Wheat lines differing in yield, root architecture and mucilage secretion show associated differences in rhizosheath microbiome composition

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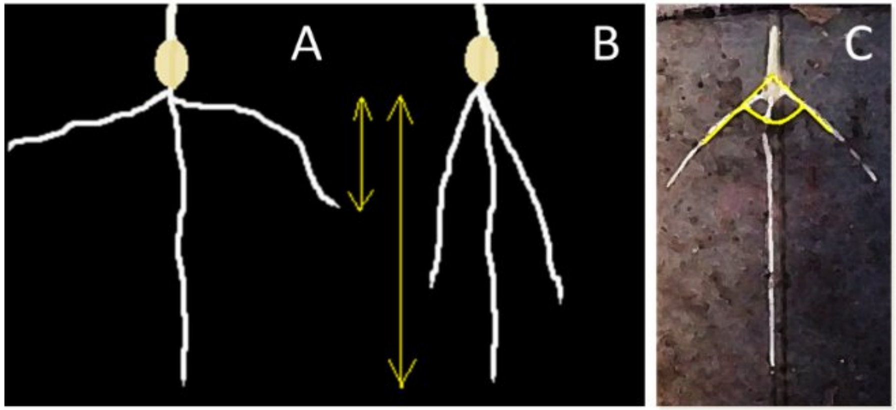
Root System Architecture

Root growth angle helps determine overall root system architecture and acquisition of soil water and nutrients

Avalon x Cadenza (AxC) Doubled Haploid population
Measured seminal root angle using clear pot method and ImageJ

Roots were photographed after 7 days growth in soil



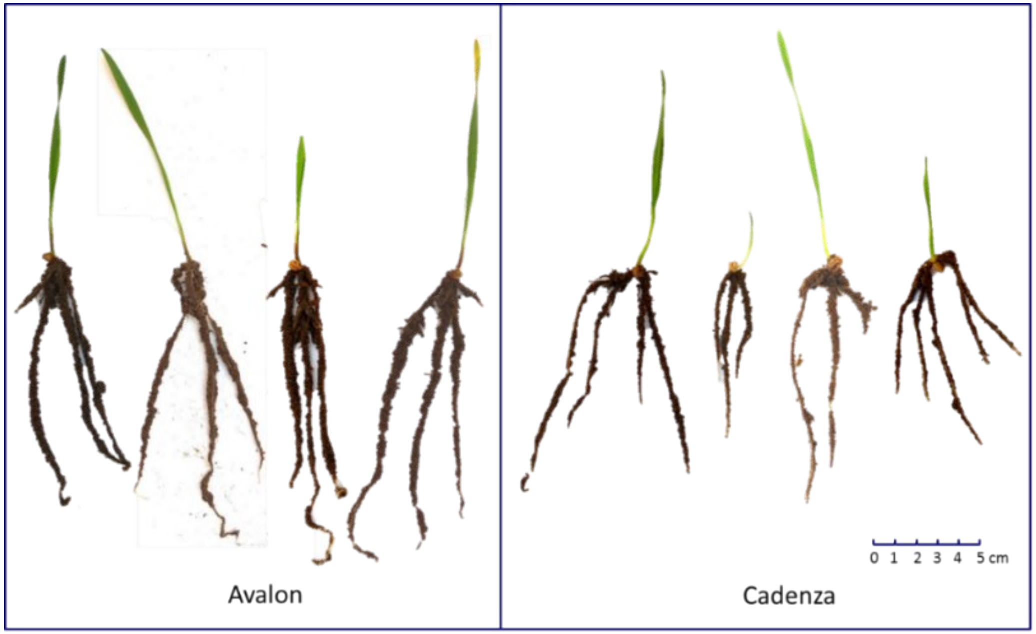
Quantifying root angle using image analysis

Emily Marr (2020). *Unlocking the genetic potential of the root system, rhizosheath mucilage and microbiome of wheat (Triticum aestivum L.)* (PhD thesis, Univ. Cambridge). https://doi.org/10.17863/CAM.69285

Richard et al. (2015). High-throughput Phenotyping of Wheat Seminal Root Traits in a Breeding Context. https://doi.org/10.1016/j.proenv.2015.07.179

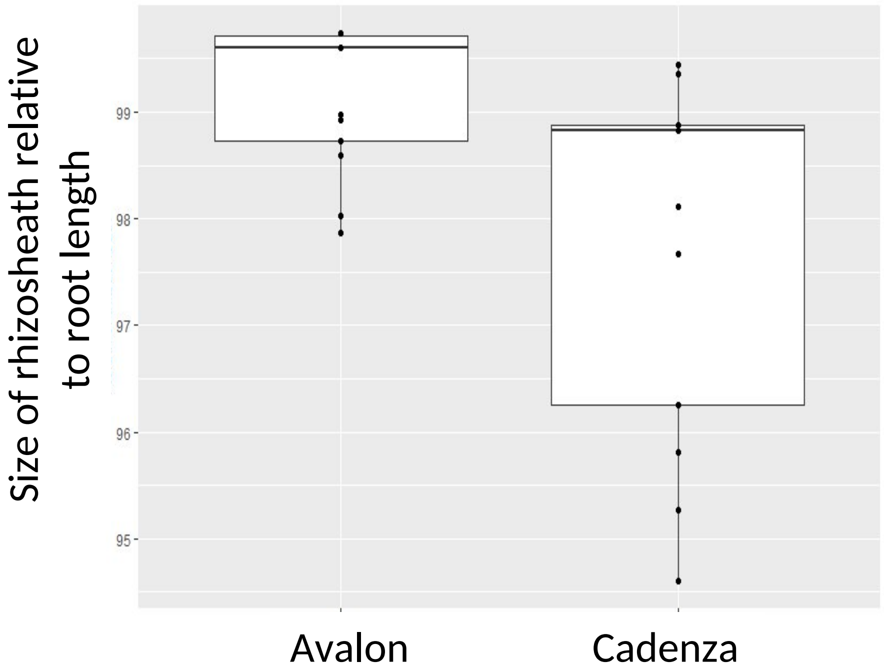
Avalon and Cadenza differ in rhizosheath size

- Avalon has a larger rhizosheath than Cadenza
  - Estimated by image analysis of 2D projected soil area, normalised by root length



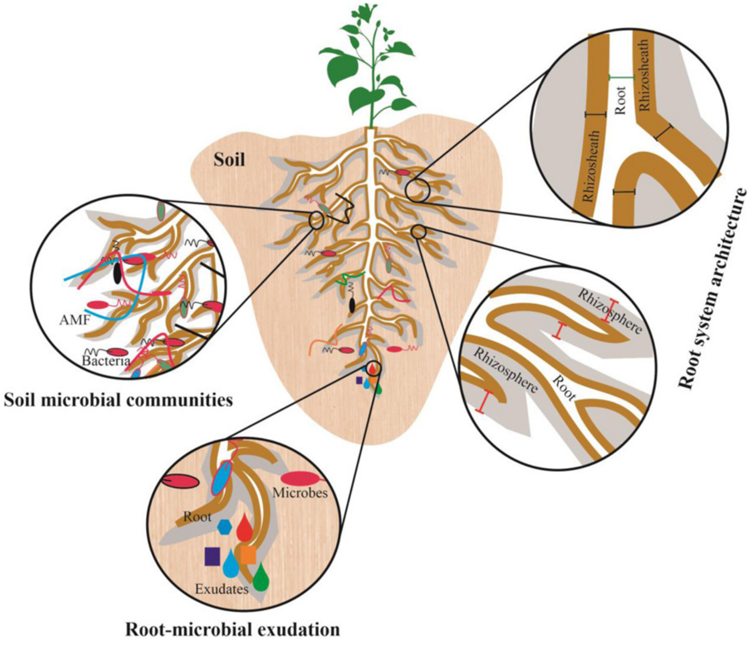
Avalon

Cadenza



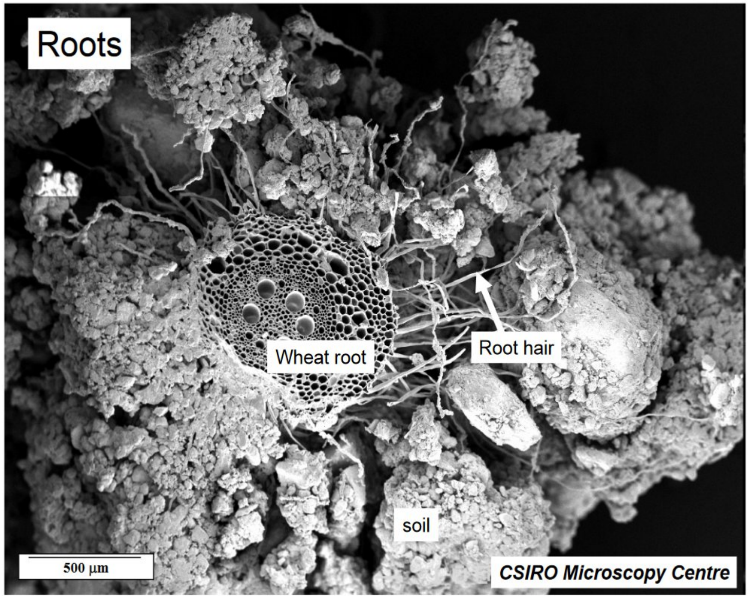
The importance of the rhizosheath

- Increases hydraulic conductivity between root surface and bulk soil
    - aids uptake of water and nutrients
    - formation stimulated under drought conditions
- Formation depends on root hairs + polymeric root exudates (mucilage)
- Soil/root interface rhizoplane harbours microbial activity



