Electronic Supplementary Information

Application of vibrational spectroscopy techniques to non-destructively monitor plant health and development

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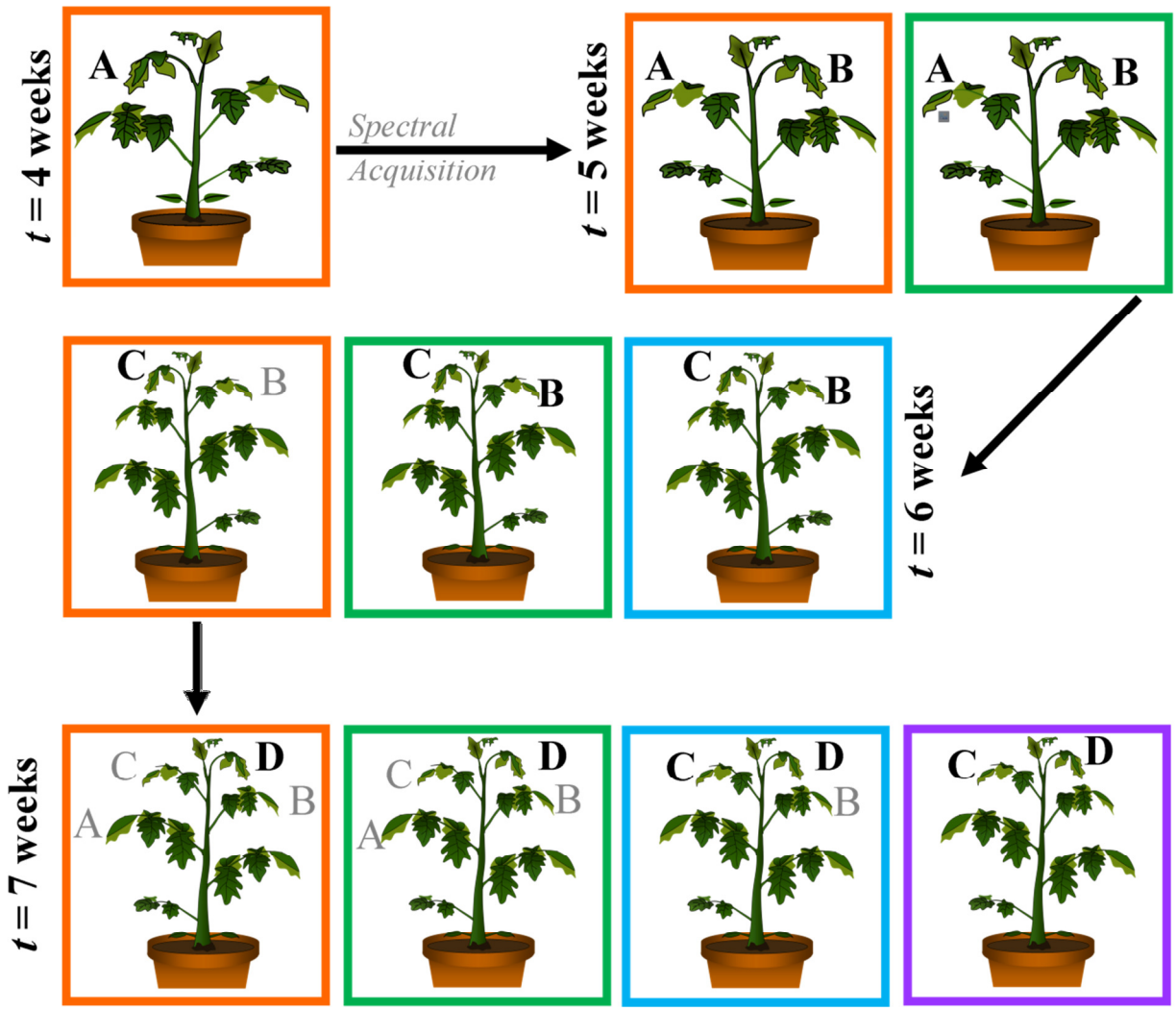

Figure S1. An overview schematic of the experimental procedure for the review of non-destructive analysis. A new set of plants was introduced each week and is symbolised by coloured boxes ('Plant Set 1' orange, 'Plant Set 2' green, 'Plant Set 3' blue and 'Plant Set 4' purple). Individual leaflets are labelled and represent equivalent leaflets between plant sets. Greyed out labels show previously analysed leaflet, which are no longer being interrogated at that particular week of analysis.

