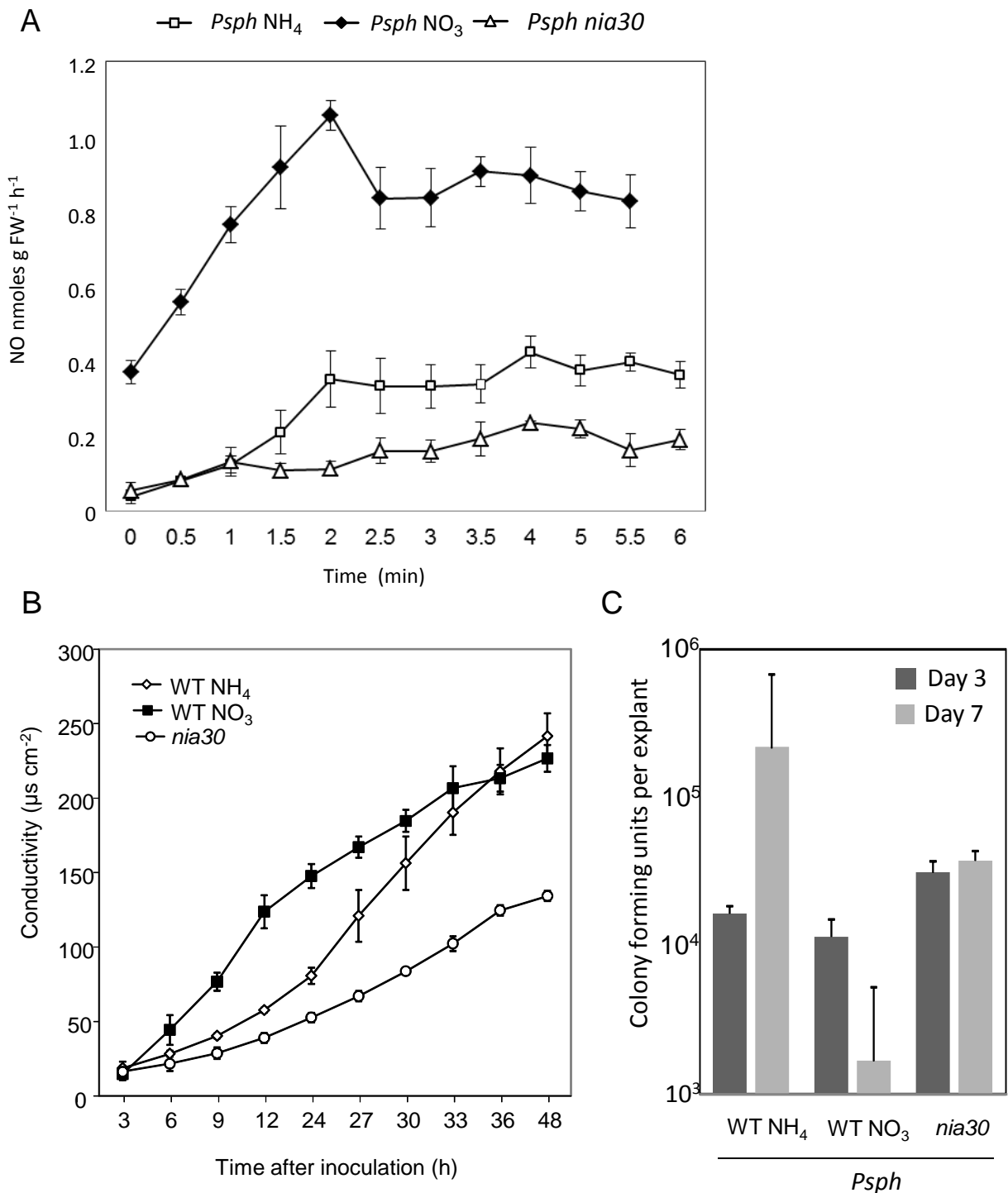


# The form of nitrogen nutrition affects resistance against *Pseudomonas syringae* pv. *phaseolicola* in tobacco

Kapuganti J. Gupta, Yariv Brotman, Shruthi Segu, Tatiana Zeier<sup>5</sup>, Jürgen Zeier, Stefan T. Persijn, Simona M. Cristescu, Frans J. M. Harren, Hermann Bauwe, Alisdair R. Fernie, Werner M. Kaiser, Luis A. J. Mur

