Using GC-MS based metabolite profiling to compare the effects of different management practices on the composition of cereal crops.




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Introduction

We are investigating the effects of conventional vs integrated management practices on the chemical composition & agronomic properties of six important Scottish crop species :
Potato, Spring & Winter Barley, Field Beans, Winter Wheat and Winter Oilseed Rape.

This is being conducted using a long-term rotation at the James Hutton Institute's **C**entre for **S**ustainable **C**ropping (CSC) at Balruddery farm in Angus.

For each crop: one industry standard variety + 3-4 others selected for optimum performance under reduced input &/or for differing end use sector requirements.

First 6 year rotation ran from 2011 – 2016; Second rotation began in 2017.

This presentation: Compositional analysis of Winter Barley 2011 – 2015

Questions being asked:

Are there any differences between Varieties, Input & Years?

Are any difference between Variety & Input consistent over the 5 years?

What are the metabolic processes that drive variation?

Harvesting & Sample Preparation

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Road (BK) - Winter OSR:
1. **Excalibur (a)**
2. Cracker 1 (b)
3. Cracker 2 (c)
4. Catana 1 (d)
5. Catana 2 (e)

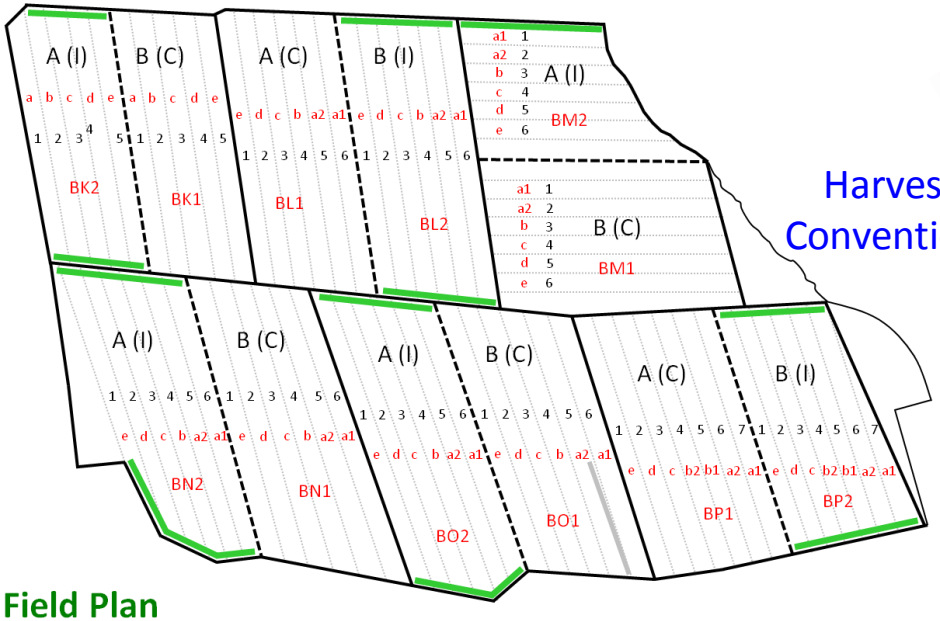
Middle East (BL) - Spring Barley:
1. 4 Component Mix (e)
2. Waggon (d)
3. Concerto (c)
4. Westminster (b)
5. **Optic 2 (a2)**
6. Optic 1 (a1)

Den South (BM) – Field Beans:
1. Fuego 1 (a1)
2. **Fuego 2 (a2)**
3. Pyramid (b)
4. Fanfare (c)
5. Boxer (d)
6. Babylon (e)

Milling (powdered)

Freeze-drying

Harvest (5 field replicates)
Conventional (C); Integrated (I)



2015 Field Plan

Pylon (BN) - Winter Barley:
1. 4 Component Mix (e)
2. Saffron (d)
3. Sequel (c)
4. Cassata (b)
5. **Retriever 2 (a2)**
6. Retriever 1 (a1)

Kennels (BO) – Potatoes:
1. Lady Balfour 2 (e)
2. Lady Balfour 1 (d)
3. Vales Sovereign (c)
4. Cabaret (b)
5. **Maris Piper 2 (a2)**
6. Maris Piper 1 (a1)

Estate (BP) - Winter Wheat:
1. Viscount (e)
2. Beluga (d)
3. Istabraq (c)
4. Alchemy 2 (b2)
5. Alchemy 1 (b1)
6. **Consort 2 (a2)**
7. Consort 1 (a1)

Black Codes = CSC IDs
Red Codes = Farm IDs
Blue BOLD = Industry Standard

Metabolite Profiling by GC-MS

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Freeze-dried powder

+ added Internal standards (IS)

Extraction of metabolites

Methanol/Water/Chloroform

Polar Extract

(Methanol/ Water)

Amino acids;
Organic acids;
Sugars etc.

Non-polar Extract

(Chloroform)

Fatty Acids;
Fatty Alcohols;
Alkanes;
Terpenes (+Sterols)

Chemical Derivatisation

Analysis by GC-MS

Chemical Derivatisation

Abundance values for every sample:

136 polar metabolites (54 known, 82 unknowns)

74 non-polar metabolites (58 of known, 16 unknowns)

Statistical Analysis

Principal **C**omponents **A**nalysis (PCA)

Restricted **M**aximum **L**ikelihood (REML)

(ways of visualising differences)

