Class of lipid		TAG	DAG	FFA
Scan type		Neutral loss	Neutral loss	Q1 ESI-MS
NL Mass	Fatty acid	Fatty acids mass		
	FA 15:0 standard	NL259.0		
	FA16:0	NL273.2	NL273.2	
	FA18:0	NL301.2	NL301.2	
	FA18:1	NL299.2	NL299.2	
	FA18:2	NL297.2	NL297.2	
	FA18:3	NL295.2	NL295.2	
	FA20:0	NL329.2	NL329.2	
	FA20:4 standard		NL321.2	
Mode		Positive	Positive	Negative
Curtain Gas (arbitrary units)		10	20	10
Ion Source 1 (arbitrary units)		12	45	12
Ion Source 2 (arbitrary units)		0	45	off
Declustering Potential		+120V	+100V	-125V
Entrance Potential		+15V	+10V	-10V
Collision energy		+37V	+40V	-
Collision cell exit potential		+6.5V	+10V	-
Ion spray Voltage		+5500V	+5500V	-4500V
Interface heater		ON	ON	ON
Number of cycles		40	100	25
Delay time (sec)		0	0	200

Table S1. ESI-MS/MS methods and parameters for molecular species identification of TAG, DAG and FFA. Specific acyl groups present in TAG and DAG were identified by Neutral loss masses as indicated in the table. The Q1 mass range for TAG was 500-1100, DAG: 500-800 and FFA: 100-500. Lipidomic analysis was performed using the 4000 Qtrap System, AB Sciex (Framingham, MA, U.S.A.).

	F-test p-values		
Term	Cultivar	N	Cultivar x N
LPC_16_0	0.389	0.251	0.954
LPC_18_0	**0.036**	0.080	**0.018**
LPC_18_1	0.218	0.108	0.992
LPC_18_2	0.393	0.503	0.961
LPC_18_3	0.068	0.449	0.913
PC_32_0	**<0.001**	0.762	0.204
PC_34_1	**0.014**	0.328	0.536
PC_34_2	**<0.001**	0.202	0.455
PC_34_3	**<0.001**	0.331	**0.009**
PC_36_2	**0.008**	0.642	0.230
PC_36_3	**0.003**	0.530	0.357
PC_36_4	**<0.001**	0.666	0.433
PC_36_5	**<0.001**	0.551	**0.041**
PC_36_8	**<0.001**	**<0.001**	**<0.001**
PE_34_2	**<0.001**	0.161	**0.044**
PE_36_3	**<0.001**	0.092	**<0.001**
PE_36_4	**<0.001**	**0.010**	**0.014**
DAG_32_0	**<0.001**	0.622	**<0.001**
DAG_34_1	**0.010**	0.058	0.414
DAG_34_2	0.901	0.631	0.452
DAG_36_2	0.136	**0.024**	0.058
DAG_36_3	0.585	0.205	0.515
DAG_36_4	0.096	0.467	0.807
DAG_36_5	**0.004**	**0.003**	0.722
DAG_38_0	**<0.001**	**0.019**	0.067
DAG_40_2	**0.003**	**0.035**	0.575
TAG_50_1	**0.018**	**<0.001**	**0.014**
TAG_50_2	0.149	0.236	0.560
TAG_52_2	**0.001**	0.053	0.214
TAG_52_3	0.230	0.674	0.288
TAG_52_4	0.677	0.919	0.184
TAG_52_5	**0.023**	**0.002**	**0.040**
TAG_54_3	**<0.001**	0.599	0.321
TAG_54_4	0.084	0.382	0.827
TAG_54_5	0.871	0.286	0.627
TAG_54_6	0.363	0.421	0.516
TAG_54_7	0.293	0.053	0.688
TAG_56_5	**0.003**	**0.003**	**0.002**
FFA_16_0	0.224	0.065	0.090
FFA_16_1	0.493	**0.005**	0.069
FFA_18_0	0.557	0.344	0.900
FFA_18_1	0.170	0.472	0.464

FFA_18_2	0.127	**0.008**	0.130
FFA_18_3	0.109	**0.004**	0.232
FFA_20_0	0.671	0.072	0.986
FFA_21_0	0.079	0.405	0.544
FFA_22_0	0.678	0.064	0.737
FFA_24_0	0.649	**0.029**	0.221

Table S2 A list of the p-values for F-test of cultivar, nitrogen and cultivar by nitrogen interaction of all the lipid species analysed in the study. Significant effects ($p<0.05$, F-test) for each of the lipids are highlighted in bold.

