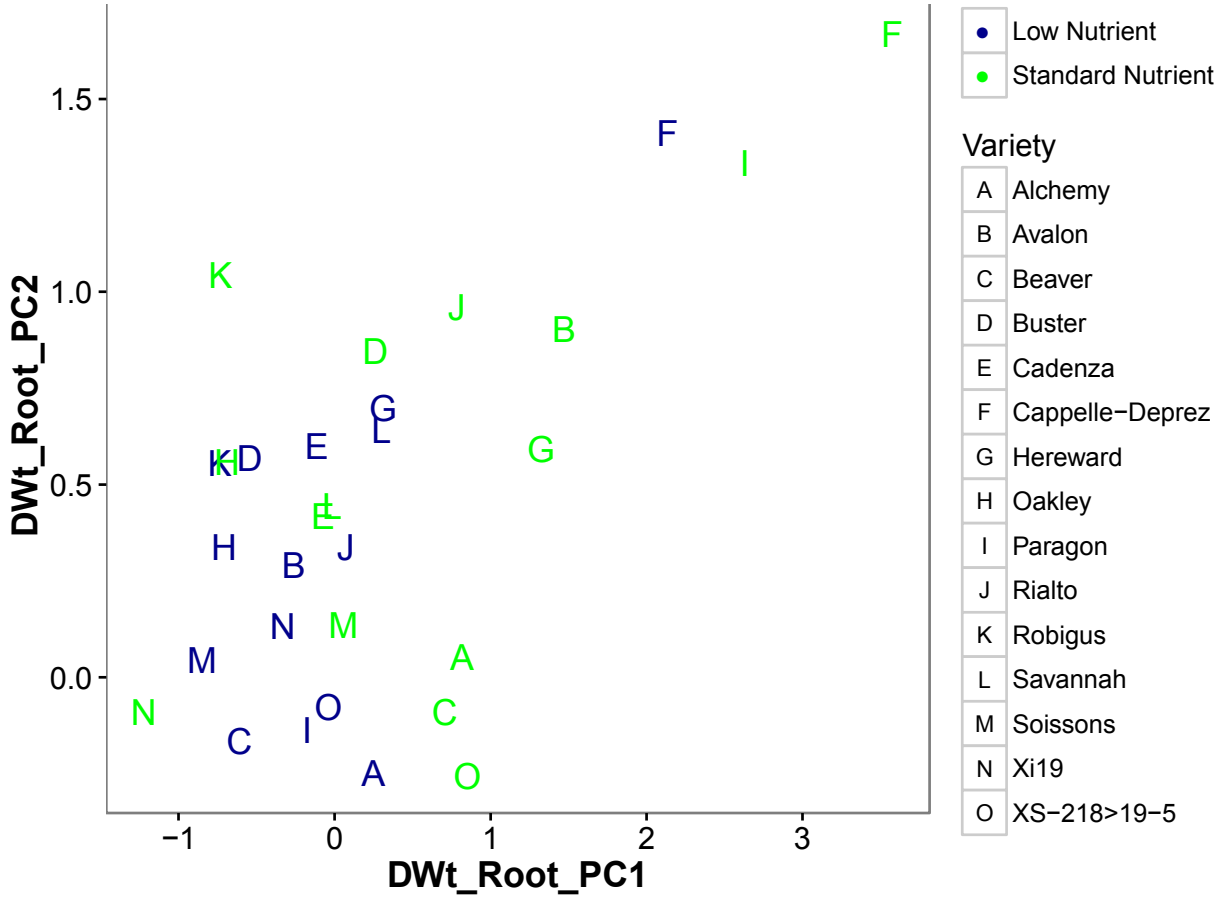

Figure 4.3.9 Principal components analysis of root sections in the 2013 experiment. Principal components analysis of the root dry mass partitioning in 15 wheat varieties grown in rhizotubes with standard nitrogen or low nitrogen nutrient feed at the three different stages of growth when the plants were destructively harvested. Colour coding = Nutrient treatment: blue low nitrogen nutrient treatment, green = standard nitrogen nutrient treatment. Letters represent different varieties. The larger the distance between same letter the larger the effect of the nitrogen treatment on the root system.