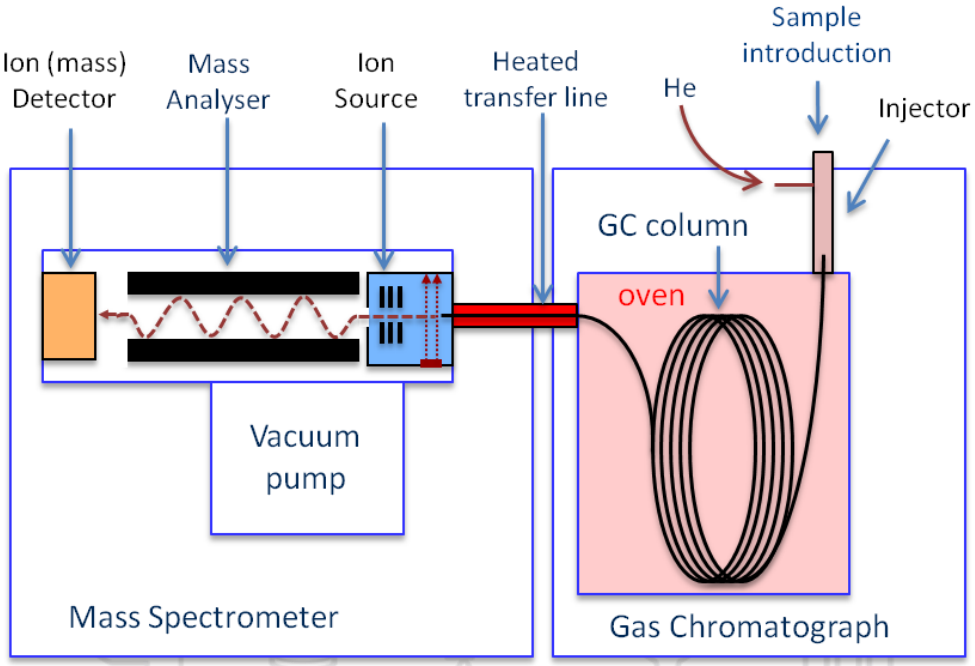
GC-MS Instrumentation

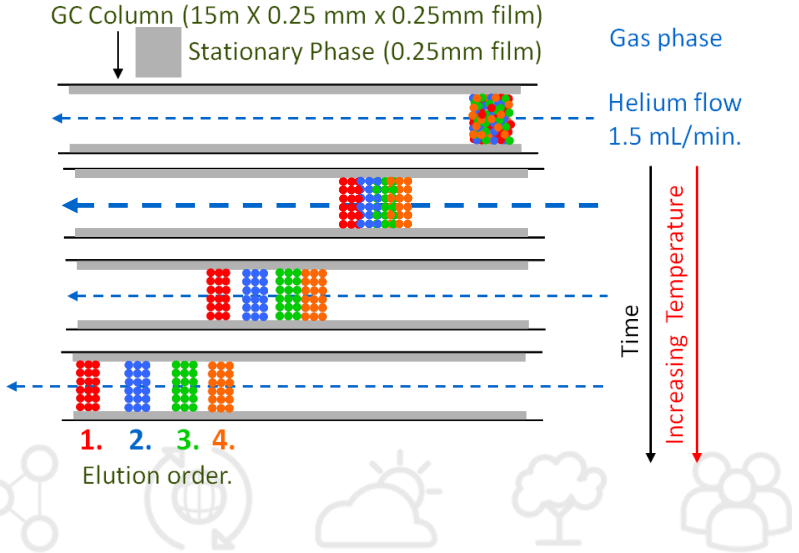
Gas Chromatograph (GC)

Separates complex mixtures into individual components on passage through a GC column.

Mass Spectrometer (MS)

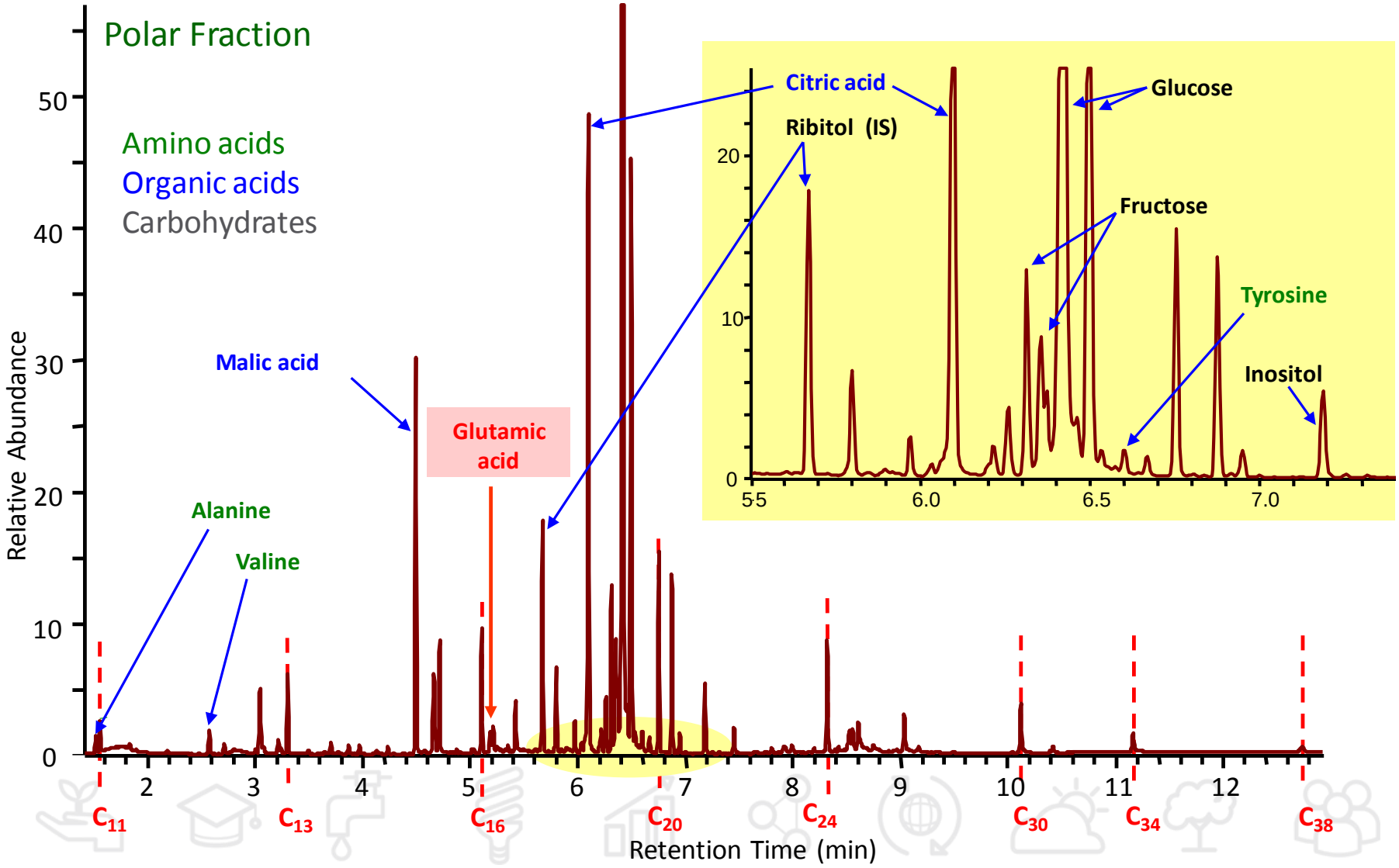
Ionises molecules which break up into smaller fragments. Measures mass of the fragments & produces a **Mass Spectrum** – a chemical finger print.