Percentage variation

CV1	CV2	CV3
33.48	21.36	11.33

Latent vectors (loadings)

Column1	CV1	CV2	CV3
ILPC_16_0	-2.906	*3.515*	5.688
ILPC_18_0	1.871	-0.758	0.539
ILPC_18_1	0.934	-0.799	0.727
ILPC_18_2	-2.007	-2.343	-5.515
ILPC_18_3	3.431	0.145	0.553
IPC_32_0	-0.175	-2.873	2.211
IPC_34_1	**-4.23**	2.988	-4.486
IPC_34_2	-1.025	-1.167	1.454
IPC_34_3	-0.801	-2.328	-2.905
IPC_36_2	2.94	2.611	3.312
IPC_36_3	-0.858	0.218	0.742
IPC_36_4	1.589	0.251	-1.997
IPC_36_5	2.492	-0.446	2.263
IPC_36_8	2.42	0.821	-0.201
IPE_34_2	-0.95	-2.605	1.092
IPE_36_3	3.18	2.059	2.191
IPE_36_4	2.255	1.875	-2.462
IDAG_32_0	-0.886	0.255	3.498
IDAG_34_1	1.862	2.366	-2.268
IDAG_34_2	1.69	-1.807	-1.845
IDAG_36_2	0.43	0.414	-0.614
IDAG_36_3	**5.076**	-1.107	5.291
IDAG_36_4	**10.016**	*3.428*	1.611
IDAG_36_5	-1.366	*-3.063*	-3.995
IDAG_38_0	1.825	-1.167	-0.014
IDAG_40_2	1.546	-1.126	-2.876
ITAG_50_1	1.342	0.162	-0.11
ITAG_50_2	0.004	*-3.702*	-1.055
ITAG_52_2	-1.372	-0.161	0.275
ITAG_52_3	-0.714	1.252	0.016
ITAG_52_4	-0.019	0.286	0.299
ITAG_52_5	1.449	*4.459*	4.467
ITAG_54_3	0.32	*3.88*	-1.748

ITAG_54_4	1.038	-0.354	0.374
ITAG_54_5	0.211	1.311	1.558
ITAG_54_6	-0.389	-1.137	-0.404
ITAG_54_7	-0.605	*-4.083*	-0.329
ITAG_56_5	-3.265	-2.119	-0.779
IFFA_16_0	2.706	0.336	2.755
IFFA_16_1	**-4.31**	0.216	-2.064
IFFA_18_0	-0.624	2.524	1.851
IFFA_18_1	1.972	-1.919	-3.017
IFFA_18_2	-0.428	*4.095*	3.715
IFFA_18_3	0.873	0.829	-1.195
IFFA_20_0	-1.385	-1.174	-1.699
IFFA_21_0	-2.585	-2.679	2.03
IFFA_22_0	3.659	2.854	1.508
IFFA_24_0	-3.165	*-3.87*	-3.704

Table S3 The Canonical Variates latent (loading) vector values on the variables (quantified lipids). The magnitude of the loadings indicates the relative importance of the original lipids in providing the discrimination seen in the CVA plot (Figure 4). Significant loading values for **CV1** are highlighted in **bold**, *CV2* are placed in *italics*, and CV3 values are underlined. In this case, within each CV, these are selected as being greater than half the maximum magnitude loading.