Differences in PC1 were significant for Harvest Growth Stage ($P<0.01$), Variety ($p<0.001$), Treatment ($p<0.001$), H x V ($p = 0.021$), and HxVxT ($p = 0.047$) but not for HxT ($p = 0.432$) or VxT ($p = 0.090$. PC2 was significant for Harvest Growth Stage ($p = 0.024$), Variety $p<0.001$), HxV ($p = 0.008$).

Examples of varieties with root systems that were more responsive vs. less responsive to N treatment are given in Figure 4.3.10. For example, Beaver increased the root mass across most of its root system in response to a higher N supply, whereas Oakley was found to have limited response in root size to increased N supply (Figure 4.3.10 Top). Similarly, overall root shape could change. Figure 4.3.10 Bottom shows two varieties that responded differently in this respect, with Robigus increasing root mass in the deeper sections in response to low Nitrogen supply, compared with the proliferation pattern found in the standard nitrogen treatment, whereas Paragon decreased root mass in shallower sections of the profile, in response to the reduced nitrogen in the nutrient feed.

Percentage N and total N were also measured in the shoot partitions of the final harvest of the 2013 experiment. The nitrogen nutrient treatment significantly affected the % N in the grain ($p=0.004$) and the total N in the grain ($p=0.015$), but not the stem or leaf percentage N or total N. There was also a significant interaction between variety and Nitrogen nutrient treatment for grain %N ($p=0.014$). Varieties Alchemy, Beaver, Rialto, Soissons and XS-218>19-5 had significantly higher %N in the grain in the standard nitrogen treatment than in the low nitrogen treatment, whereas, Savannah and Xi19 had higher %N in the grain in the low nitrogen treatments than in the standard nitrogen treatment.