Example of Total Ion Chromatogram (TIC)

Each peak = One compound (sometimes more)



Using Mass Spectral Data to Quantify Metabolite Abundance

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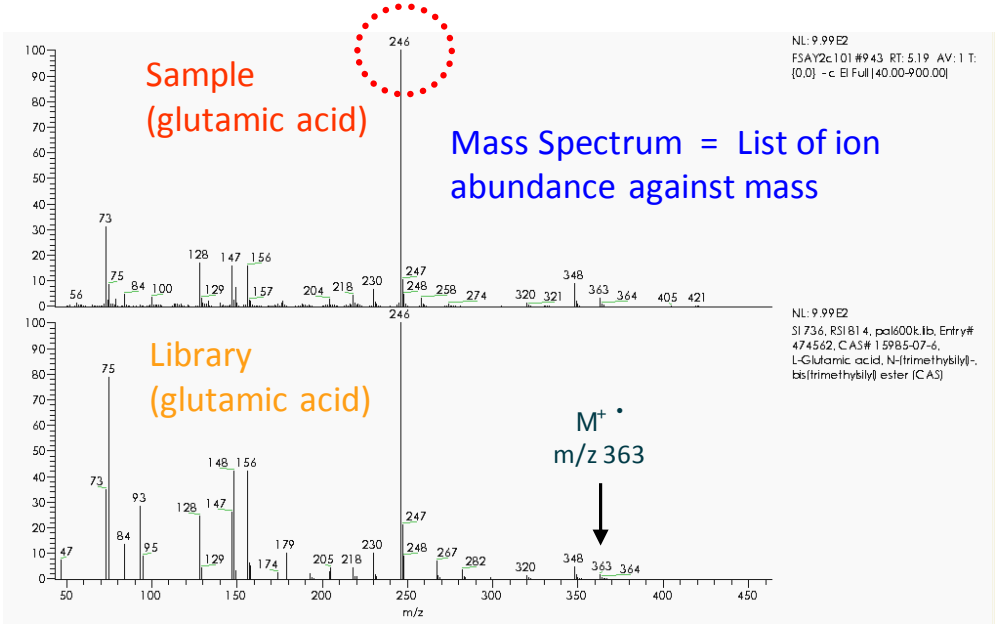
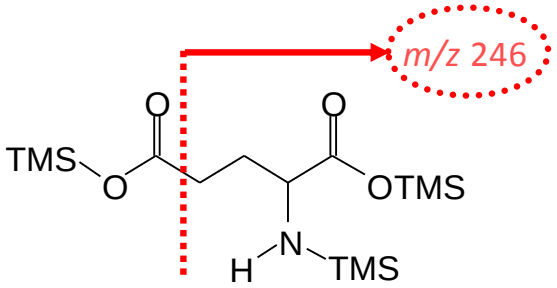
e.g. Glutamate $(TMS)_3$





Ion selection for Identification and Quantification of metabolites

- If possible Ion chosen should be:
  - Of high abundance
  - Absent from spectra of other nearby metabolites

Relative Quantification

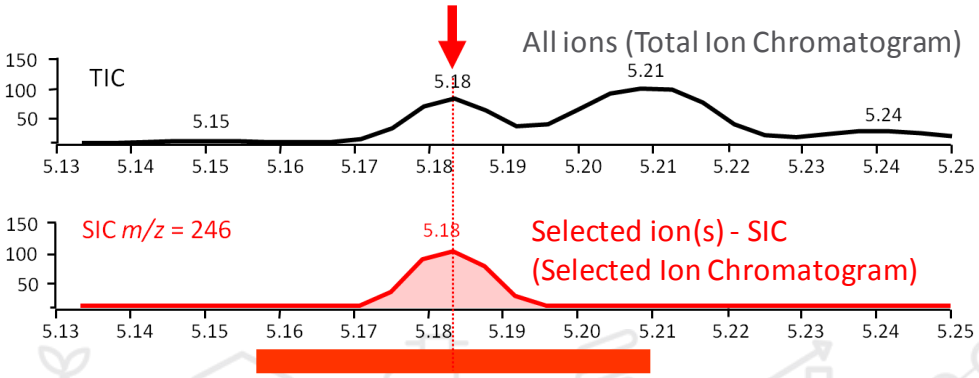
- Integrate area under SIC peak

  = Analyte abundance (or IS abundance)

- For each analyte calculate:

$$\frac{\text{Analyte abundance}}{\text{IS abundance}}$$

= Response Ratio (RR)



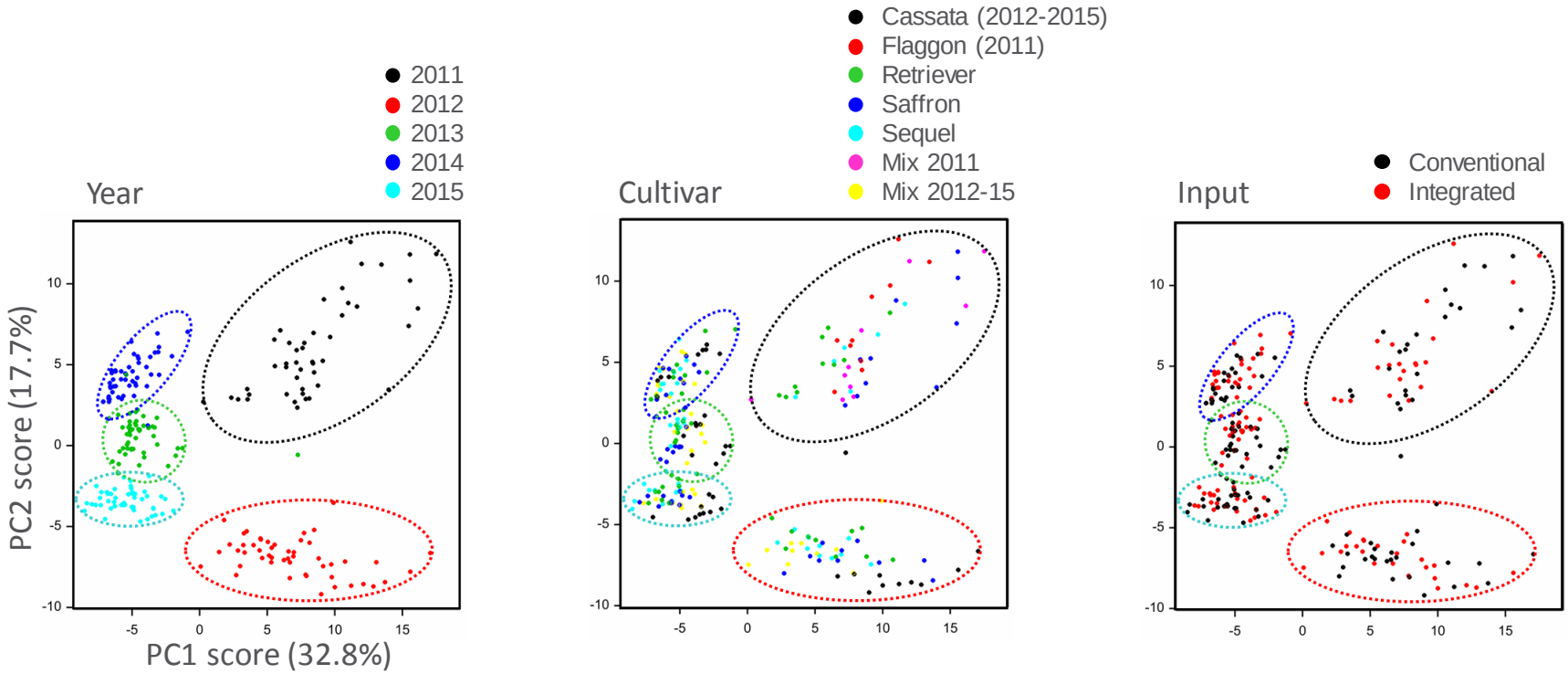
Data Analysis 1 – Principal Components Analysis

Principal Components Analysis (PCA)- This looks at the overall picture

Are distinct groupings separating?