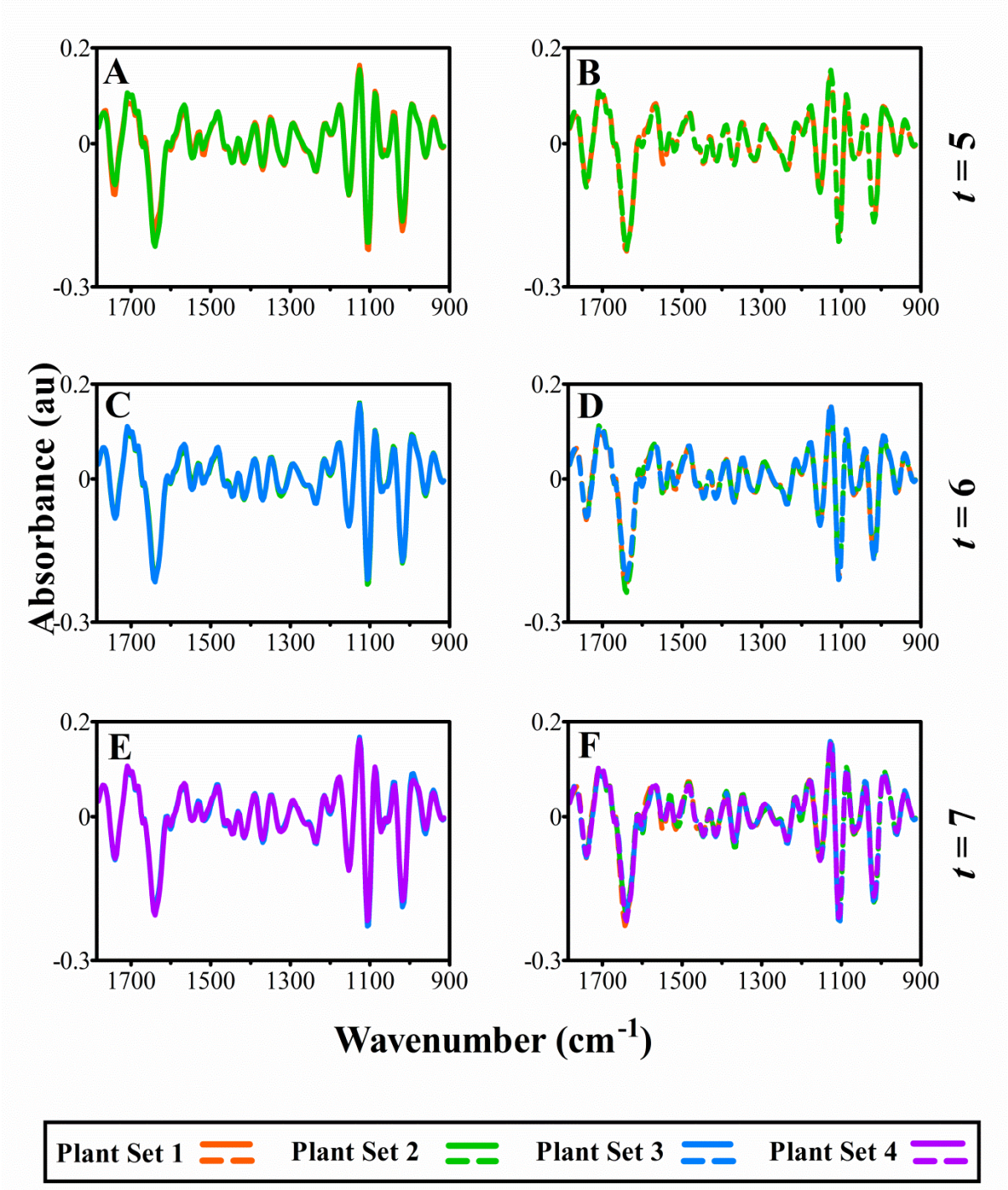

Figure S2. ATR-FTIR class means spectra to compare previously analysed and equivalent leaflets (full line), as well as differences in systemic leaflets (dashed line). At week 5 (t = 5) of development, plants were compared at mature leaflet A (**A**) and newly expanded leaflet B (**B**); at week 6 (t = 6), plants were compared at leaflet B (**C**) and newly expanded C (**D**); and finally at week 7 (t = 7), leaflet C (**E**) and D (**F**) were compared. Spectra were processed with second order differentiation baseline correction and vector normalisation.