Graphic: Aslam et al. 2022 doi:10.1111/pce.14395

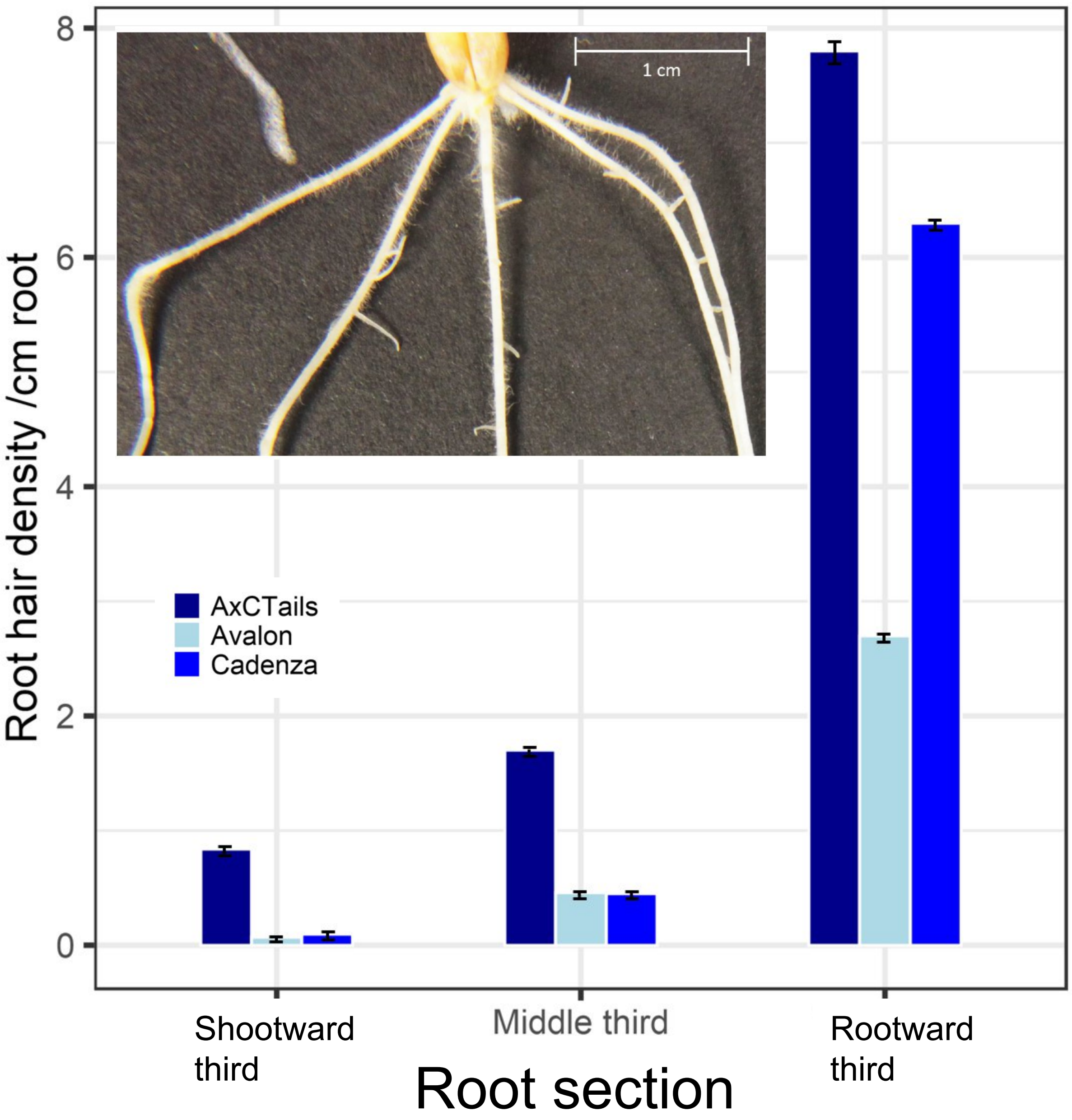
Field sample - drought
Hinxton, 2017



Root hair (RH) density in tails of the A x C population (25 lines)

Root section	Root hair density (number of hairs cm^{-1} root)			p-value	
	AxC Tails	Avalon	Cadenza	AxC Tails	Avalon and Cadenza
Total root length	3.4	1.1	2.3	0.001***	0.067
Top third	7.8	2.7	6.3	0.001***	0.040*
Middle third	1.7	0.46	0.45	0.001***	0.14
Bottom third	0.84	0.072	0.097	0.029*	0.63

- Genetic variation for RH density among A x C tails
- Avalon (larger rhizosheath) has smaller or little difference in RH density than Cadenza
- Other factors may contribute to A vs C differences in rhizosheath size



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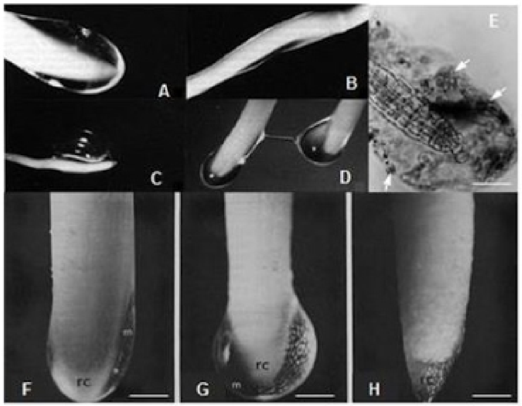
Production of root exudates benefit crops

But little is known about the genetic control, and how varieties can be developed that have optimal properties and production of exudates

- 5-21% of photoassimilate ends up in soil through root exudates
- Root secretions forming mucilage help:
  - aggregate soil particles via the soil-binding properties of polymeric gels:
    - More favourable distribution of aggregate sizes
    - better crumb structure, soil tilth
    - Increases compressive strength of aggregates to resist mechanical shearing via tillage, breakdown by raindrops (seedbed capping)
    - improves hydration and soil wetting characteristics, important for light-textured soils
  - helps form rhizosheaths, particularly in dry conditions-improves hydraulic connections from soil to root surface
  - Helps lubricate movement of the root tip into cracks within soil peds, between particles in tight soils
  - provides C and N source for microbial activity at root-soil interfaces



https://phys.org/news/2013-09-secret-life-underground-microbes-root.html

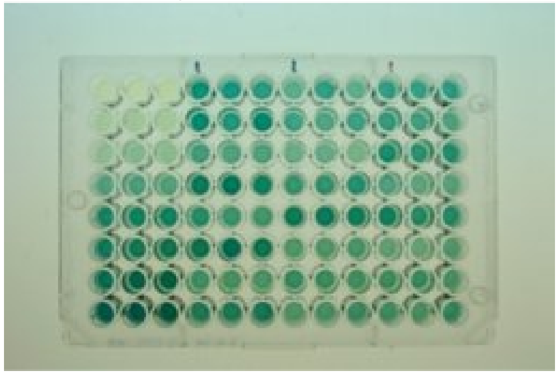
The rhizosheath microbiome

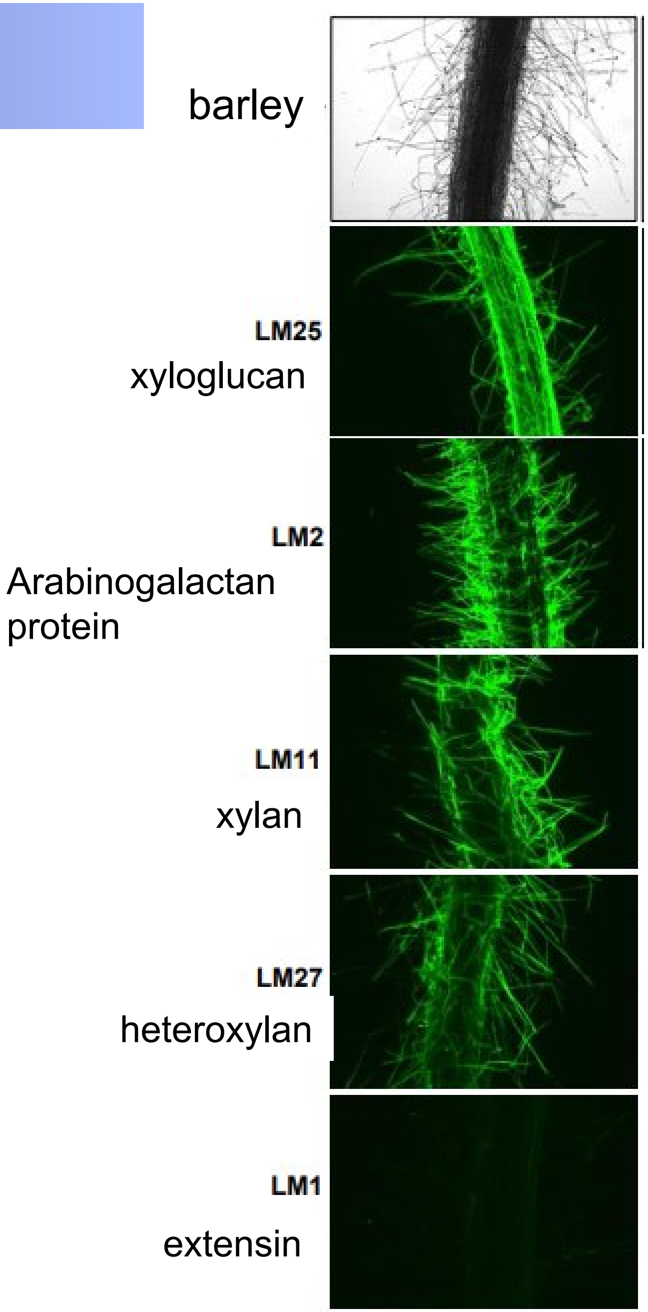
Research questions:

- Is there genetic variation in mucilage abundance and composition in this wheat population?
- Is there genetic variation in the composition of the microbial populations in the rhizosheath of different wheat lines
- What is the functional and taxonomic diversity of the wheat rhizosheath microbiome
- Are there associations between rhizosheath characteristics and field performance?