Winter Barley Polar Metabolites 2011-2015

Variability between Year, Cultivar, Input (PC1 vs PC2)



Variation by year ?

Yes

Variation by cultivar?

Yes - some

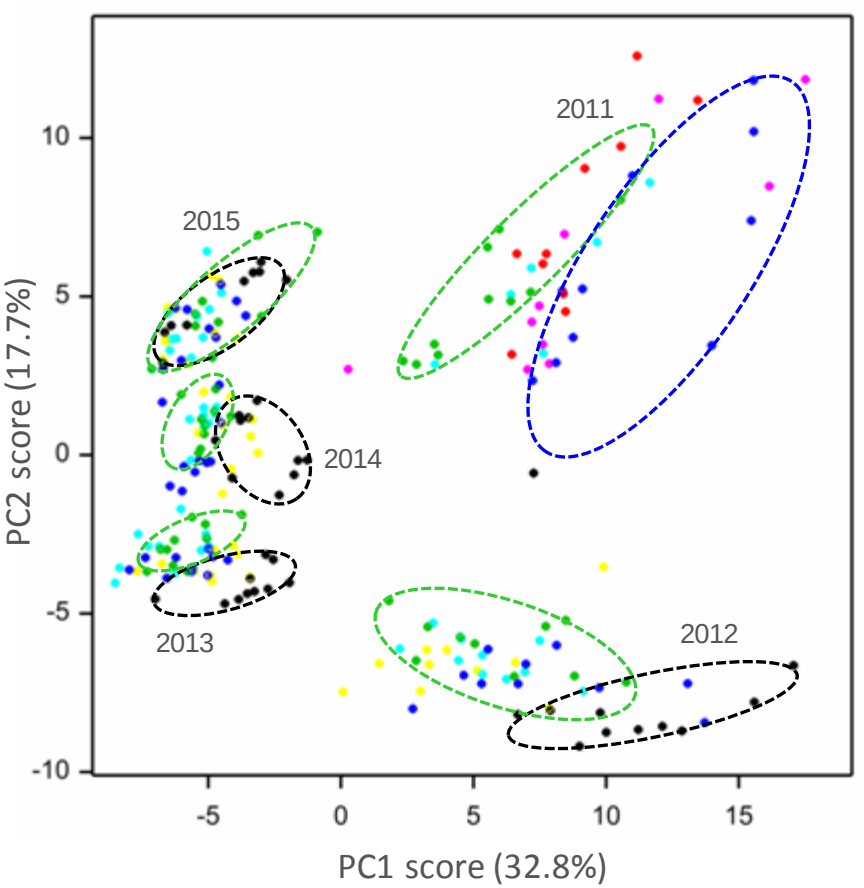
Variation by input?

No

Data Analysis 1 – Principal Components Analysis

Winter Barley Polar Metabolites 2011-2015

Variability between Cultivar (PC1 vs PC2)



Cultivar

- Cassata (2012-2015)
- Flaggon (2011)
- Retriever
- Saffron
- Sequel
- Mix 2011
- Mix 2012-15

Variation by cultivar?

2011:

Saffron separates from Retriever

2012-2014:

Cassata separates from Retriever and most other cultivars

Data Analysis 1 – Principal Components Analysis

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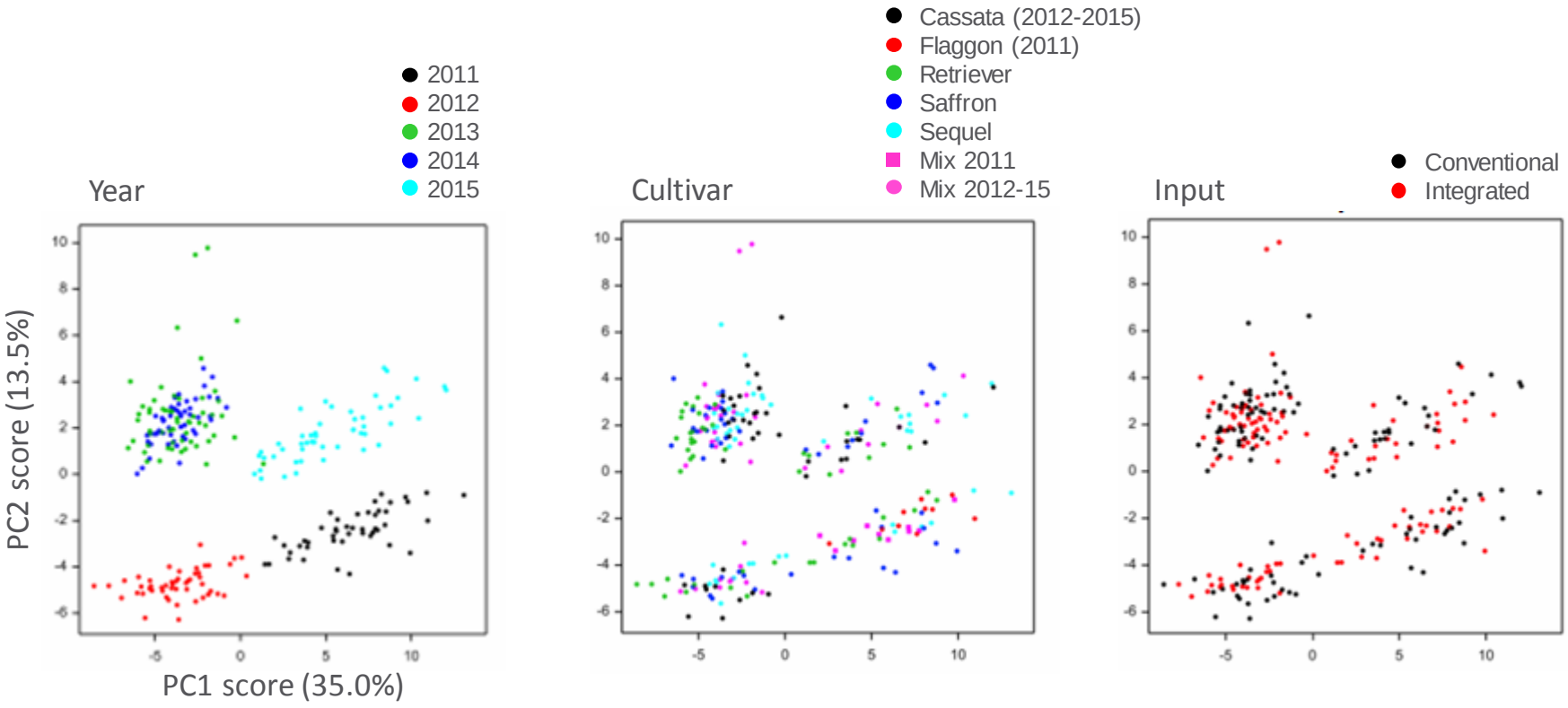
Winter Barley Non-Polar Metabolites 2011-2015

Variability between Year, Cultivar, Input (PC1 vs PC2)



Variation by year ?