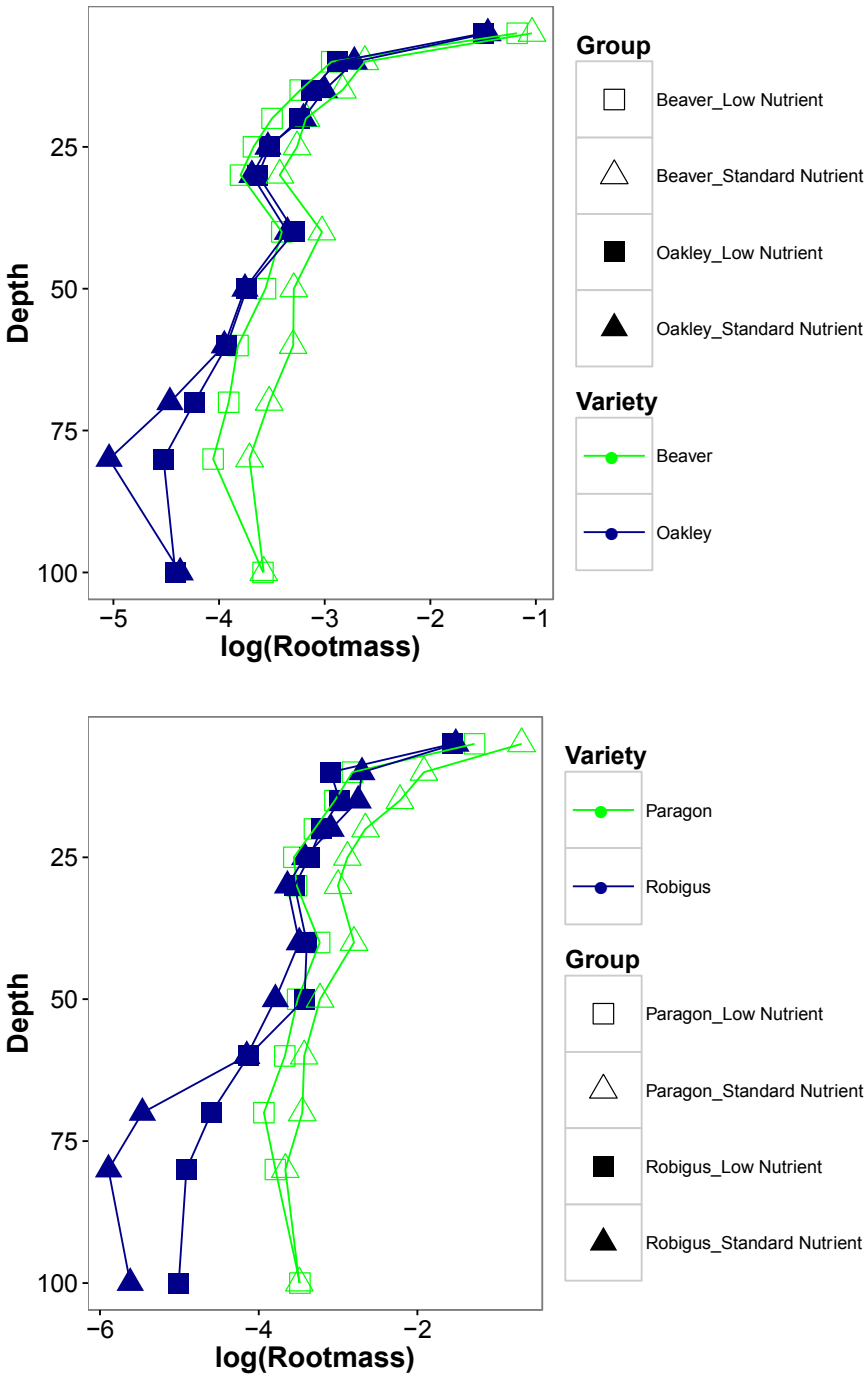

Figure 4.3.10: Examples of root profiles under different Nitrogen nutrient treatments.
Top: Root mass profiles of Beaver (green) and Oakley (blue). While the roots of Oakley are similar in both standard (triangles) and low nitrogen nutrient treatments (squares), Beaver shows reduced root mass production in the Low Nitrogen nutrient treatments (squares) compared with the standard nitrogen nutrient treatment (triangles)
Bottom: Differential variety responses to nitrogen supply for Paragon (green) and Robigus (blue). Under low nitrogen nutrient treatment (squares) Paragon loses root proliferation in the mid root sections, whereas Robigus proliferated roots in the bottom 40cm of the rhizotubes in response to the low nitrogen nutrient treatment (squares) compared with the standard nitrogen nutrient treatment.

5. Discussion

5.1. Semi quantitative estimation of wheat roots in soil using DNA

Results show that it is possible to quantify root biomass in soil using DNA based methods. The method was capable of discriminating wheat and barley from among the major agricultural crops and showed no reaction with DNA extracted from black grass (*Alopecurus myosuroides*). The inability to discriminate between wheat and barley is not ideal; however, as this method would be applied in well managed agricultural trials where only one cereal is present, this does not represent a practical problem. The ability to exclude weed roots or other extraneous fibres from the total measured root density represents an advance over conventional root washing methods.

It has been demonstrated that the method is capable of discriminating between wheat varieties for their root biomass phenotype. The calculated least significant differences are less than observed differences in phenotype in many cases though it is clear that the assay does not offer sufficient discrimination for all cases. However, high variances generated by field replication and laboratory batch membership are also seen. Neither of these observations is surprising. Spatial variation of soil has long been recognised (Oliver & Carroll, 2004). Variation within a single field may arise from small differences in the underlying substrate, the effects of slope and the effects of cultivation or compaction. The laboratory batch membership represented each 384 well PCR plate used to process DNA extracts for qPCR. The relatively small volumes used for each reaction (5µl) may be subject to pipetting errors and small variations in instrument response will result in large variations in calculated DNA concentrations due to the log-linear response of the qPCR system (Karlen *et al*., 2007). For these reasons it is recommended that any experiment using this method recognises its limitations and treats the results as semi quantitative.