Mucilage analysis

- 25 lines from Avalon x Cadenza doubled haploid mapping population (202 lines)
  - tails selected from ~8400 individuals representing top/bottom 1.1% of BLUPS for seminal root angle
- Grow individuals 7 days in multiplexed 50 mL field soil-filled tubes
- Shake off excess soil and measure root length and sample mass
- Gently vortex roots/rhizosheath and extract mucilage in water
- Assay extract aliquots to detect carbohydrate epitopes using ELISA with specific monoclonal antibodies
- Quantify using standard curves using pure carbohydrate standards



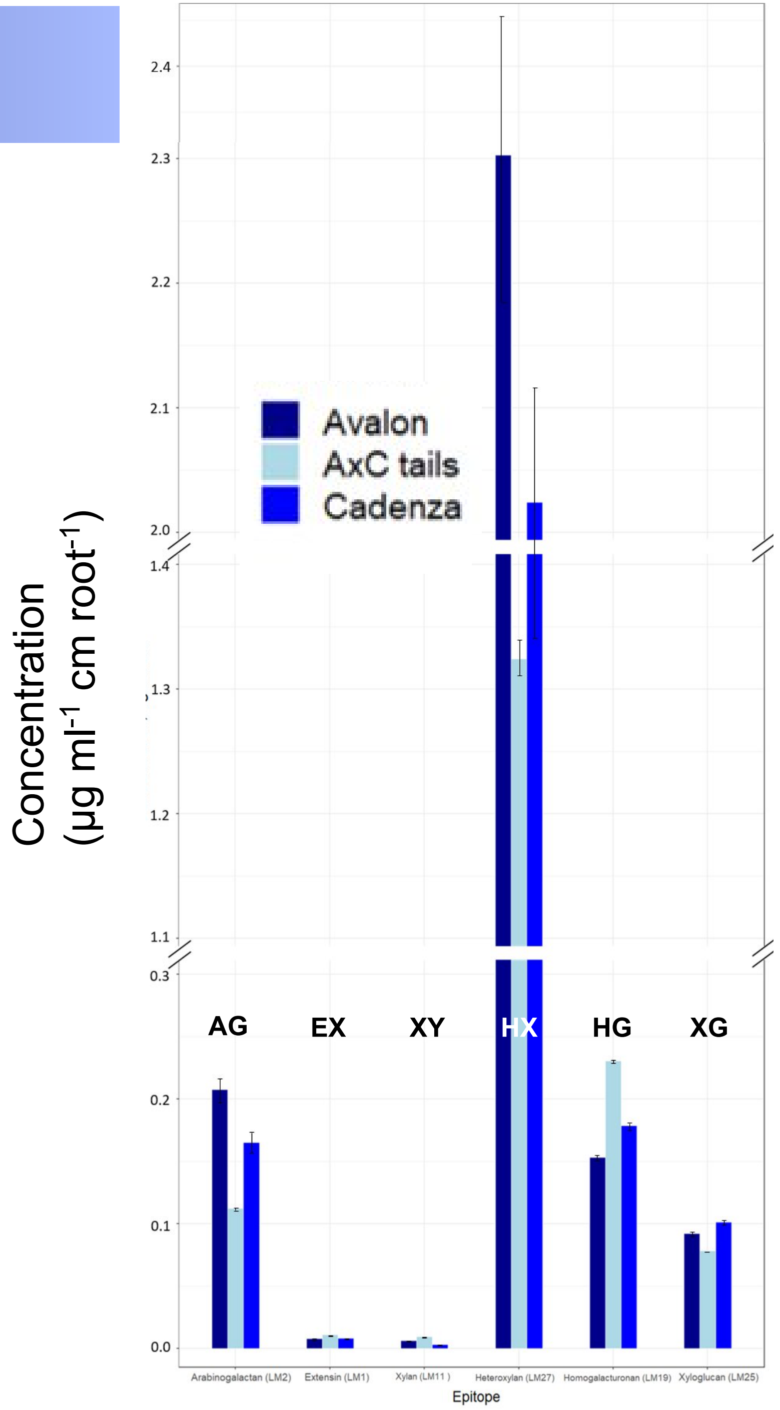


Galloway et al. 2022
https://doi.org/10.1101/2021.04.15.440065

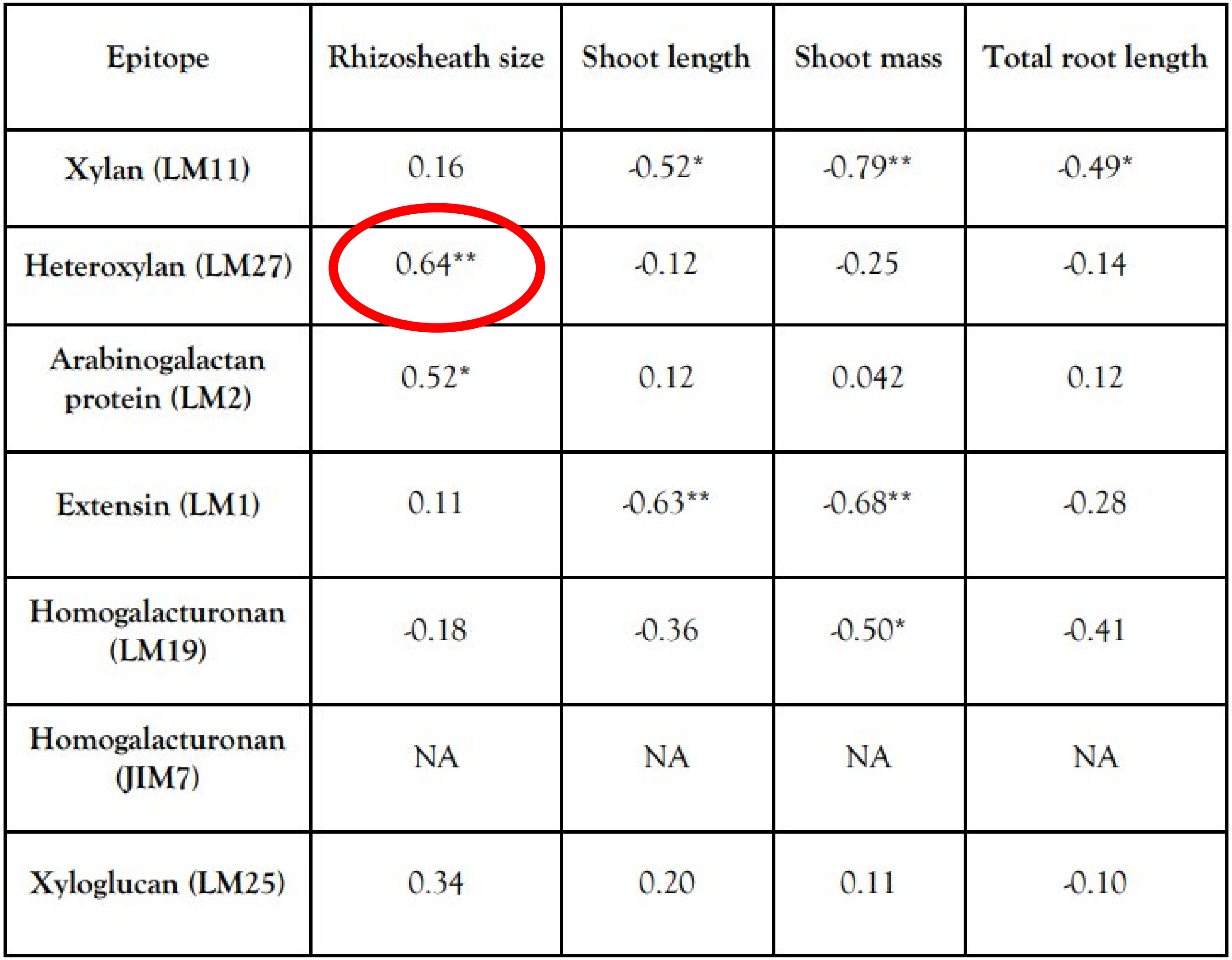
Rhizosheath mucilage composition

Epitope (µg ml-1 cm-1)	Xylan	Hetero xylan	Arabino galactan	Extensin	Homo galact uronan19	Homo galact uronan7	Xylo glucan
AxC tails	0.0096	1.3	0.11	0.011	0.23	0.0014	0.078
Avalon	0.0064	2.3	0.21	0.0078	0.15	0.00083	0.092
Cadenza	0.0033	2	0.16	0.0081	0.18	0.00068	0.1
Genotypic variation p-value	0.022 *	<0.001 ***	0.001 **	<0.001 ***	<0.001 ***	0.0015 **	0.004**
Heritability	0.38	0.40	0.40	0.050	0.66	NA	0.56