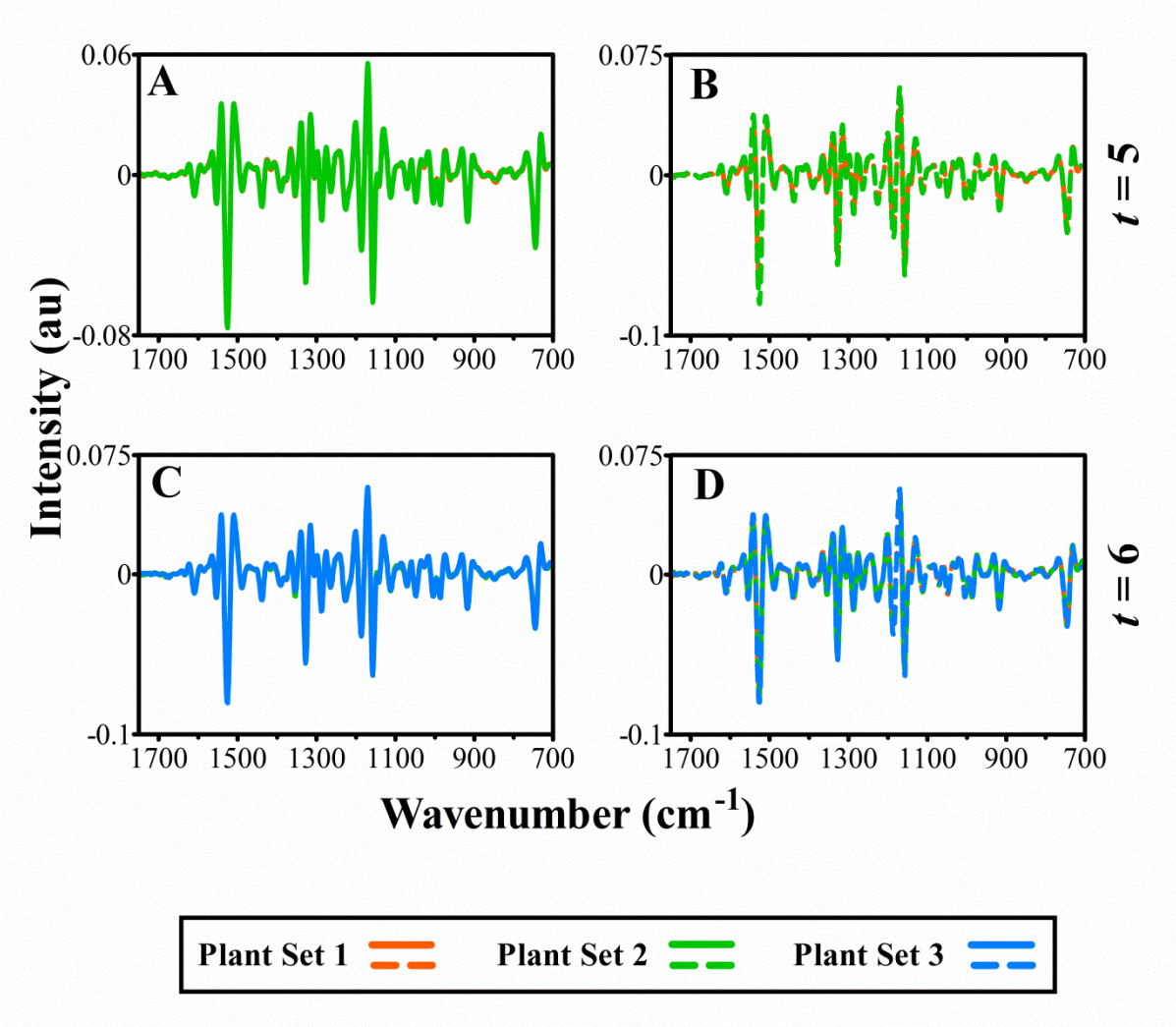

Figure S3. Raman class means spectra to compare previously analysed and equivalent leaflets (full line), as well as differences in systemic leaflets (dashed line). At week 5 (t = 5) of development, plants were compared at mature leaflet A (**A**) and newly expanded leaflet B (**B**); at week 6 (t = 6), plants were compared at leaflet B (**C**) and newly expanded C (**D**). Spectra were processed with first order differentiation baseline correction and vector normalisation.