Despite the caveats in the previous paragraph, where both soil washing results and predictions made using a DNA based assay are available, the correlation between the two methods is high. Having calculated the statistical power derived from these results it is clear that it is possible to discriminate between varieties with extreme values for the predicted root mass within the soil profile. This success opens the door to further investigations of root phenotypes, either by variety, by agronomic treatment or to explore variety by environment interaction.

When compared with current methods it is clear that the assay has strengths and weaknesses. Clearly this method is a blunt instrument in comparison with a detailed dissection of root architecture in a rhizotron where, for example, rooting angles or the ratio of fine to coarse roots can be explored. Unlike a rhizotron the method can be implemented in field conditions in unmodified soils. Unlike observation tubes the method can be implemented in any field without preparation before the crop is sown. There is no reason why cores should not be taken at any point in the growing season and stored before analysis; this would allow root development to be studied in field situations. In common

with root washing or observation trenches, sampling is destructive. Processing time for a batch of samples is likely to be comparable, or less, than that required for soil washing assays. Apart from a soil mill, the equipment needed is likely to be available in many molecular biology facilities.

Despite its limitations, this method allows cost effective estimation of partitioning of roots within the soil profile, comparison of different genotypes in their rooting behaviour and an exploration of the effects of differing agricultural practices on root phenotypes.

5.2. Characterising the mycorrhizal fungal community associated with wheat roots

5.2.1. Evaluation of the protocol for fungal diversity analysis

The objective of a high-resolution method to characterise the species diversity of AMF communities in plant roots and soil was achieved. The new protocol proved robust in amplifying DNA from wheat roots and a range of soils, with only a few samples failing to provide sufficient amplified product for sequencing. The Ion Torrent produced ample numbers of good quality reads (e.g. 100,000 – 200,000 identified reads per sample in the first run of 16 samples, Figure 4.2.1) to give a clear view of fungal diversity down to species that formed less than a thousandth of the community. Barcodes were included in the PCR primers, which simplifies the protocol relative to the alternative of adding barcodes in a separate step afterwards, but requires a separate barcoded primer for each sample, which adds to the initial cost.

The amplification of AMF sequences using AML1 and AML2 primers was very specific, with more than 99% of identified reads falling into the Glomeromycota. It also appeared to be comprehensive in covering the diversity of AMF, since sequences were amplified from most of the diverse families that make up the Glomeromycota (e.g. Figure 4.2.6). It is encouraging that the relative abundance of AMF species amplified with AML1-AML2 and with AU2-AU4 is very similar (Figure 4.2.2). This suggests that neither amplification is strongly biased or missing major groups of AMF.

The AU2-AU4 amplification of fungi in general appeared initially to be successful, too, as the sequences covered a wide taxonomic range, including Basidiomycetes, Ascomycetes, Mucoromycotina and Chytridiomycota in addition to Glomeromycota (Figures 4.2.1 and 4.2.6). In order to gain additional information about fungi in general, these primers were used rather than AML1-AML2 in the later studies, even though AMF were the main focus of the project. However, closer inspection revealed a problem. There was an expectation that *Gaeumannomyces graminis*, the causative agent of take-all disease in wheat, would be present in some of these samples, but the corresponding sequence was never found. Upon investigating the target sequence, it became clear that the *Gaeumannomyces* sequence was divergent in the primer binding region and would

not be expected to amplify with the AU2-AU4 primer pair. The same is true of some other common plant-associated fungi. In other words, the AU2-AU4 pair is not truly universal for all fungi, even though some fungal sequences in all major fungal clades are amplified. These primers were originally designed more than a decade ago, when the database of available fungal sequences was much more restricted. Potentially, the protocol could be adapted to survey all fungi by redesigning the primers appropriately, but the issue did not come to light early enough to take this route within the current project.