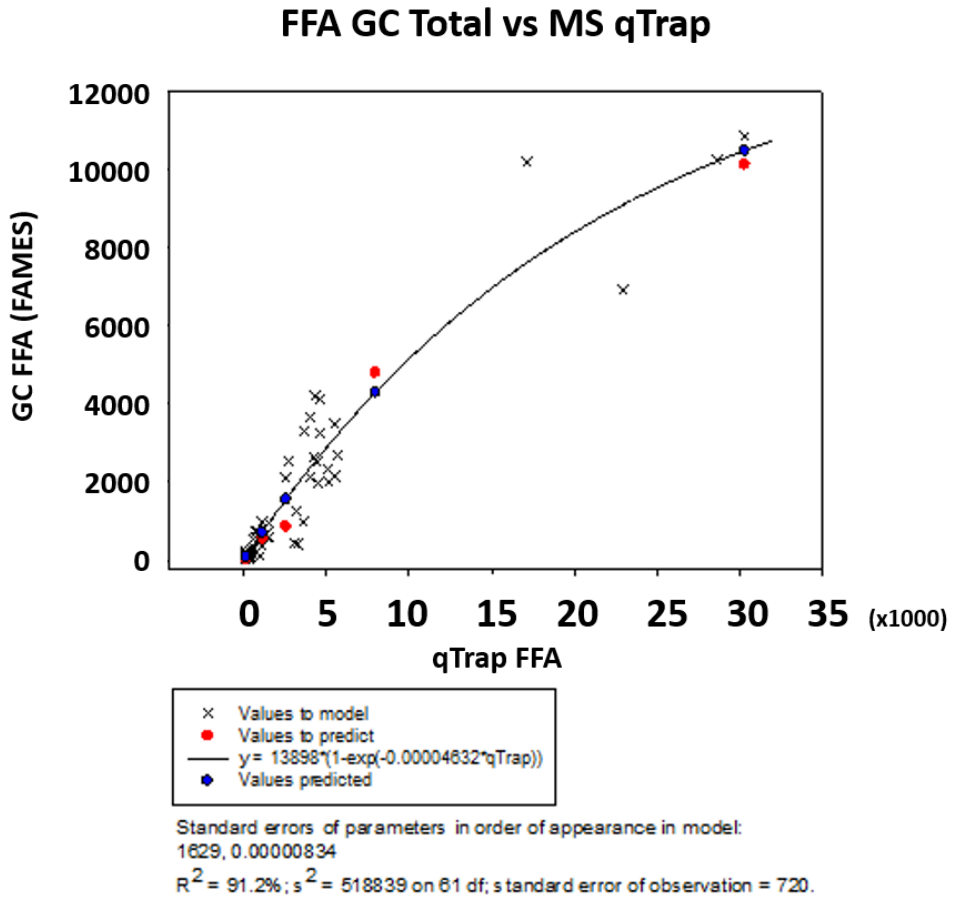

Figure S1A – The fitted asymptotic exponential curve for the FFA data from the ESI-MS/MS and GC-FID. The equation of the curve used to model FFA FAMES GC (y) on QTRAP MS (x) was

$y = A(1 - \exp(-Cx))$

where A is the upper asymptote of the curve, and C is the exponential rate of approach to A. Note that the curve goes through the origin.