Yes

Variation by cultivar?

Yes - some

Variation by input?

No

Data Analysis 1 – Principal Components Analysis

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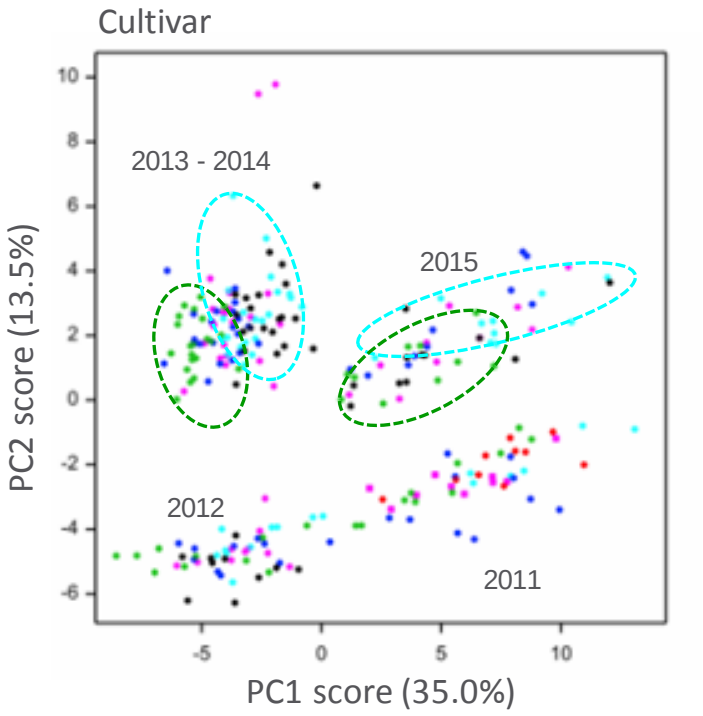
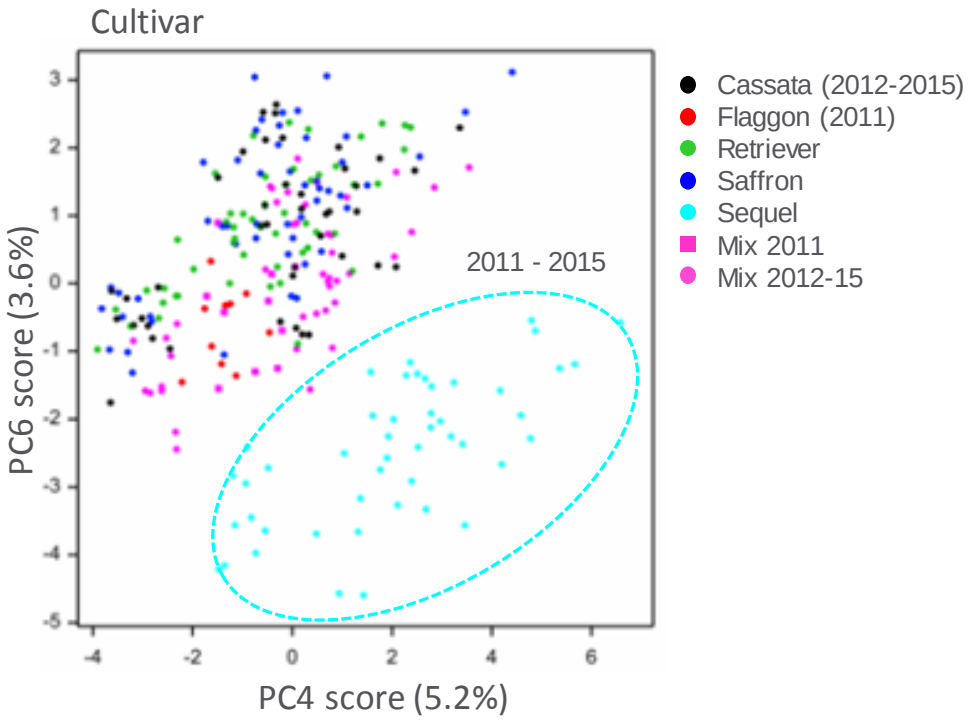
Winter Barley Non-polar Metabolites 2011-2015

Variability between Cultivar (PC 1 vs PC2; PC6 Vs PC4)





Variation by cultivar?

2013-2015:
Sequel separates from Retriever

Variation by cultivar?

2011-2015:
Sequel separates from the other cultivars

Data Analysis 2 – Restricted Maximum Likelihood

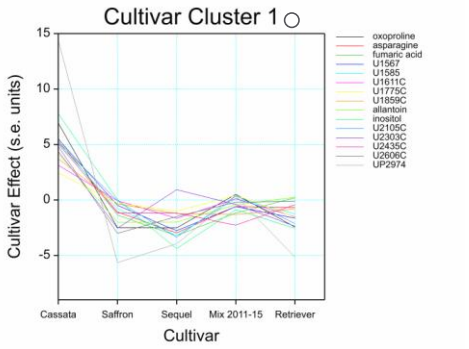
Restricted Maximum Likelihood (REML) Over-Years Cluster Analysis

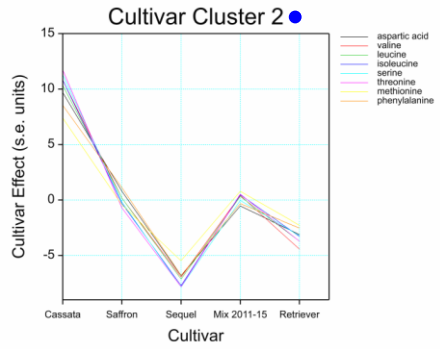
Looks at similarity in patterns of inter-cultivar variability

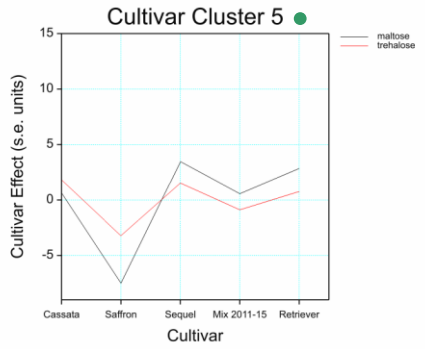
Are there any groupings of individual compounds that show similar patterns of inter-cultivar variability over 2011-2015?