5.2.2. AMF diversity in wheat roots and the surrounding soils

Although the studies described here were based on a limited set of wheat varieties, it was clear from the findings that the AMF communities associated with wheat in the field are highly diverse, both within communities (alpha diversity) and between them (beta diversity). Very different community compositions are detected even among replicate plots that share the same treatment and wheat variety, and this is true both for fungal sequences in roots and those in the soil. In light of this high heterogeneity that appears to be random, or at least unexplained by recorded factors, there is a need to average over a larger spatial scale. For example, multiple small samples could be taken across a trial plot to ensure that the sample was representative. Thorough mixing is then important because the size of the sample that can be analysed is small (e.g. 0.25 g of soil for a typical DNA extraction kit).

In the light of this high diversity between replicates, it is not surprising that effects of treatment, depth or cultivar are hard to detect. In one study, at least, AMF in roots were less diverse than in the surrounding soil (Figure 4.2.5), which is to be expected if the plants are being colonised only by some of the fungi from the available soil community.

5.2.3. Future directions

This study has revealed the diversity of AMF in arable soil and crop plants at an unprecedented level of resolution. The heterogeneity that it has revealed certainly raises some interesting questions as well as some technical challenges. It seems unlikely that all the organisms that are detected have equivalent effects on the plant, whether these are positive or negative. Does the apparent homogeneity of a wheat crop reflect microbial heterogeneity that occurs on such a small spatial scale that each plant samples a smoothed average over its root system, or can plant homeostasis compensate for differences in the microbial community? The real challenge for the future, though, is to move from measures of taxonomic diversity to measures of functional diversity.

5.3. Dissecting root traits at key development stages

Overall the rhizotube system proved useful in assessing differences in root traits in wheat. Further analysis will be needed to understand where relationships exist between seedling, stem elongation, anthesis and maturity depending on the length of the growth season, as some root traits appear to be more affected by length of season than others. Analysis will also be needed to understand differences and similarities between field grown and rhizotube root shape.

Both the growing season and growth media, including nutrient supply, have an impact on the root and shoot biomass produced in rhizotubes and in field. The rate of acquisition and allocation of biomass, C and N are important as the build up of these resources during development phases can act as a pool of resources that can be reallocated during seed production. The optimal allocation will depend on putting the appropriate balance of resources between roots and shoots to acquire resources from the growth media (particularly water and N) *vs.* allocation to leaves and shoots for photosynthesis to acquire C. The level of N availability particularly influences the concentration of grain storage proteins (Foulkes *et al*., 2009; Shewry *et al*., 2001) and affects grain processing quality (Dai *et al*., 2015; Nuttall *et al*., 2016). Further uptake and allocation potentially affects trophic interactions and has implications for pest control and the wider farm ecosystem (Chesnais *et al*., 2016; Butler *et al*., 2012; Aqueel and Leather, 2011).

Experiments with longer (pre-anthesis) growing season enabled higher production of root mass at anthesis and at maturity as previously noted by Barraclough and Leigh (1984). Further analysis is thus needed to assess which parts of the roots are most affected by seasonal length, and what proportions of the phenotypes are more driven by variety differences. Root parameters overall were more sensitive to positional effects than shoot parameters, and root models showed a lower % explanation of the variation in root responses than shoot parameters (usually c.60% of the variation is accounted compared with 80-90% for shoot statistics).

Differences in response to N supply between varieties were found, with varieties responding to nitrogen differences in the root sections between 5 and 30 cm deep. Interestingly, these match with the most common inversion plough depth used in the farming systems under which these varieties have been bred and suggests that some varieties may be better at expanding roots in this plough layer in response to available nitrogen. Although no interactions were found with regards to overall grain weight produced per plant, there was an effect on %N in the grain. Further analysis of differences in N use efficiency from the rhizotubes studies can now be used to compare with varietal differences in N use efficiency and yield from field studies such as Barraclough *et al.* (2010). Further, assessment of hypotheses relating to root traits such as greater root length at depth can now be tested using varieties that are known to bulk or maintain root length at lower parts of the profile particularly under reduced nutrient condition (Foulkes *et al*, 2009) .