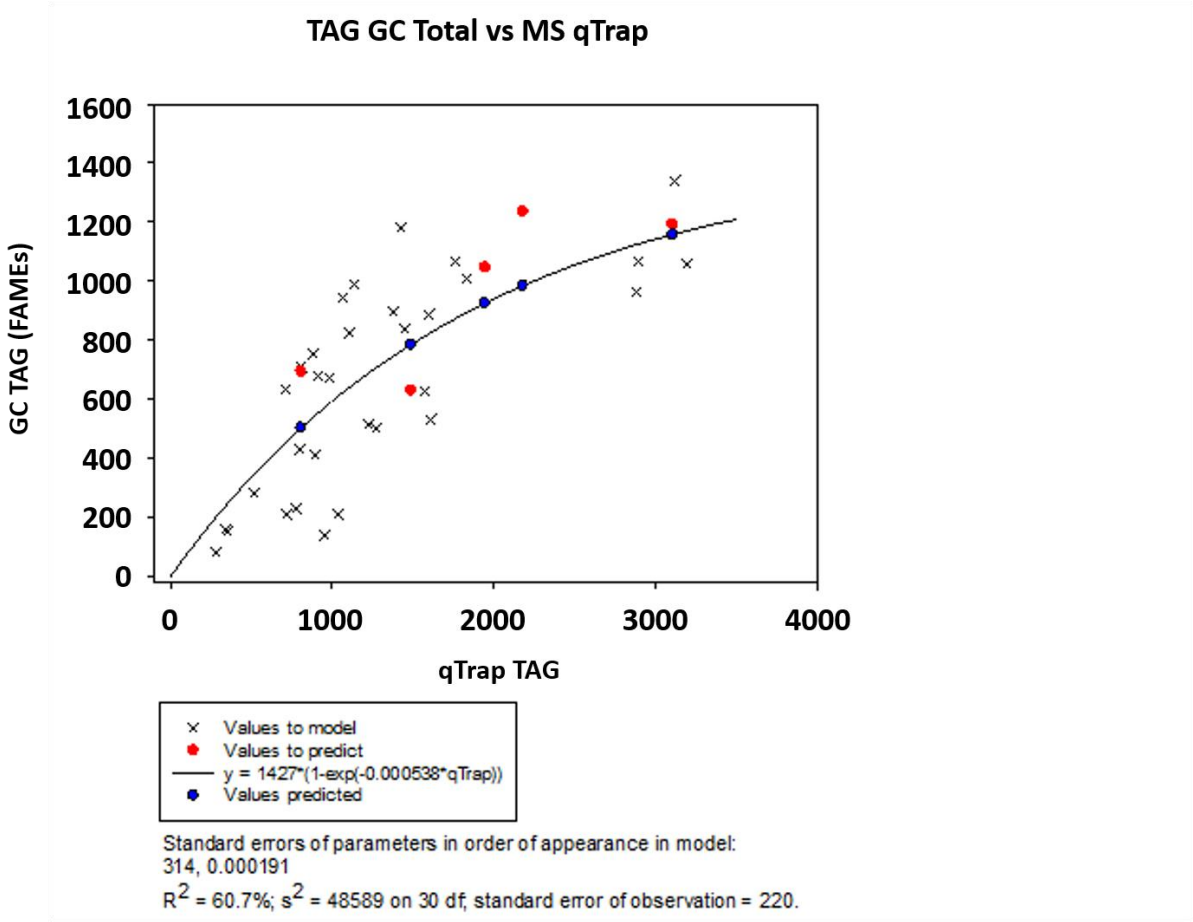

Figure S1B- The fitted asymptotic exponential curve for the TAG data from the ESI-MS/MS and GC-FID. The equation of the curve used to model TAG FAMES GC (y) on QTRAP MS TAG (x) was

$y = A(1 - \exp(-Cx))$

where A is the upper asymptote of the curve, and C is the exponential rate of approach to A. Note that the curve goes through the origin.