Table S1.A. Average rate of H_2O assimilation (mmol H_2O m^{-2} s^{-1} ± standard error) for equivalent leaflets (A-D) in four plant sets over a time course of three weeks (*t* = 5-7) to determine any detrimental effects of ATR-FTIR spectroscopy interrogation. Leaflets previously analysed using the technique are shown in italic. No significant responses were determined by statistical analyses.

	***t* = 5 weeks**		***t* = 6 weeks**		***t* = 7 weeks**	
Leaflet	**A**	**B**	**B**	**C**	**C**	**D**
Plant Set						
1	*0.84 ± 0.12*	1.92 ± 0.18		2.04 ± 0.23		1.58 ± 0.16
2	2.08 ± 0.40	2.40 ± 0.62	*1.09 ± 0.23*	1.31 ± 0.18		2.02 ± 0.32
3			2.32 ± 0.30	1.47 ± 0.26	*1.32 ± 0.25*	1.63 ± 0.29
4					0.77 ± 0.27	1.18 ± 0.21

Table S1.B. Average rate of stomatal conductance (mmol m^{-2} s^{-1} ± standard error)

	***t* = 5 weeks**		***t* = 6 weeks**		***t* = 7 weeks**	
	A	**B**	**B**	**C**	**C**	**D**
1	*89.25 ± 27.16*	106.50 ± 19.37		47.56 ± 8.45		73.83 ± 8.49
2	64.50 ± 16.59	70.83 ± 13.59	*120.67 ± 20.59*	70.56 ± 14.73		101.83 ± 20.23
3			93.67 ± 18.49	42.78 ± 8.50	*62.33 ± 15.83*	80.67 ± 17.95
4					33.33 ± 13.14	50.83 ± 10.54

Table S1.C. Average rate of internal CO_2 (µmol mol^{-1} ± standard error)

	***t* = 5 weeks**		***t* = 6 weeks**		***t* = 7 weeks**	
	A	**B**	**B**	**C**	**C**	**D**
1	*206.36 ± 30.66*	108.22 ± 9.30		135.00 ± 29.00		122.33 ± 23.98
2	133.11 ± 19.57	187.25 ± 37.30	*155.71 ± 24.78*	214.75 ± 43.17		293.33 ± 132.29
3			208.57 ± 42.27	267.67 ± 38.40	*77.75 ± 9.26*	191.75 ± 39.32
4					372.33 ± 99.15	289.17 ± 64.62

Table S1.D. Average rate of vapour pressure deficit (mPa Pa^{-1} ± standard error)

	***t* = 5 weeks**		***t* = 6 weeks**		***t* = 7 weeks**	
	A	**B**	**B**	**C**	**C**	**D**
1	*25.27 ± 0.73*	23.22 ± 0.99		24.09 ± 0.42		23.08 ± 0.32
2	26.87 ± 0.63	23.67 ± 0.87	*25.67 ± 0.57*	23.24 ± 0.63		22.13 ± 1.03
3			23.29 ± 0.59	21.99 ± 1.01	*23.17 ± 1.03*	22.45 ± 0.72
4					25.57 ± 0.77	24.38 ± 0.68

Table S2. *P*-values corresponding to cross validated PCA-LDA scores plots in Figure 3 derived by one-way ANOVA with Tukey's multiple comparison test. (**A**) week 5; (**B**) week 6; (**C**) week 7 of plant development. Significant values are highlighted in bold type and colours correspond to specific plants sets highlighted in Figure 3 (red = 'Plant Set 1', green 'Plant Set 2', blue = 'Plant Set 3', purple = 'Plant Set 4'). Columns and rows in full colour represent comparison of leaves previously analysed and bordered cells represent comparisons of systemic leaves.

	Leaflets					
(A) Week 5		**A**	**A**	**B**	–	–
	A	*P* >0.05	–	–	–	–
	B	*P* >0.05	*P* >0.05	–	–	–
	C	*P* >0.05	*P* >0.05	*P* >0.05	–	–
(B) Week 6		**B**	**B**	**C**	**C**	–
	B	*P* >0.05	–	–	–	–
	B	*P* >0.05	*P* >0.05	–	–	–
	C	*P* >0.05	*P* >0.05	*P* >0.05	–	–
	C	*P* >0.05	*P* >0.05	*P* >0.05	*P* >0.05	–
(C) Week 7		**C**	**C**	**D**	**D**	**D**
	C	*P* >0.05	–	–	–	–
	D	*P* >0.05	*P* >0.05	–	–	–
	D	*P* >0.05	*P* >0.05	*P* >0.05	–	–
	D	*P* >0.05	*P* >0.05	*P* >0.05	*P* >0.05	–
	D	*P* >0.05	*P* >0.05	*P* >0.05	*P* >0.05	*P* >0.05