- **Genotypic variation for each carbohydrate epitope**
- **Profile dominated by heteroxylan**



Exudate composition correlation with root system traits



Epitope	Rhizosheath size	Shoot length	Shoot mass	Total root length
Xylan (LM11)	0.16	-0.52*	-0.79**	-0.49*
Heteroxylan (LM27)	0.64**	-0.12	-0.25	-0.14
Arabinogalactan protein (LM2)	0.52*	0.12	0.042	0.12
Extensin (LM1)	0.11	-0.63**	-0.68**	-0.28
Homogalacturonan (LM19)	-0.18	-0.36	-0.50*	-0.41
Homogalacturonan (JIM7)	NA	NA	NA	NA
Xyloglucan (LM25)	0.34	0.20	0.11	-0.10

Heteroxylan levels positively associated with rhizosheath size amongst AxC tails (25 lines)

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Metagenomic methods

- 25 lines from Avalon x Cadenza doubled haploid mapping population
  - tails selected from ~8400 individuals representing top/bottom 1.1% of BLUPS for seminal root angle
- Grow individuals 7 days in multiplexed 50 mL field soil-filled tubes
- Shake and extract DNA from adhering rhizosheath soil
- PCR amplification and sequencing
  - bacterial 16S rRNA V5-V7 hypervariable region
  - fungal ITS1 rRNA gene
  - paired-end Illumina sequencing (2 x 250 bp) NovaSeq 6000 platform
- Operational taxonomic unit (OTU) processing UPARSE 10.0 clustering pipeline using 97% similarity
- QC
- Reads assigned to taxonomic ranks: UTAX algorithm and fungal (Unite V7); bacterial (RDP set 15) reference databases
- Alpha diversity (diversity within a sample) indices:
  - Chao1, ACE, Shannon-Weaver, Simpson
- Beta diversity (difference in microbial community composition between samples)
  - Weighted and unweighted UniFrac distances analysed using PERMANOVA
- OTU lifestyle assignments: FUNGuild (probable/highly probable)

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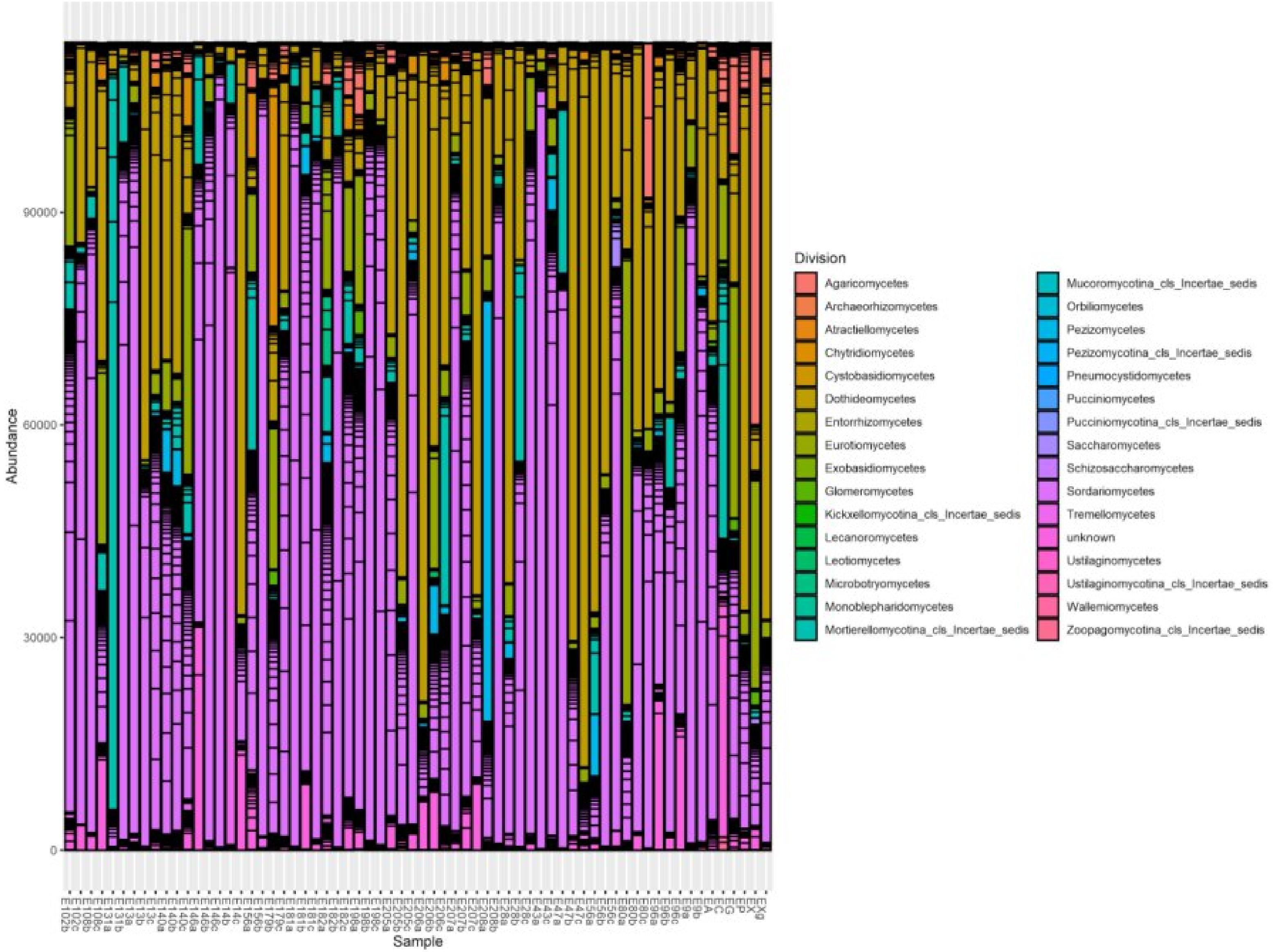
Abundance of fungal phyla in rhizosheath soil



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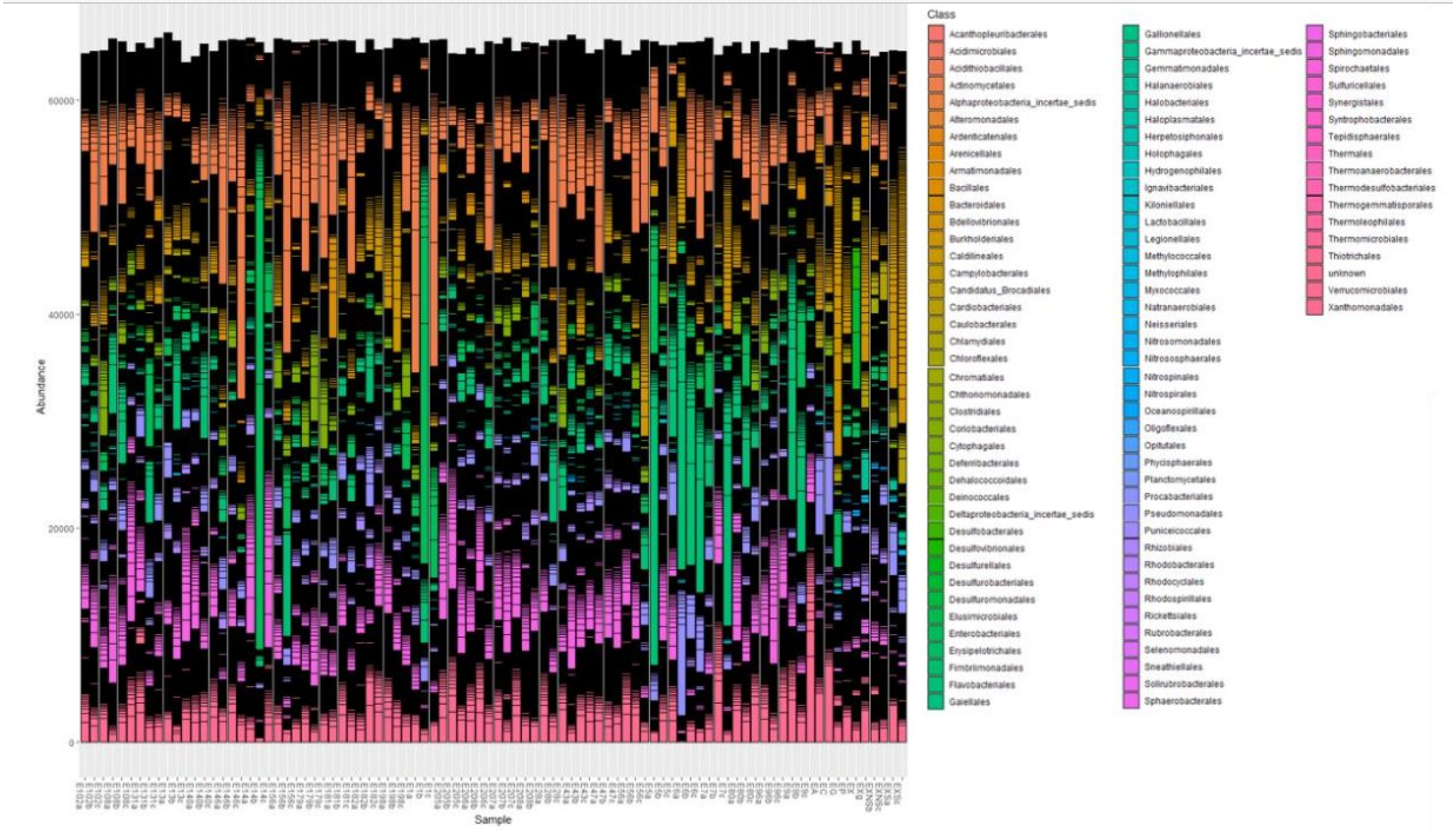
Abundance of bacterial phyla in rhizosheath soil