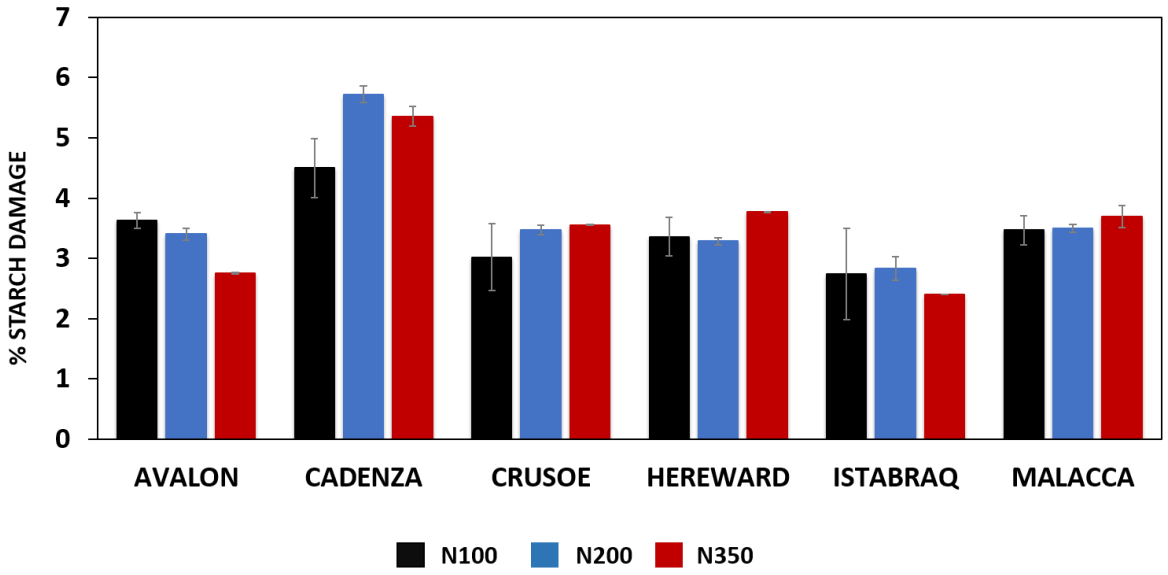

Figure S2 Starch damage analysis of the six cultivars grown in three nitrogen conditions. Starch damage levels were low being below 6% in all cases. Data are mean values of ±SE of three independent samples analysed using the Megazyme starch damage kit (Megazyme Bray, Ireland).

LPC total

Mean	SE
7.024	0.055

PC total

Cultivar	
Avalon	6.178
Cadenza	5.304
Crusoe	5.455
Hereward	5.583
Istabraq	5.232
Malacca	6.063

SED = 0.1879 on 72 df; LSD (5%) = 0.3746

PE total

Cultivar	100	200	350
Avalon	2.599	3.276	1.938
Cadenza	2.088	2.159	1.784
Crusoe	1.939	1.614	1.853
Hereward	1.550	1.874	1.733
Istabraq	0.665	0.857	0.685
Malacca	2.068	2.176	2.086

SED = 0.2811 on 72 df; LSD (5%) = 0.5603

DAG total

Mean	SE
6.186	0.033

TAG total

Cultivar	
Avalon	6.885
Cadenza	7.295
Crusoe	6.792
Hereward	6.943
Istabraq	6.643
Malacca	7.180

SED = 0.1123 on 72 df; LSD (5%) = 0.2240

N	
100	6.803
200	6.943
350	7.124

SED = 0.0794 on 72 df; LSD (5%) = 0.1584

FFA total

Cultivar	100	200	350
Avalon	7.198	7.116	7.236
Cadenza	6.798	7.589	7.412
Crusoe	7.096	7.435	6.594
Hereward	7.158	7.409	7.174
Istabraq	7.192	7.207	7.090
Malacca	7.307	7.434	7.355

SED = 0.2339 on 72 df; LSD (5%) = 0.4662

Polar lipids total

Mean	SE
7.302	0.043

Neutral lipids total

Cultivar	100	200	350
Avalon	7.920	7.941	8.072
Cadenza	7.770	8.359	8.365
Crusoe	7.879	8.005	7.774
Hereward	7.869	8.174	8.022
Istabraq	7.884	7.874	7.759
Malacca	8.117	8.146	8.231

SED = 0.1359 on 72 df; Max LSD (5%) = 0.2709

Figure S3 Relevant means on the log scale for the WGIN lipid totals. The p-values for F-test of cultivar, N and cultivar by N interactions are shown along with standard error of the difference (SED) and least significant difference (LSD) values for comparison of the means.