Further, while some seedling root traits might be amenable to breeding improvement and differences in early rooting e.g. number of seminal roots, root length (Richards *et al*, 2007) , there was only limited evidence of relationships between early seedling growth and overall mature root mass of plants, presumably because growing conditions and length of growing season had a large effect on the mature root traits investigated. Further investigations are needed to assess whether any correlations can be found between early seedling root traits and specific growth stage root traits rather than the overall mass, particularly after differences in length of the growth season in the different experiments has been taken into account. Since this relationship will also be affected by soil conditions care must be taken in extrapolating seedling variation to mature plant production. It will therefore also be important to matching root traits to different growing (soil) conditions (Richards *et al.*, 2007, White *et al.* , 2013).

6. Conclusions

1.1 A new DNA method for quantifying root biomass within soil samples was developed and successfully tested.

1.2 Variety has a significant effect on the quantified root biomass in the soil column. This result suggests this new assay will prove to be useful to plant breeders.

1.3 This method can readily be extended to other crop species or used to dissect crop-weed interactions.

2.1 A new DNA method for characterising the mycorrhizal fungal diversity in field root or soil samples was developed and successfully tested.

2.2 This method revealed that the mycorrhizal fungal populations are highly diverse on and around individual wheat plants and variable on a small spatial scale.

2.3 The method could be extended to examine other plant-associated fungi, including pathogens.

3.1 The use of rhizotubes was assessed for growing wheat to maturity and assessing root and shoot biomass partitioning at different growth stages.

3.2 Large variations in root biomass distribution and shoot biomass were found between varieties and in response to reduced nutrient particularly in the 5-30cm zone.

3.3 There was evidence of differential responses of varieties to the reduced N supply. While some varieties reduced their root biomass under reduced N others were able to maintain and or redistribute their root systems.

7. Messages for growers

7.1 The difficulties of measuring root phenotypes has led to their neglect by researchers. Novel methods, including this new DNA based assay will enable researchers and plant breeders to measure and optimise crop root systems and thus improve crop performance.

7.2 Wheat roots in the field are colonised by a complex mixture of mycorrhizal fungal species that are present in the soil. While the methods are now available to identify them, more research is needed to understand which are most beneficial to the crop in terms of phosphorus nutrition, drought tolerance and protection against pathogens, and how to encourage these.

7.3 Parallel systems such as the rhizotube system enable plant breeders to have a more detailed assessment of root efficiency under different environmental conditions such as N supply as demonstrated here, and this will assist in selection of varieties with different root properties for use in breeding.

8. References

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9. Appendices

Appendix 1: Germplasm used within the "New wheat root ideotypes for improved resource use and yield performance in reduced input agriculture" project

	JHI trait dissection				NIAB
Variety	Seedling	2011	2012	2013	DNA based assay
1RSv.1BLp (921/94)		Y			
Access		Y			
Alchemy	Y	Y	Y	Y	Y
Alixan		Y			
Andalou		Y			
Apache		Y			
Aszita		Y			
Avalon	Y	Y	Y	Y	Y
Babax		Y			
Bacanora		Y			
Battalion		Y			
Beaver	Y	Y	Y	Y	Y
Brompton		Y			
Buster	Y	Y	Y	Y	Y
Cadenza	Y	Y	Y	Y	Y
Caphorn		Y			
Cappelle Desprez	Y	Y	Y	Y	Y
Cezanne		Y			
Chinese Spring (A. J. Worland)		Y			
Claire		Y			
Consort		Y			
Cordiale		Y			
Deben		Y			
Dover		Y			
Einstein		Y			
Equinox		Y			
Exotic		Y			
Exsept		Y			