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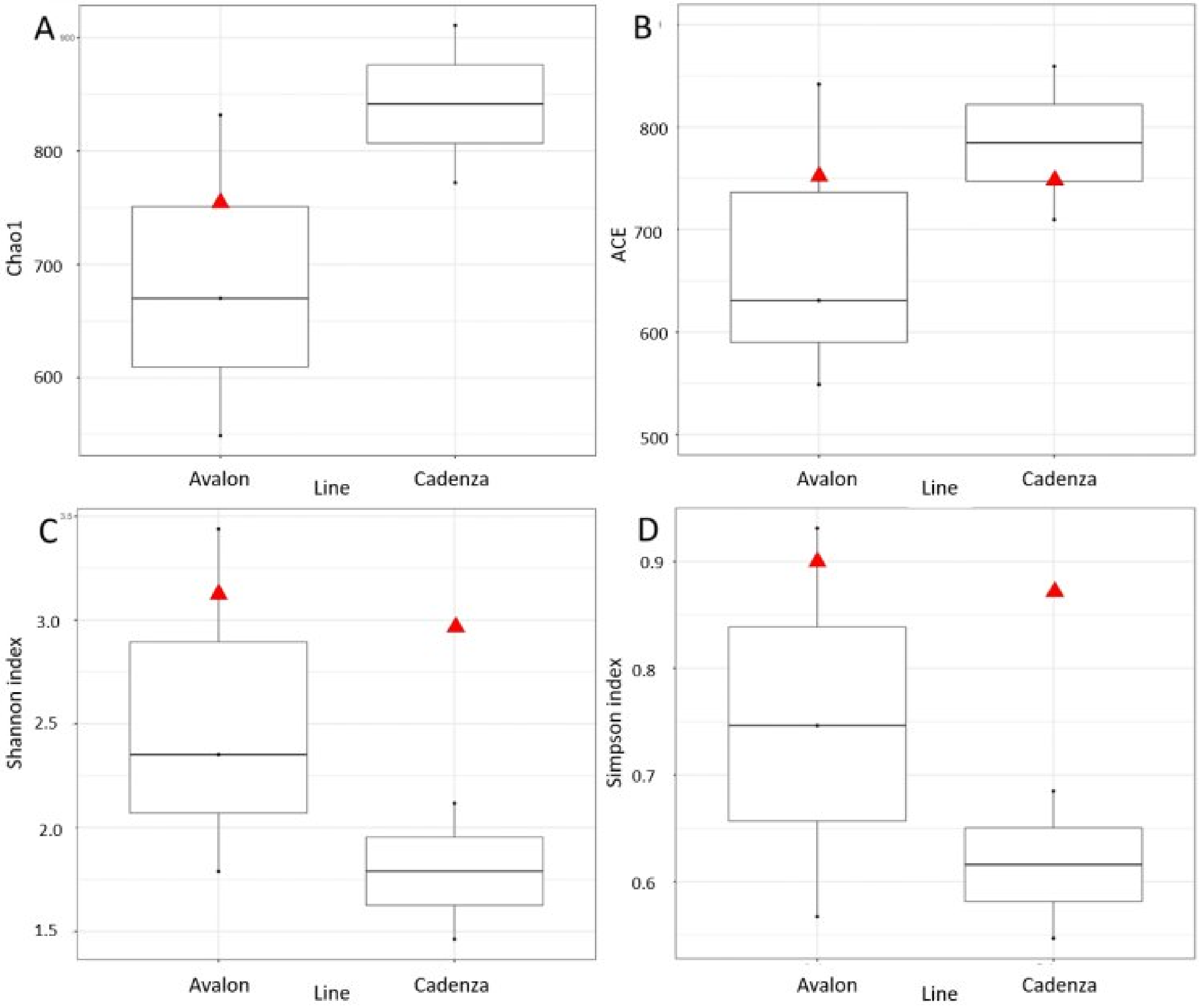
Fungal alpha diversity indices



- No difference in diversity between rhizosheath samples of parental lines
- In absence of plants, no difference between soils treated with root exudates from each line (red triangles)

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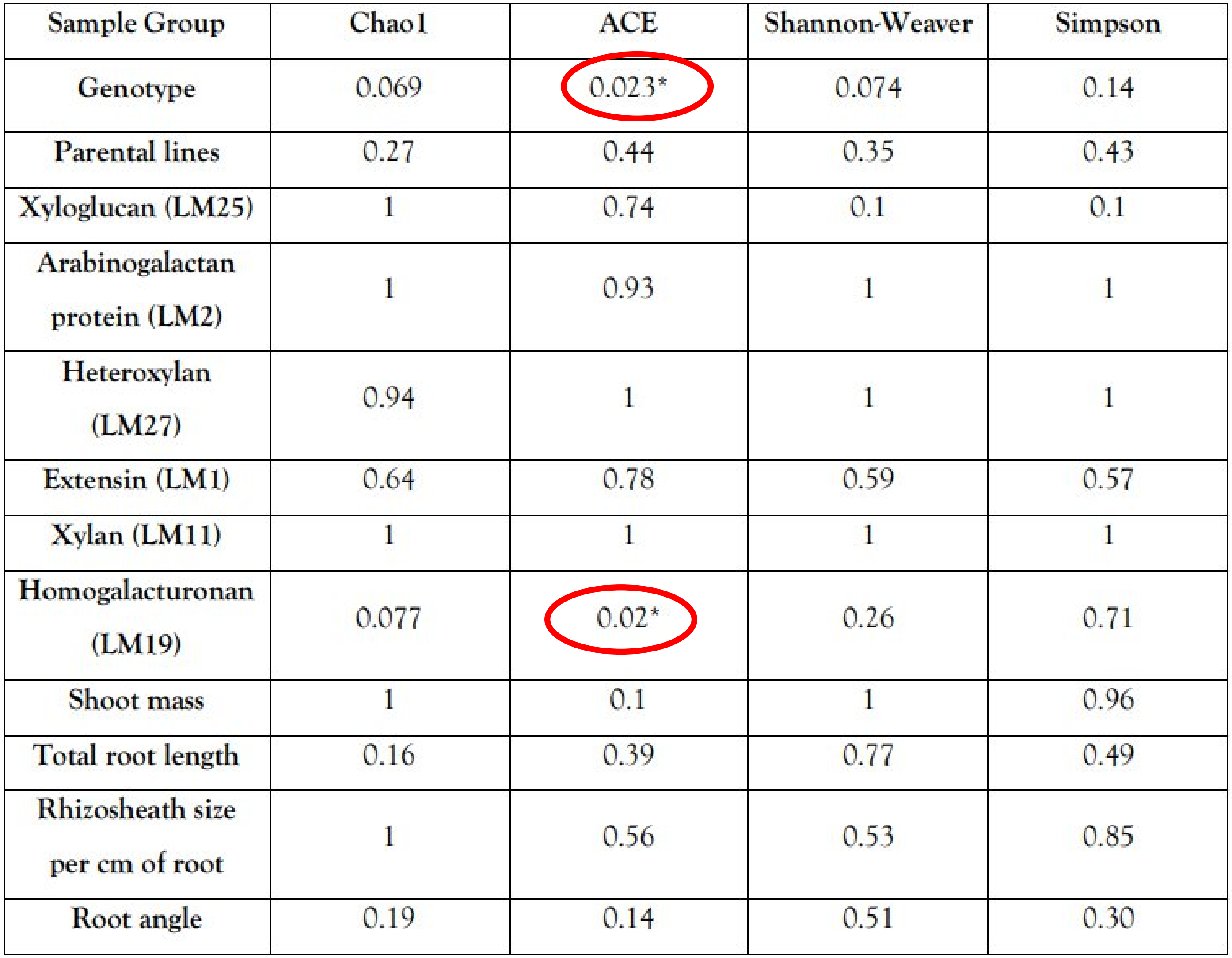
Fungal alpha diversity indices (*p*-values)



Sample Group	Chao1	ACE	Shannon-Weaver	Simpson
Genotype	0.069	0.023*	0.074	0.14
Parental lines	0.27	0.44	0.35	0.43
Xyloglucan (LM25)	1	0.74	0.1	0.1
Arabinogalactan protein (LM2)	1	0.93	1	1
Heteroxylan (LM27)	0.94	1	1	1
Extensin (LM1)	0.64	0.78	0.59	0.57
Xylan (LM11)	1	1	1	1
Homogalacturonan (LM19)	0.077	0.02*	0.26	0.71
Shoot mass	1	0.1	1	0.96
Total root length	0.16	0.39	0.77	0.49
Rhizosheath size per cm of root	1	0.56	0.53	0.85
Root angle	0.19	0.14	0.51	0.30