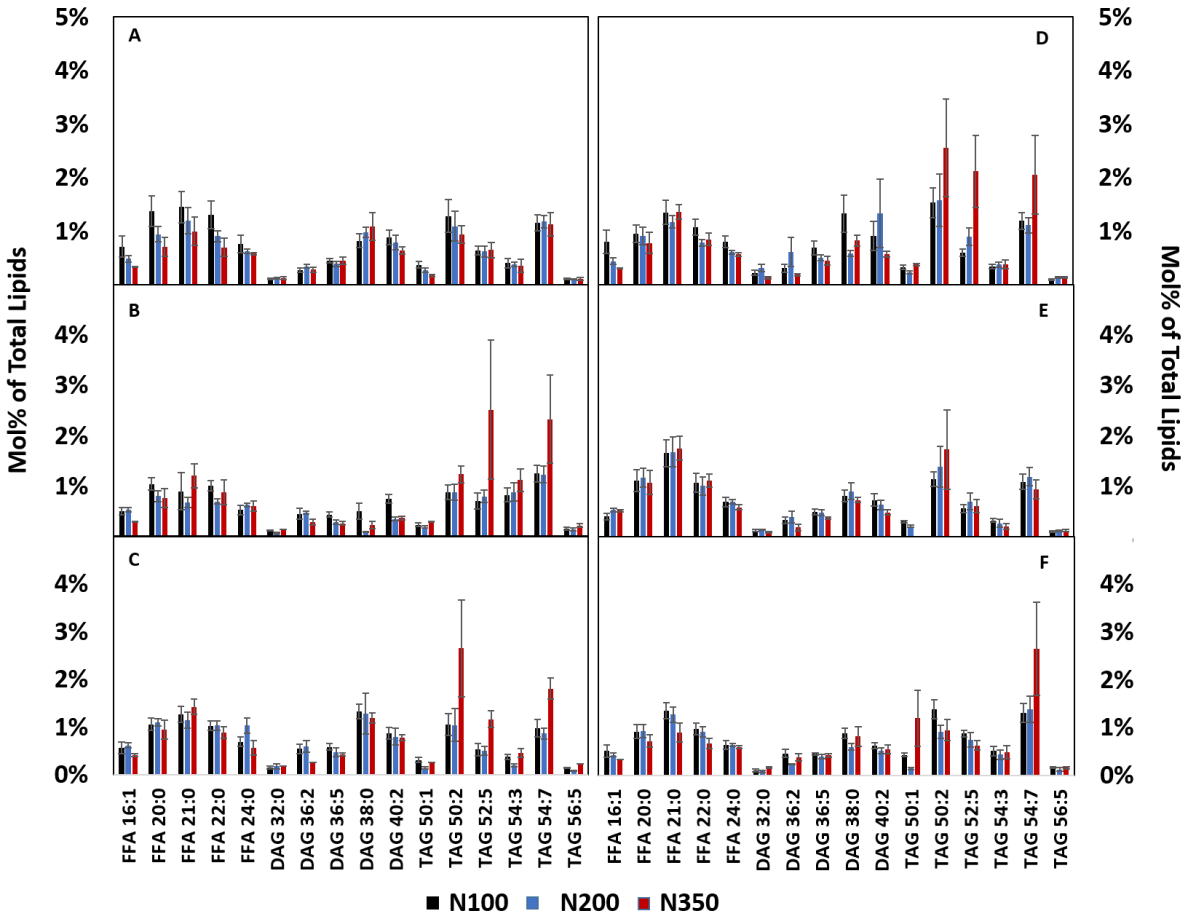

Figure S4A Lipid composition of selected minor neutral lipids between the three nitrogen conditions. The lipids displayed in this figure include 5 free fatty acids (FFA 16:1, 20:0, 21:0, 22:0, 24:0), 5 diacylglycerols (DAG 32:0, 36:2, 36:5, 38:0, 40:2) and 6 triacylglycerols (TAG 50:1, 50:2, 52:5, 54:3, 54:7, 56:5). A, Avalon; B, Cadenza; C, Crusoe; D, Hereward; E, Istabraq; F, Malacca. Data are mean values of ±SE of five independent samples analysed via ESI-MS/MS.