	JHI trait dissection				NIAB DNA
Variety	Seedling	2011	2012	2013	based assay
Galahad		Y			
Gatsby		Y			
Gladiator		Y			
Glasgow	Y	Y	Y		Y
Glenson	Y	Y	Y		
Gulliver		Y			
Haven		Y			
Hereward	Y	Y	Y	Y	Y
Hobbit		Y			
Humber		Y			
Huntsman		Y			
Hustler		Y			
Hyperion		Y			
Istabraq		Y			
Laurin		Y			
Longbow		Y			
Malacca		Y			
Maris Widgeon		Y			
Marksman		Y			
Mendel		Y			
Mercato		Y			
Mercia	Y	Y	Y		Y
Mercia-Rht12		Y			
Mercia-Rht8	Y	Y	Y		Y
Mercia-Rht-B1c		Y			
Mercia-Rht-D1b	Y	Y	Y		Y
Mercia-Rht-D1d		Y			
MEX-19		Y			
Norman	Y	Y	Y		Y
Oakley	Y	Y	Y	Y	Y
Paragon	Y	Y	Y	Y	Y
Pavon 1 BS 1 BLv		Y			
Pavon 1 Rsv 1Alp		Y			
Pavon-76		Y			

	JHI trait dissection				NIAB
Variety	Seedling	2011	2012	2013	DNA based assay
PUB-94		Y	Y		
R-34/RS-131-6 (ppd-D1.e1)		Y			
R-35/RS-111-5-8 (Ppd-D1.e1)		Y			
Recital		Y			
Rialto	Y	Y	Y	Y	Y
Riband		Y			
Rob-173-16-A-10-7 (Ppd-A1.a)		Y			
Rob-173-2-A-17-6 (ppd-A1.a)		Y			
RobChS-R/RC-211-2-10 (ppd-B1.e2)		Y			
RobChS-R/RC-211-2-11 (Ppd-B1.e2)		Y			
Robigus	Y	Y	Y	Y	Y
Royssac		Y			
Savannah	Y	Y	Y	Y	Y
Scroro		Y			
Seri-29B		Y			
Seri-32B		Y			
Seri-87B		Y			
SHW-173	Y	Y	Y		
Soissons	Y	Y	Y		Y
Solstice		Y			
Spark	Y	Y	Y		Y
Timber		Y			
Virtue		Y			
Viscount		Y			
Wiwa		Y			
Xi19	Y	Y	Y	Y	Y
XPS-058-3-19		Y			
XS-036>6-6		Y			
XS-051>14-3		Y			
XS-052>4-6		Y			

	JHI trait dissection				NIAB DNA
Variety	Seedling	2011	2012	2013	based assay
XS-063>2-4		Y			
XS-109>4-2		Y			
XS-144>44-1		Y			
XS-170>4-1		Y			
XS-218>18-1	Y	Y	Y	Y	Y
XS-218>19-5	Y	Y	Y	Y	Y
XS-441>4-1		Y			
Zebedee		Y			

Appendix 2: Data from soil cores collected from the Terrington 2014 and 2015 trials.

Tables of means

Variate: ug/g dry soil

Variety	Overall	Year	
		2014	2015
Alchemy	38.0	61.6	14.4
Avalon	55.2	29.4	80.9
Beaver	13.8	16.4	11.2
SHW Xi19 / (Xi19 // SHW-218) >18	32.0	26.3	37.8
SHW Xi19 / (Xi19 // SHW-218) >19	67.2	42.0	92.4
Buster	20.0	26.1	14.0
Cadenza	31.7	51.5	12.0
Cappelle D	25.8	20.5	31.1
Glasgow	12.0	12.4	11.7
Hereward	12.5	14.9	10.1
Mercia	12.9	14.9	10.9
Mercia Rht8	21.8	13.1	30.4
Mercia Rht8 D1B	21.4	18.6	24.2
Norman	45.6	43.3	47.8
Oakley	21.0	28.4	13.5
Paragon	47.0	43.6	50.4
Rialto	17.1	24.4	9.8
Robigus	14.7	15.0	14.4
Savannah	35.6	28.9	42.3
Soissons	23.7	14.0	33.4
Spark	39.7	65.6	13.8
Xi19	78.5	38.0	119.0
Mean	31.2	29.5	33.0

Data from soil cores collected from the Terrington 2014 and 2015 trials (continued)