Table S3. *P*-values corresponding to cross validated PCA-LDA scores plots in Figure 4 derived by one-way ANOVA with Tukey's multiple comparison test. Table (**A**) week 5; (**B**) week 4 of plant development. Significant values are highlighted in bold type and colours correspond to specific plants sets highlighted in Figure 3 (red = 'Plant Set 1', green 'Plant Set 2', blue = 'Plant Set 3', purple = 'Plant Set 4'). Columns and rows in full colour represent comparison of leaves previously analysed and bordered cells represent comparisons of systemic leaves.

	Leaflets				
(A) Week 5		**A**	**A**	**B**	–
	A	*P* >0.05	–	–	–
	B	*P* >0.05	*P* >0.05	–	–
	B	*P* >0.05	*P* >0.05	*P* >0.05	–
(B) Week 6		**B**	**B**	**C**	**C**
	B	*P* >0.05	–	–	–
	C	*P* >0.05	*P* >0.05	–	–
	C	*P* >0.05	*P* >0.05	*P* >0.05	–
	C	*P* >0.05	*P* >0.05	*P* >0.05	*P* >0.05

Table S4. *P*-values corresponding to cross validated PCA-LDA scores plots of Figure 5C (**A**) and Figure 5D (**B**) deduced by one-way ANOVA with Tukey's multiple comparison test. Significant values are highlighted in bold type and colours correspond to classes (days) as shown in Figure 5.

	Days										
(A) ATR-FTIR	**1**	**2**	**3**	**4**	**5**	**7**	**9**	**10**	**11**	**13**	**14**
2	*P* > 0.05	–	–	–	–	–	–	–	–	–	–
3	*P* > 0.05	*P* > 0.05	–	–	–	–	–	–	–	–	–
4	***P* < 0.05**	*P* > 0.05	*P* > 0.05	–	–	–	–	–	–	–	–
5	***P* < 0.05**	*P* > 0.05	*P* > 0.05	*P* > 0.05	–	–	–	–	–	–	–
7	*P* > 0.05	*P* > 0.05	*P* > 0.05	*P* > 0.05	*P* > 0.05	–	–	–	–	–	–
9	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.05**	***P* < 0.05**	***P* < 0.001**	–	–	–	–	–
10	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.05**	***P* < 0.05**	***P* < 0.001**	*P* > 0.05	–	–	–	–
11	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.01**	***P* < 0.01**	***P* < 0.001**	*P* > 0.05	*P* > 0.05	–	–	–
13	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* > 0.05	*P* > 0.05	*P* > 0.05	–	–
14	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* > 0.05	*P* > 0.05	*P* > 0.05	*P* > 0.05	–
17	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	*P* > 0.05	*P* > 0.05	*P* > 0.05	*P* > 0.05	*P* > 0.05

(B) Raman	**1**	**2**	**3**	**4**	**5**	**7**	**9**	**10**	**13**
2	***P* < 0.01**	–	–	–	–	–	–	–	–
3	*P* > 0.05	*P* > 0.05	–	–	–	–	–	–	–
4	***P* < 0.01**	*P* > 0.05	*P* > 0.05	–	–	–	–	–	–
5	***P* < 0.001**	*P* > 0.05	*P* > 0.05	*P* > 0.05	–	–	–	–	–
7	*P* > 0.05	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	–	–	–	–
9	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	–	–	–
10	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	–	–
13	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	–
15	***P* < 0.001**	*P* > 0.05	*P* > 0.05	*P* > 0.05	*P* > 0.05	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**

Table S5. *P*-values for linear regression curve analysis in most discriminating wavenumbers, calculated at 95% confidence rates, with significant values highlighted in bold, derived from Figure 10. Colours correspond to Figure 8.

	Wavenumber (cm^{-1})	NE	M	S
ATR-FTIR	1107	***P* <0.01**	*P* <0.05	*P* >0.05
	1639	***P* <0.0001**	***P* <0.0001**	*P* >0.05
	1015	***P* <0.0001**	***P* <0.001**	*P* >0.05
Raman	1328	*P* >0.05	*P* >0.05	*P* >0.05
	1158	*P* >0.05	*P* >0.05	*P* >0.05
	1529	*P* >0.05	*P* >0.05	***P* <0.05**