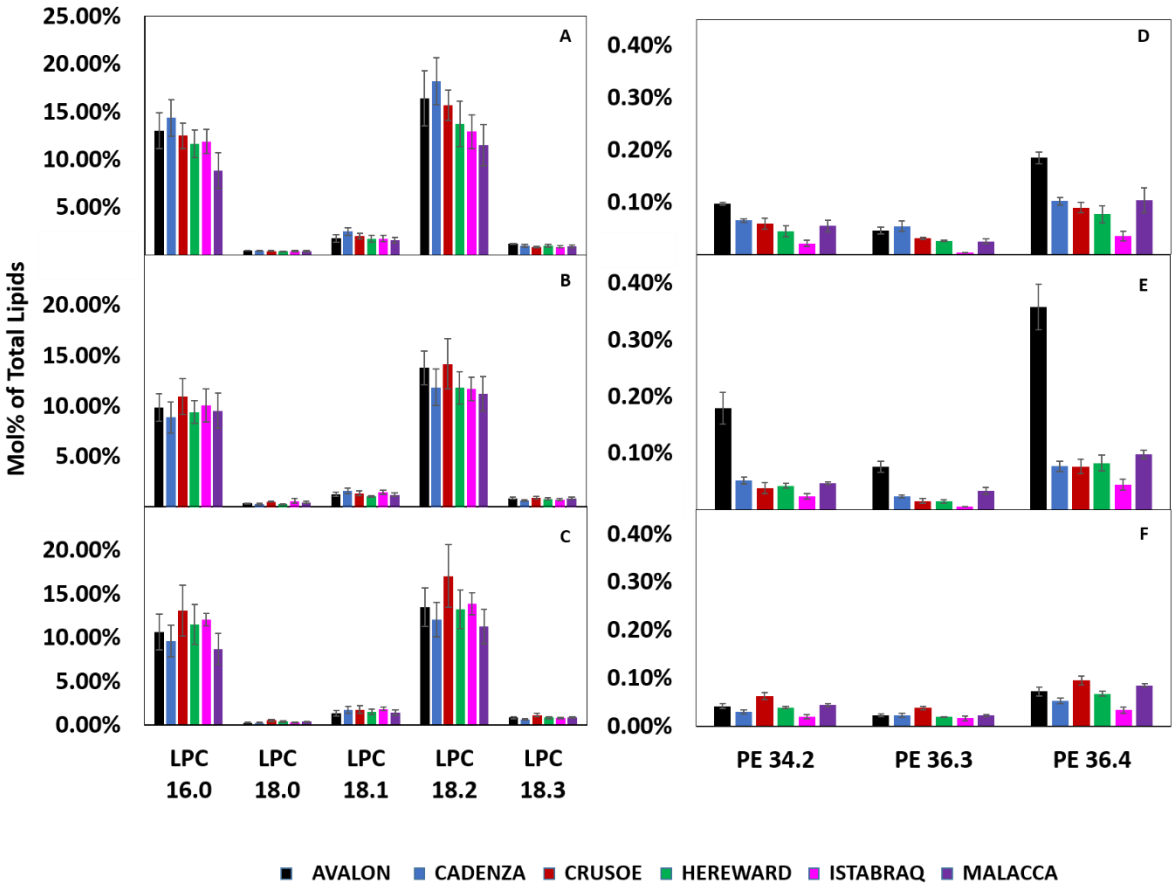

Figure S4B Comparison of LPC and PE (mol % of total lipids) between the six cultivars. The 5 species of lysophosphatidylcholine (LPC 16:0, 18:0, 18:1, 18:2 and 18:3) are displayed in this figure alongside the 3 phosphatidylethanolamines (PE 34:2, 36:3 and 36:4) which were not clearly visible in Figure 2. A&D, grown at 100kg/N/Ha; B&D, grown at 200kg/N/Ha; C&F, grown at 350kg/N/Ha. Data are mean values of ±SE of five independent samples analysed via ESI-MS/MS.