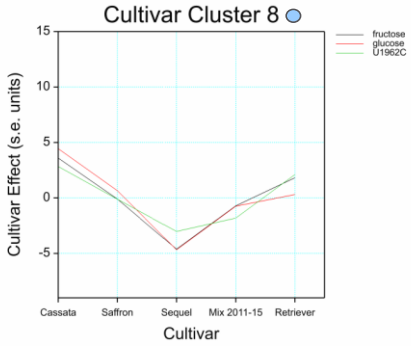
Winter Barley 2011-2015 Polar Metabolites

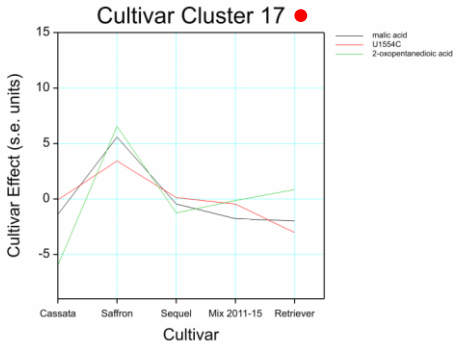
18 cluster groups identified (mainly amino acids, organic acids, carbohydrates)(5 shown)











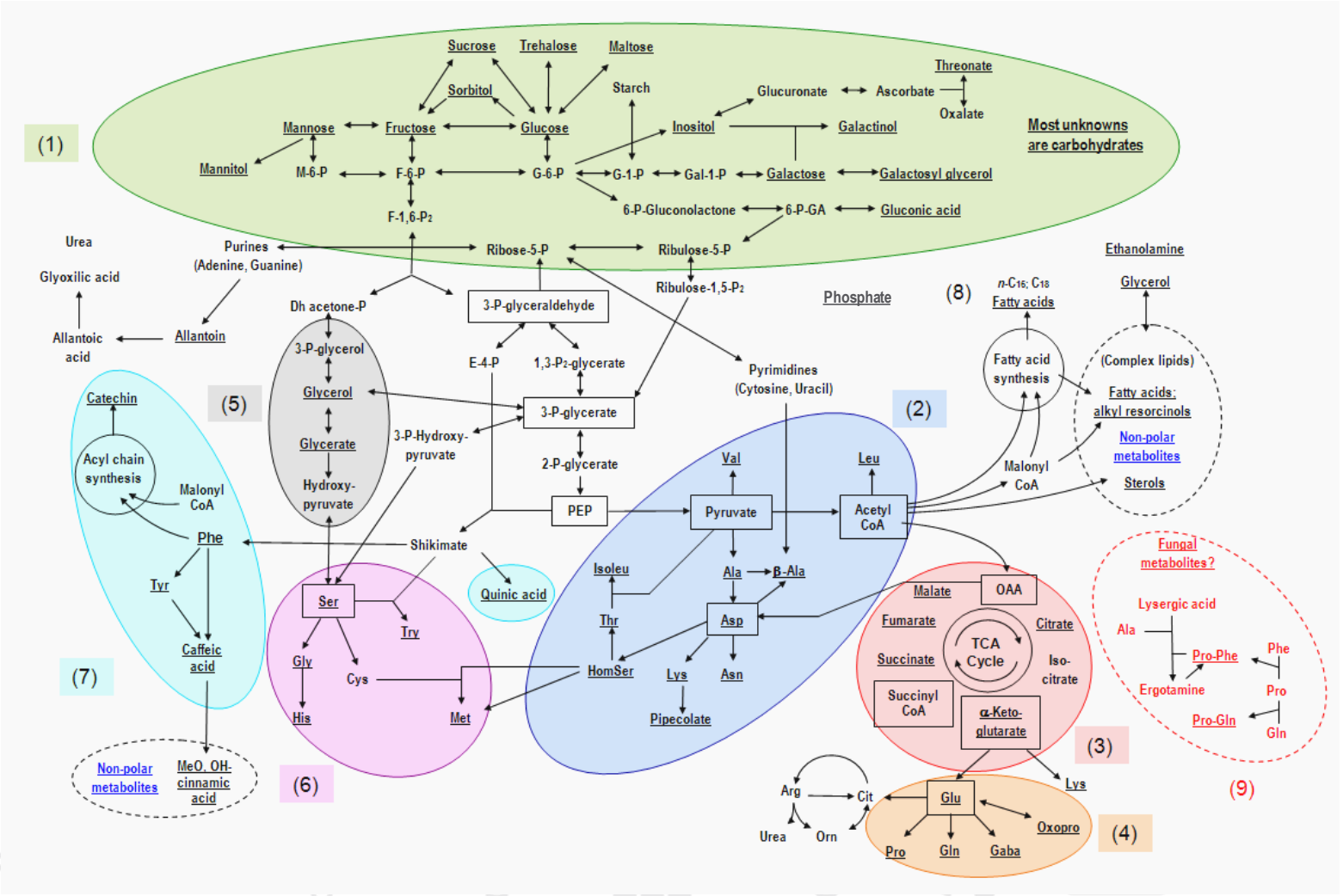
Other clusters similar to:

2 ●	17 ●	1 ○
3 ●	18 ●	5 ●
4 ●	14 ●	
8 ○	12 ●	
9 ○		
15 ●		

Data Interpretation

Winter Barley Polar Metabolites 2011-2015

Simplified general metabolite network (underlined metabolites detected in samples)



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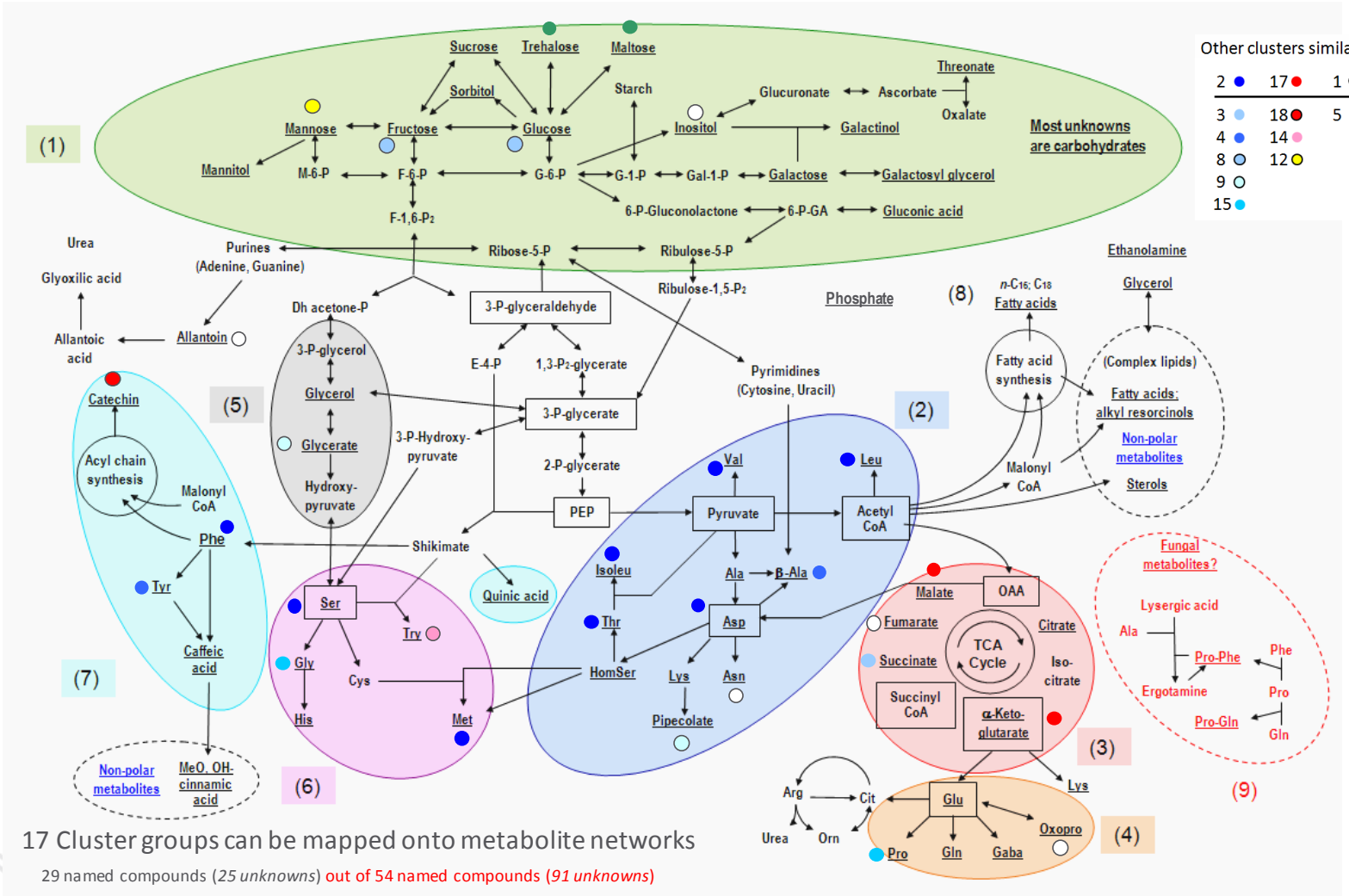