Across all AxC tails, significant differences in fungal diversity (ACE index)

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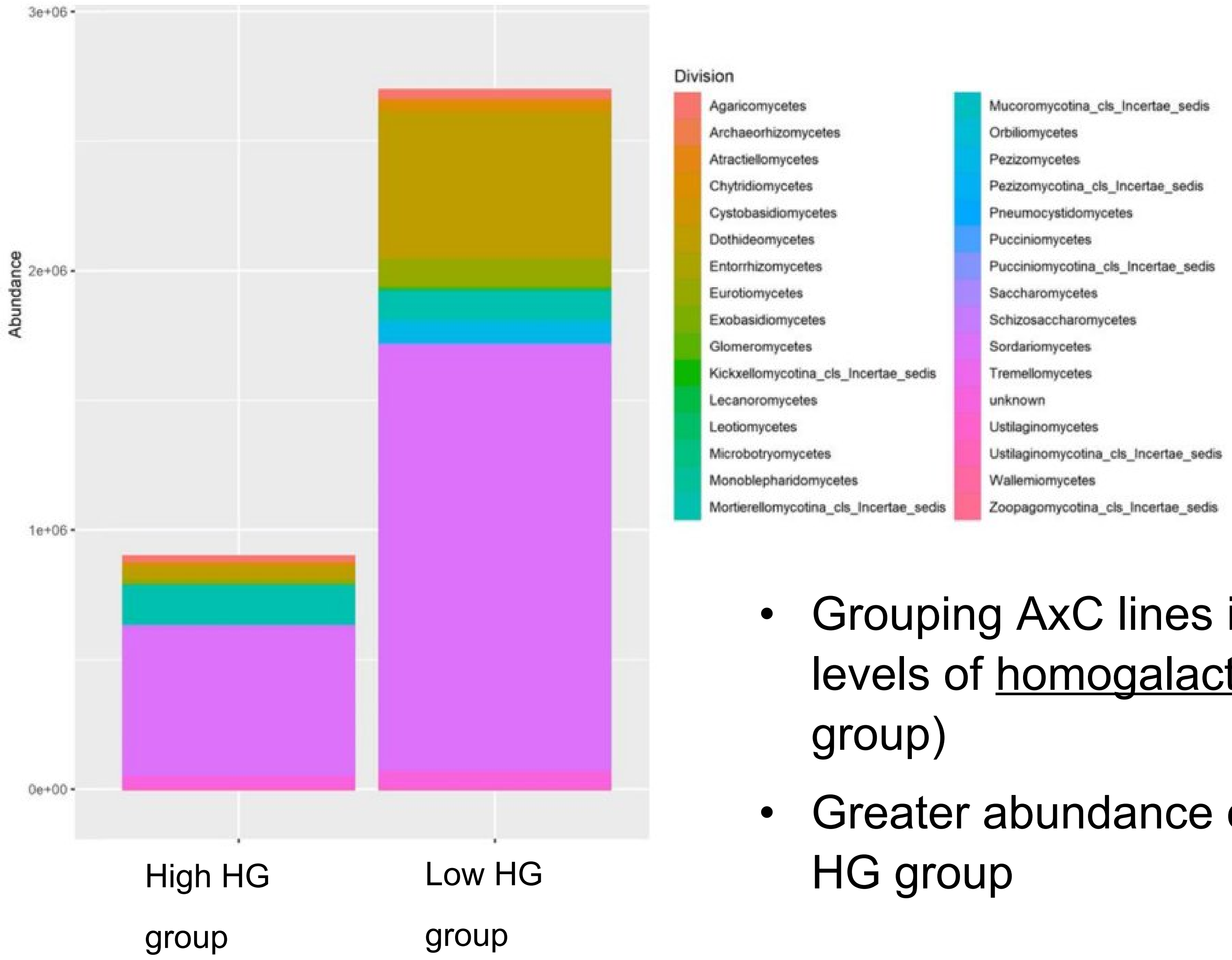
Abundance of fungal phyla in rhizosheath soil



- Grouping AxC lines into high and low levels of homogalacturonan (10 lines each group)
- Greater abundance of certain phyla in low HG group

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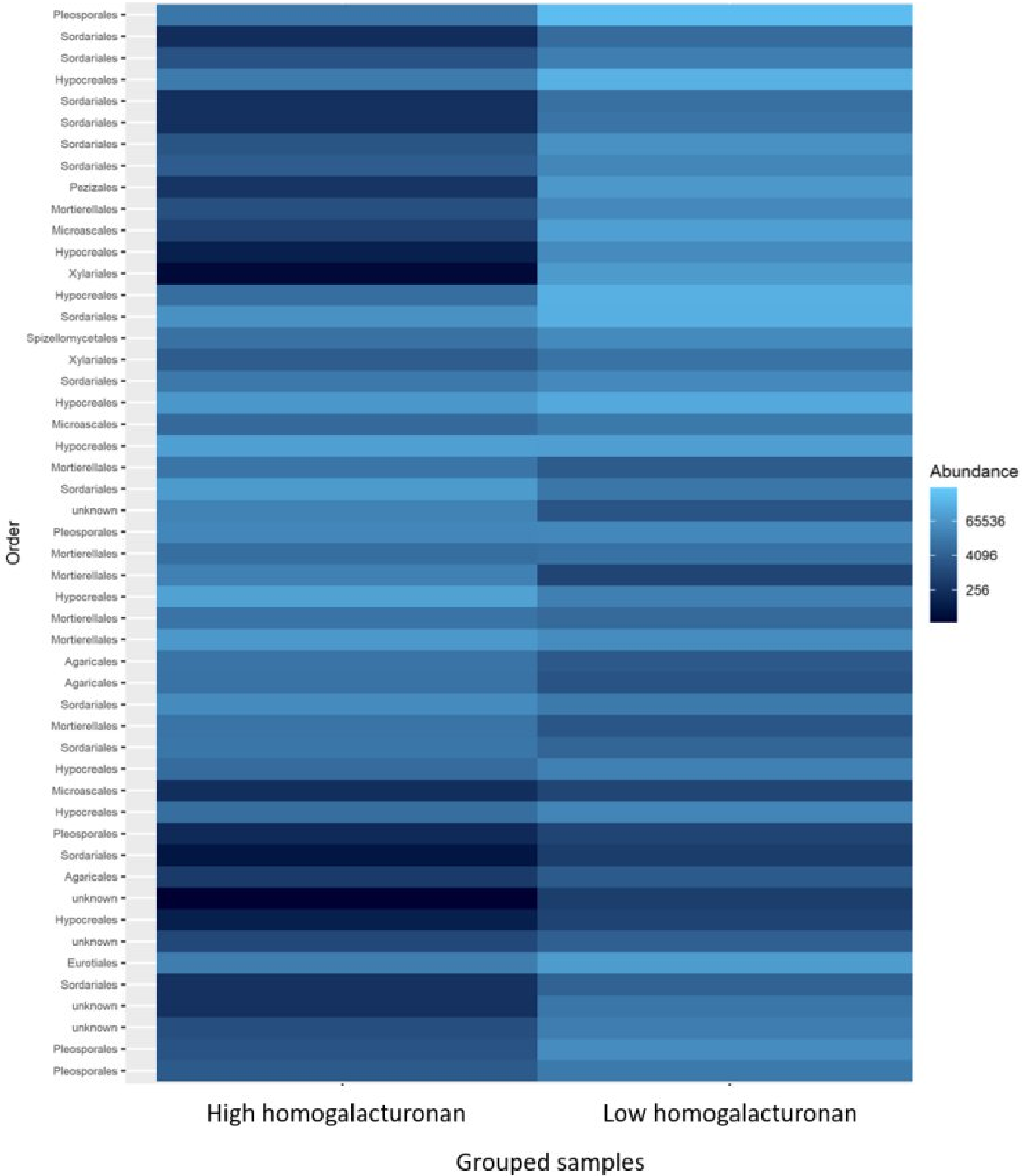
Abundance of fungal phyla in rhizosheath soil



- Grouping AxC lines into high and low levels of <u>homogalacturonan</u> (10 lines each group)
- Greater abundance of certain phyla in low HG group

Fungal beta diversity (PERMANOVA results)

Plant phenotype	Df	Sum of Squares	Mean squares	F-model	p-value	Permutations
Seedling root angle	1, 31	0.28	0.14	1.5	0.017*	999
Seedling root length	2, 37	0.12	0.12	1.3	0.096	999
Avalon vs Cadenza	3, 3	0.28	0.094	1.1	0.10	999
Extensin (LM1)	1, 38	0.089	0.089	0.94	0.56	999
Xylan (LM11)	1, 33	0.089	0.089	0.91	0.64	999
Homogalacturonan (LM19)	1, 34	0.19	0.19	2.0	0.003**	999
Xyloglucan (LM25)	1, 35	0.21	0.22	2.2	0.001***	999
Arabinogalactan protein (LM2)	1, 38	0.11	0.11	1.1	0.22	999
Heteroxylan (LM27)	1, 38	0.14	0.14	1.4	0.029*	999
Rhizosheath size	1, 33	0.098	0.098	1.0	0.42	999
Grain yield	1, 21	0.13	0.13	1.4	0.040*	999