Tables of means
Variate: ug/g dry soil

	Depth			
Variety	0-250	250-500	500-750	750-1000
Alchemy	94.3	27.7	22.7	7.3
Avalon	114.0	66.9	32.2	7.6
Beaver	36.8	12.2	3.6	2.6
SHW Xi19 / (Xi19 // SHW-218) >18	82.1	33.0	11.3	1.7
SHW Xi19 / (Xi19 // SHW-218) >19	149.1	67.6	32.6	19.4
Buster	44.2	17.4	16.4	2.1
Cadenza	51.6	29.3	28.5	17.4
Cappelle D	68.1	22.4	11.3	1.3
Glasgow	29.4	14.4	2.5	1.8
Hereward	30.2	13.3	4.3	2.2
Mercia	32.0	11.9	5.7	2.1
Mercia Rht8	60.9	20.1	4.6	1.4
Mercia Rht8 D1B	49.7	27.1	6.1	2.8
Norman	109.3	45.2	18.4	9.5
Oakley	47.5	21.3	7.6	7.6
Paragon	109.2	51.3	23.0	4.6
Rialto	35.5	25.5	6.5	0.9
Robigus	39.5	12.1	5.4	1.7
Savannah	82.9	42.8	14.2	2.4
Soissons	38.3	33.4	21.6	1.4
Spark	94.9	45.7	11.0	7.1
Xi19	212.8	87.4	6.5	7.2
Mean	73.3	33.1	13.5	5.1

Data from soil cores collected from the Terrington 2014 and 2015 trials (continued)

Tables of means

Variate: ug/g dry soil

	Year							
	Depth							
	2014					2015		
Variety	0-250	250-500	500-750	750-1000	0-250	250-500	500-750	750-1000
Alchemy	155.8	39.2	37.0	14.5	32.9	16.3	8.4	0.0
Avalon	74.4	29.5	8.5	5.4	153.6	104.3	55.9	9.8
Beaver	42.1	11.7	6.8	5.1	31.5	12.8	0.3	0.0
SHW Xi19 / (Xi19 // SHW-218) >18	70.8	20.3	10.9	3.1	93.3	45.7	11.7	0.4
SHW Xi19 / (Xi19 // SHW-218) >19	98.6	45.1	9.5	14.7	199.6	90.0	55.8	24.2
Buster	50.5	16.9	32.8	4.2	37.8	17.9	0.1	0.0
Cadenza	80.8	46.9	49.8	28.4	22.5	11.8	7.2	6.4
Cappelle D	54.8	14.7	10.0	2.6	81.5	30.2	12.6	0.1
Glasgow	27.5	14.1	4.3	3.5	31.4	14.7	0.7	0.1
Hereward	38.0	10.4	8.0	3.3	22.5	16.3	0.6	1.1
Mercia	38.8	10.4	6.3	4.1	25.2	13.3	5.1	0.1
Mercia Rht8	31.1	13.5	5.0	2.8	90.7	26.7	4.2	0.1
Mercia Rht8 D1B	38.6	20.3	9.9	5.6	60.7	33.8	2.4	0.1
Norman	93.8	34.5	28.3	16.7	124.7	55.8	8.5	2.3
Oakley	60.9	23.5	14.2	15.1	34.0	19.0	1.0	0.0
Paragon	104.8	48.2	14.1	7.3	113.5	54.3	32.0	1.8
Rialto	45.9	37.2	12.8	1.9	25.1	13.9	0.3	0.0
Robigus	34.4	11.6	10.8	3.4	44.6	12.7	0.1	0.0
Savannah	72.3	20.5	19.1	3.6	93.5	65.1	9.4	1.2
Soissons	19.0	25.9	8.7	2.2	57.6	40.9	34.5	0.6
Spark	176.4	62.4	12.3	11.3	13.5	29.0	9.7	3.0
Xi19	109.6	22.5	5.4	14.3	315.9	152.4	7.6	0.1
Mean	69.0	26.3	14.7	7.9	77.5	39.9	12.2	2.3