17 Cluster groups can be mapped onto metabolite networks

29 named compounds (*25 unknowns*) out of 54 named compounds (*91 unknowns*)

Data Analysis 2 – Restricted Maximum Likelihood

Restricted Maximum Likelihood (REML) Over-Years Cluster Analysis

Winter Barley 2011-2015 Non-Polar Metabolites

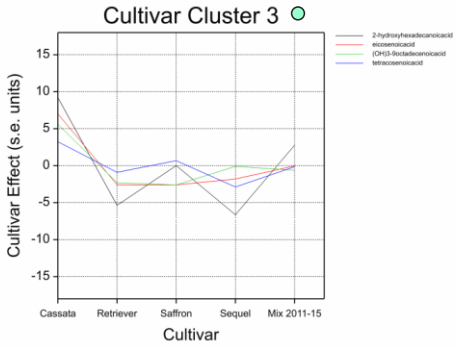
11 cluster groups identified (mainly fatty acids, sterols and alkyl resorcinols (6 shown)

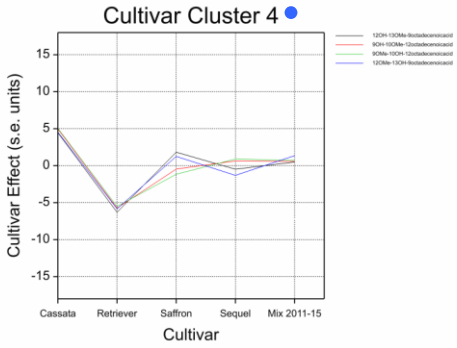
Other clusters similar to:

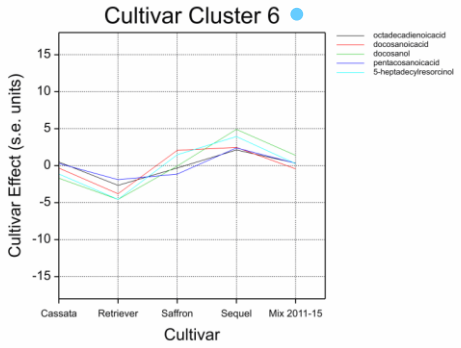
3 ● 9 ● 4 ● 6 ●

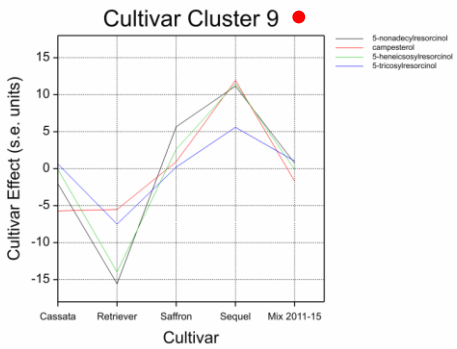
7 ● 11 ● 2 ● 5 ● 10 ● 1 ●

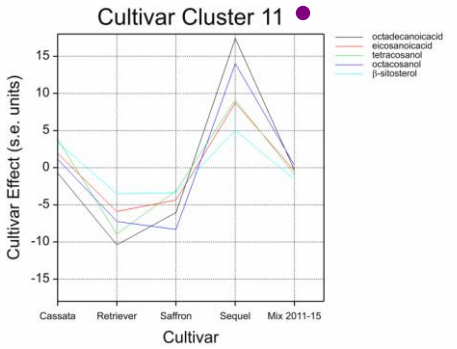
8 ●

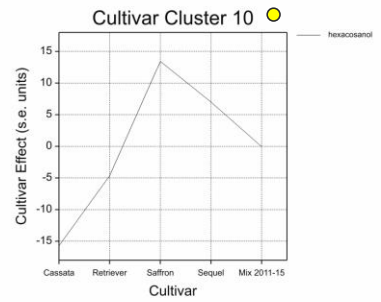








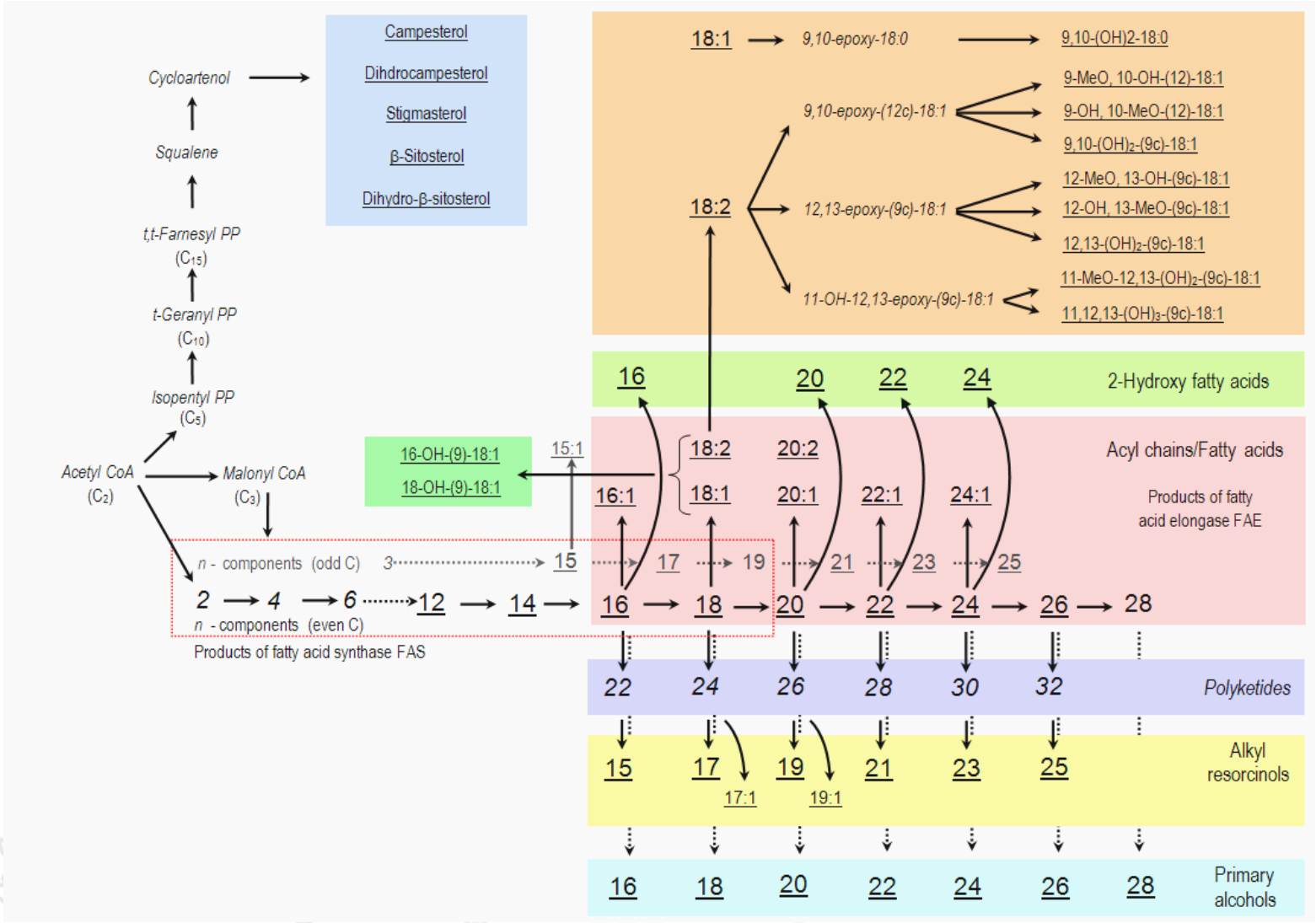




Data Interpretation

Winter Barley Non-Polar Metabolites 2011-2015

Simplified general metabolite network (underlined metabolites detected in samples)



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Winter Barley Non-Polar Metabolites 2011-2015

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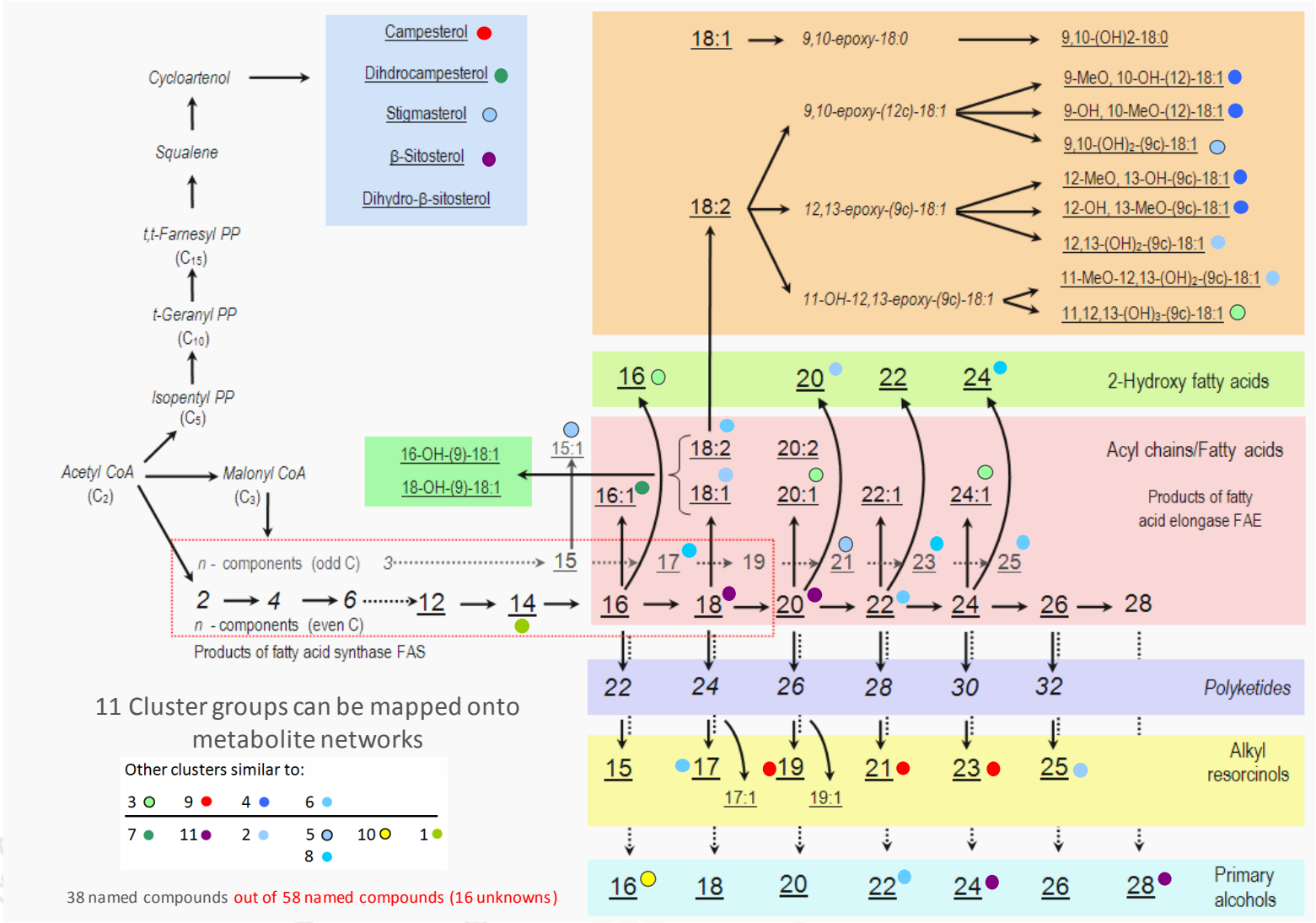

Conclusions

- Preliminary analysis of data for all three crop types over 2011-2015 using PCA indicates that year to year seasonal variation has the greatest effect on metabolite composition.
- There is evidence for cultivar-related variation in composition within individual years and consistent over years inter-cultivar variation for specific metabolite groupings.
- There is currently no evidence for consistent over-years input effects. Generally, crop growth regime (conventional or integrated) appears to have little effect on the composition of primary metabolites in cereal grains.
- The significance of any such effects will be determined following completion of in-depth across-years statistical analysis for all 3 crops over the full rotation 2011-2016.

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