- Fungal community composition shows significant dissimilarity between groupings of AxC lines according to root phenotypes

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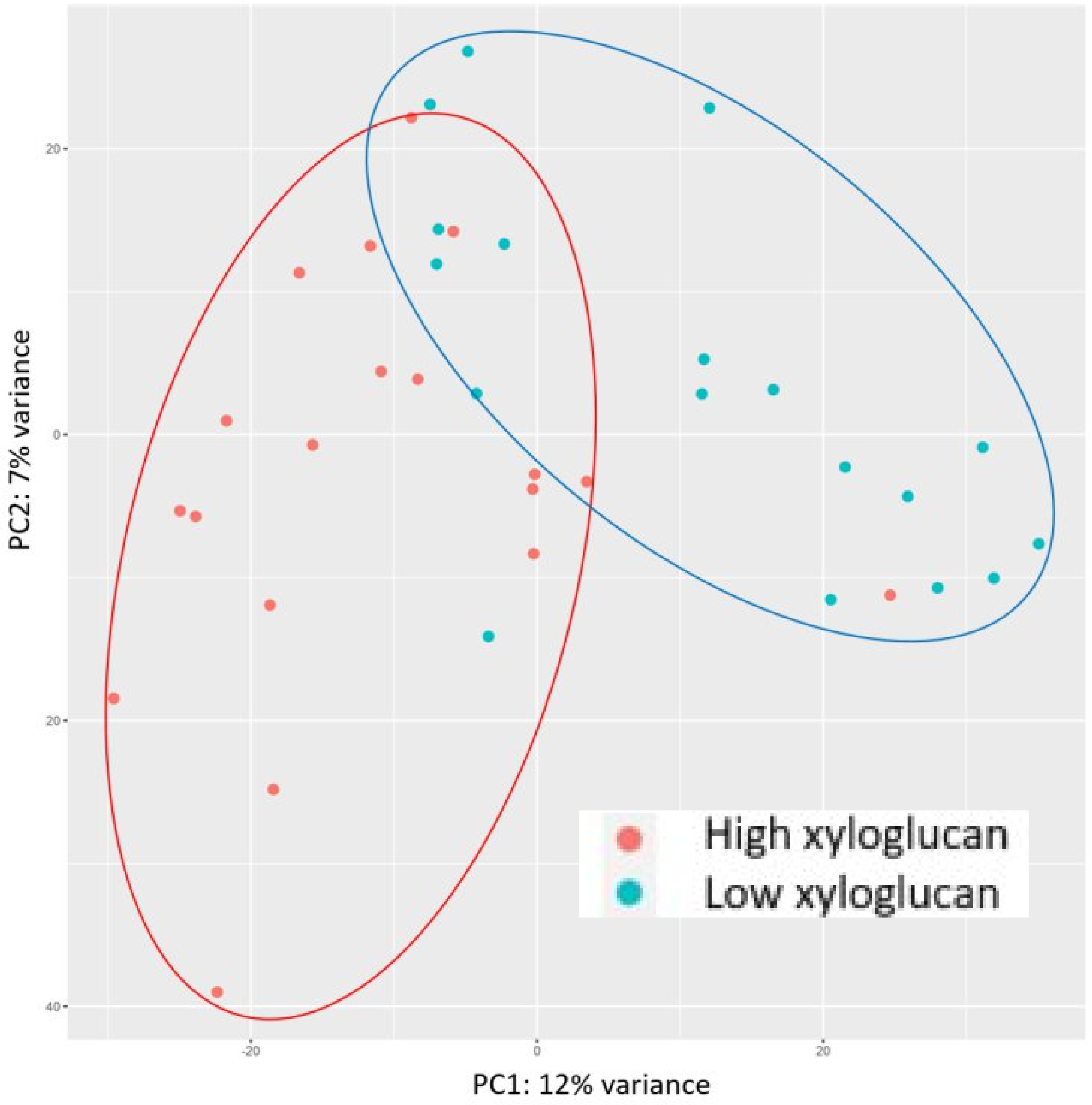
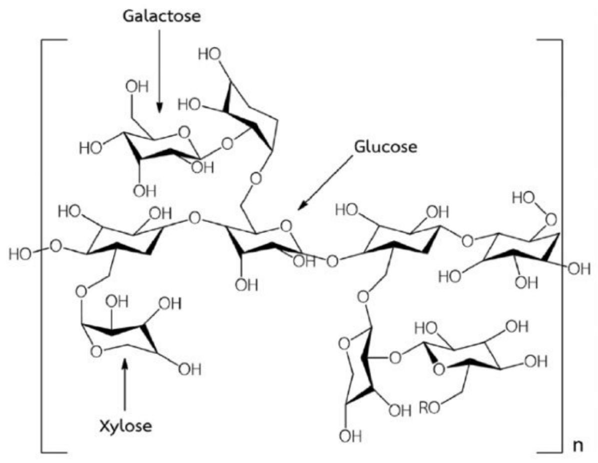
Rhizosheath diversity of fungal OTUs



- Clustering of unweighted Unifrac distances indicate differences in fungal community composition between lines with either high or low xyloglucan levels in exudate



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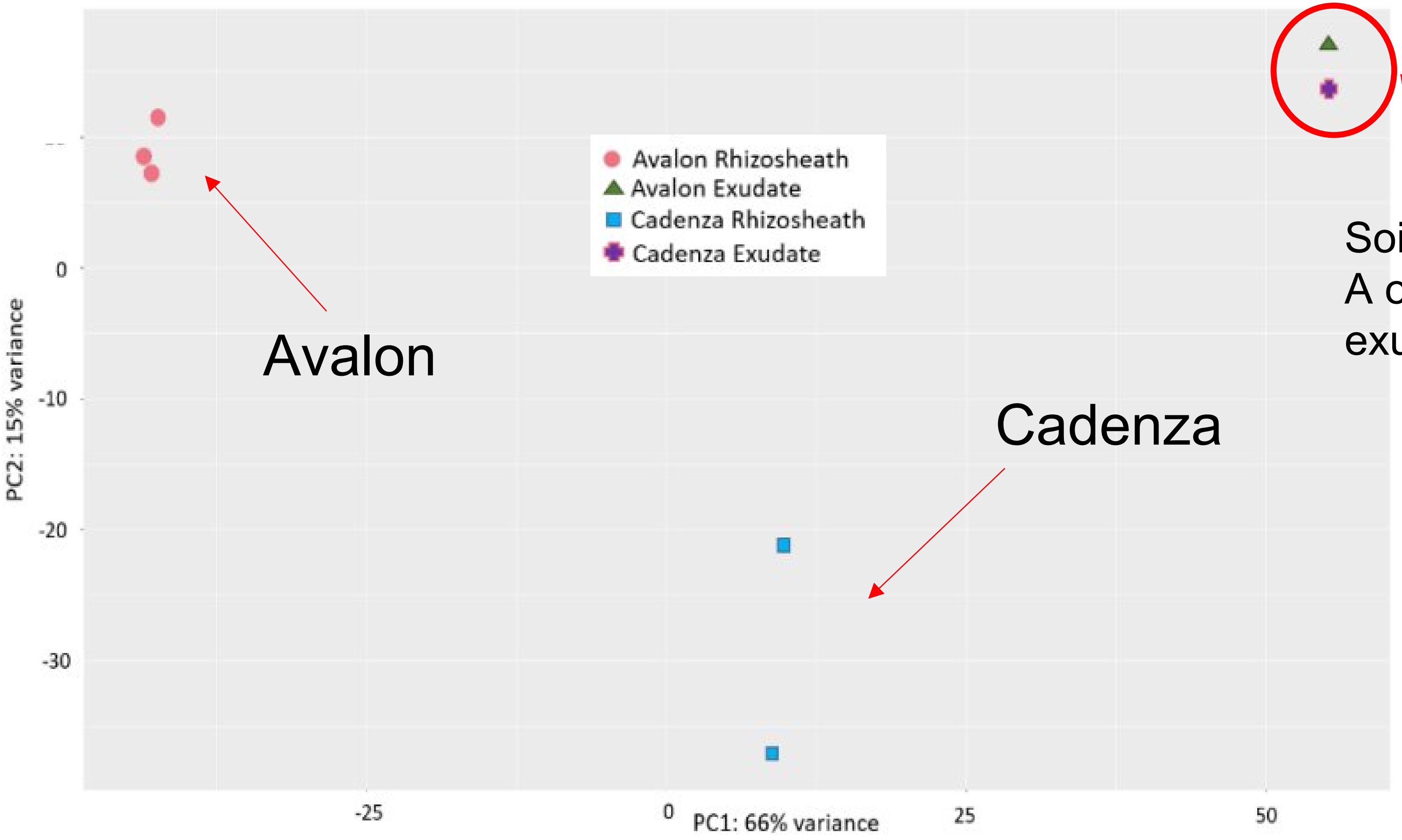
Rhizosheath diversity of bacterial OTUs



Avalon

Cadenza

Soil inoculated with A or C root exudates; no plants

- Avalon bacterial microbiome composition differs from Cadenza
- soil (without plants) supplemented with Avalon and Cadenza exudates clustered together
- differences in bacterial diversity between lines not solely due to differences in exudates?

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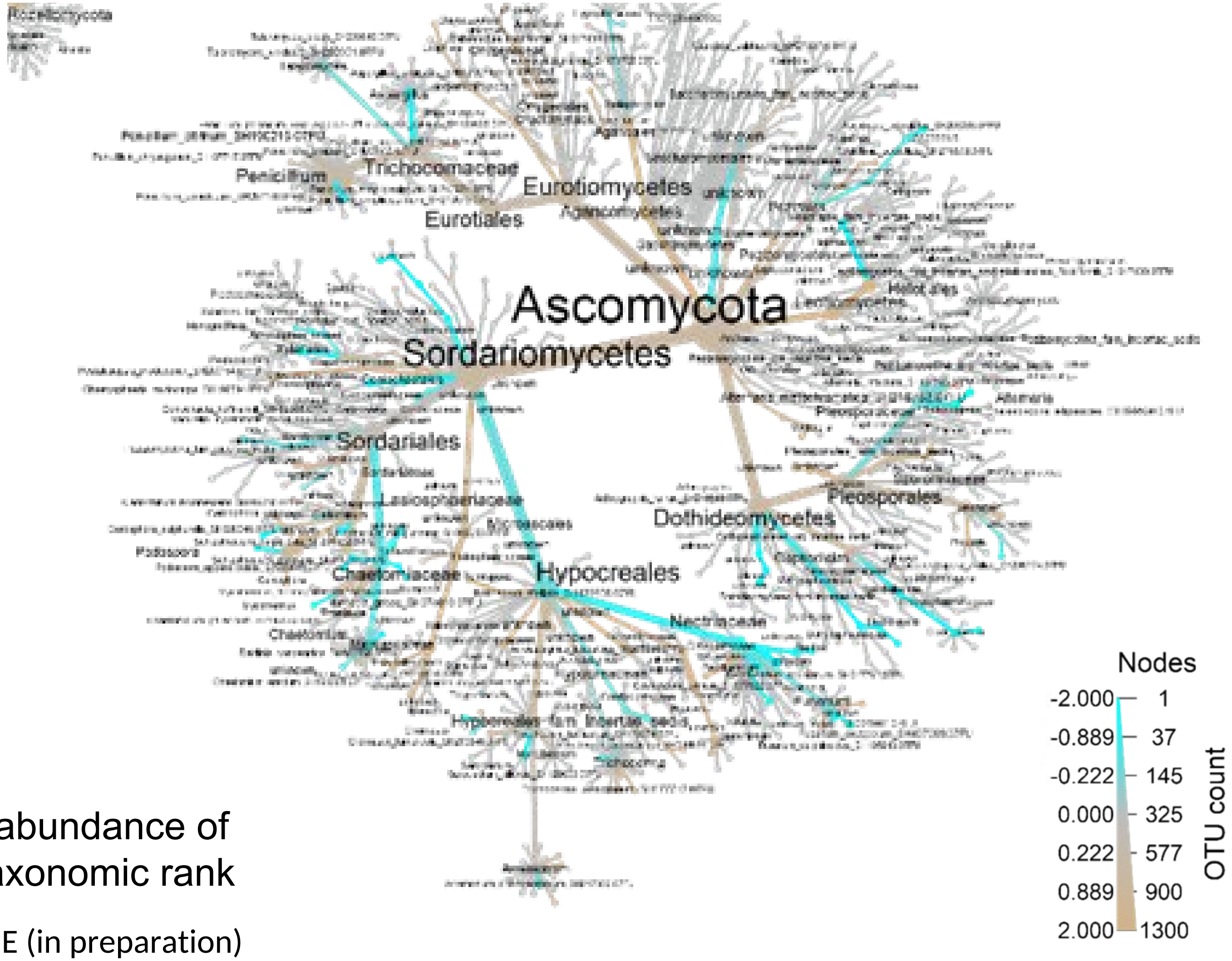
Rhizosheath diversity of fungal OTUs

Heat tree of the relative abundance of fungal species between wheat genotypes with contrasting grain yield

🟦 Relative abundance greater in lines with **higher** grain yield

🟨 Relative abundance greater in lines with **lower** grain yield



The size of nodes represents the abundance of rhizosheath fungi at the specific taxonomic rank

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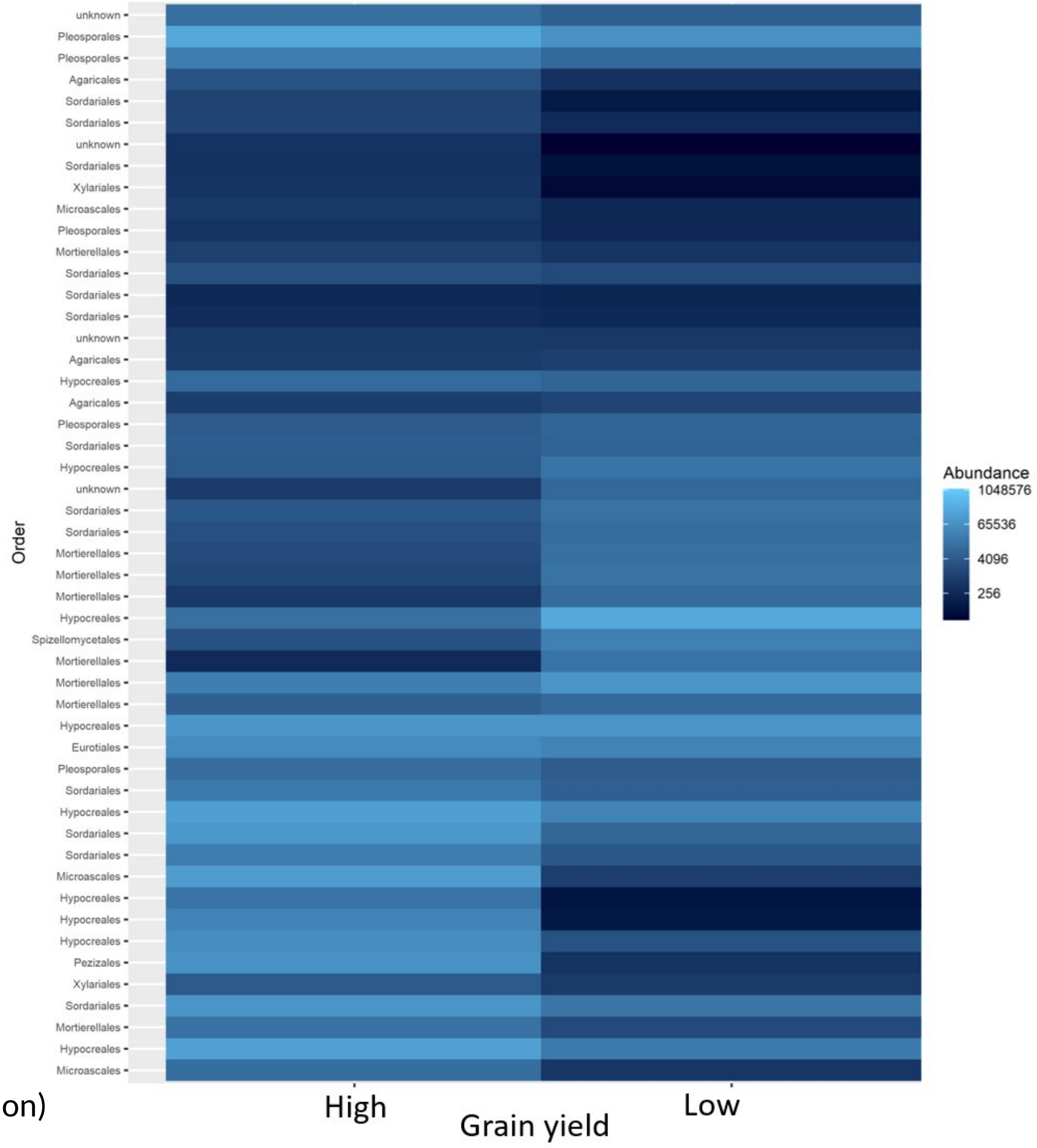
Abundance of fungal phyla in rhizosheath soil

Heat map of the 50 most abundant fungal species between groups of AxC lines with <u>contrasting grain yield</u>

Blocks represent species, labelled by the order to which the species belong

What could be the possible <u>functional connections</u> (if any) between microbiome composition and yield?



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Abundance of fungal phyla in rhizosheath soil

- High yielding wheat genotypes:
  - greater abundance of Glomeromycota
- Low yielding wheat genotypes:
  - greater abundance of saprophytic species:
    - *Spizellomyces* (Chytridiomycota)
    - *Nectria*
    - *Alternaria* (e.g. *A. metachromatica* (causal pathogen of leaf spot in tomato)

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Conclusions

- Significant genotypic variation among wheat lines (tails of AxC pop) for:
  - seminal root angle
  - rhizosheath size
  - Composition and concentration of polysaccharides in the rhizosheath
- Rhizosheath size positively correlated with arabinogalactan protein (AGP) and heteroxylan

Conclusions

- Genotypic differences in root system architecture and levels of root polysaccharide exudates were associated with differences in bacterial and fungal species composition
- Lines differing in grain yield in the field showed differences in fungal (but not bacterial) beta diversity
- Lines with narrow seminal root angle appeared to have a more beneficial root microbiome, based on the known lifestyle of constituent microbes in the rhizosheath soil

Conclusions

Can we someday engineer beneficial crop rhizospheres by choice of crop variety?

--a variety with a root system architecture and exudate profile that favours and/or recruits beneficial microbes

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