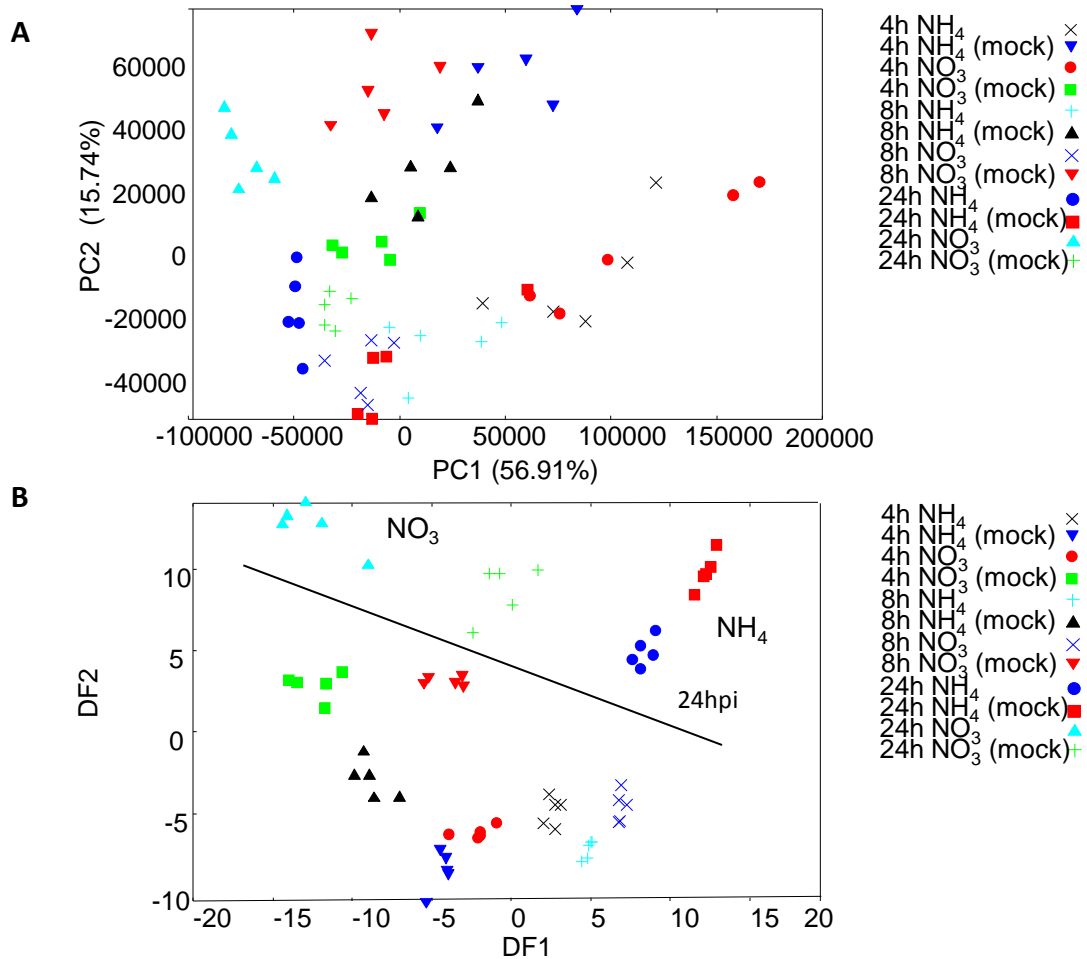
Supplementary Table 1: Loading vectors for PCA models shown in Fig. 5A, B and C which analyse metabolite changes occurring in NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> in tobacco leaves following mock and actual inoculation with *Pseudomonas syringae* pathovar *phaseolicola*

	Fig. 5A		Fig. 5B		Fig. 5C	
	Mock		NO <sub>3</sub> <sup>-</sup> -treated		NH <sub>4</sub> <sup>+</sup> -treated	
	PC1	PC2	PC1	PC2	PC1	PC2
4-aminobutyric acid	0.189908	0.077565	0.08243	0.08763	<b>0.1452</b>	<b>0.146334</b>
Galactose	<b>0.05578</b>	<b>0.674438</b>	<b>0.053127</b>	<b>0.65843</b>	<b>-0.2877</b>	<b>0.274565</b>
Fumarate	<b>0.6234</b>	<b>0.16878</b>	<b>0.602465</b>	<b>0.09085</b>	<b>0.52104</b>	<b>-0.19512</b>
Glutamine	<b>0.2147</b>	<b>0.6877</b>	<b>0.192641</b>	<b>0.535432</b>	<b>-0.09445</b>	<b>0.579814</b>
Glutamate	<b>0.04377</b>	<b>0.5432</b>	<b>-0.15874</b>	<b>0.2874</b>	<b>0.095644</b>	<b>0.200134</b>
Inositol	<b>0.05324</b>	<b>0.3348</b>	<b>-0.00747</b>	<b>0.4015</b>	<b>-0.18766</b>	<b>0.146521</b>
Malate	<b>0.6114</b>	<b>-0.5687</b>	<b>0.388412</b>	<b>0.1657</b>	<b>0.44351</b>	<b>-0.11745</b>
Proline	<b>0.061011</b>	<b>0.4125</b>	<b>-0.20876</b>	<b>0.209144</b>	<b>0.10457</b>	<b>0.19674</b>
Putrescine	0.0954	0.11636	<b>0.19833</b>	<b>-0.7223</b>	0.401121	0.501247
Spermidine	-0.04714	0.02751	<b>0.212</b>	<b>0.2247</b>	0.004574	0.00784



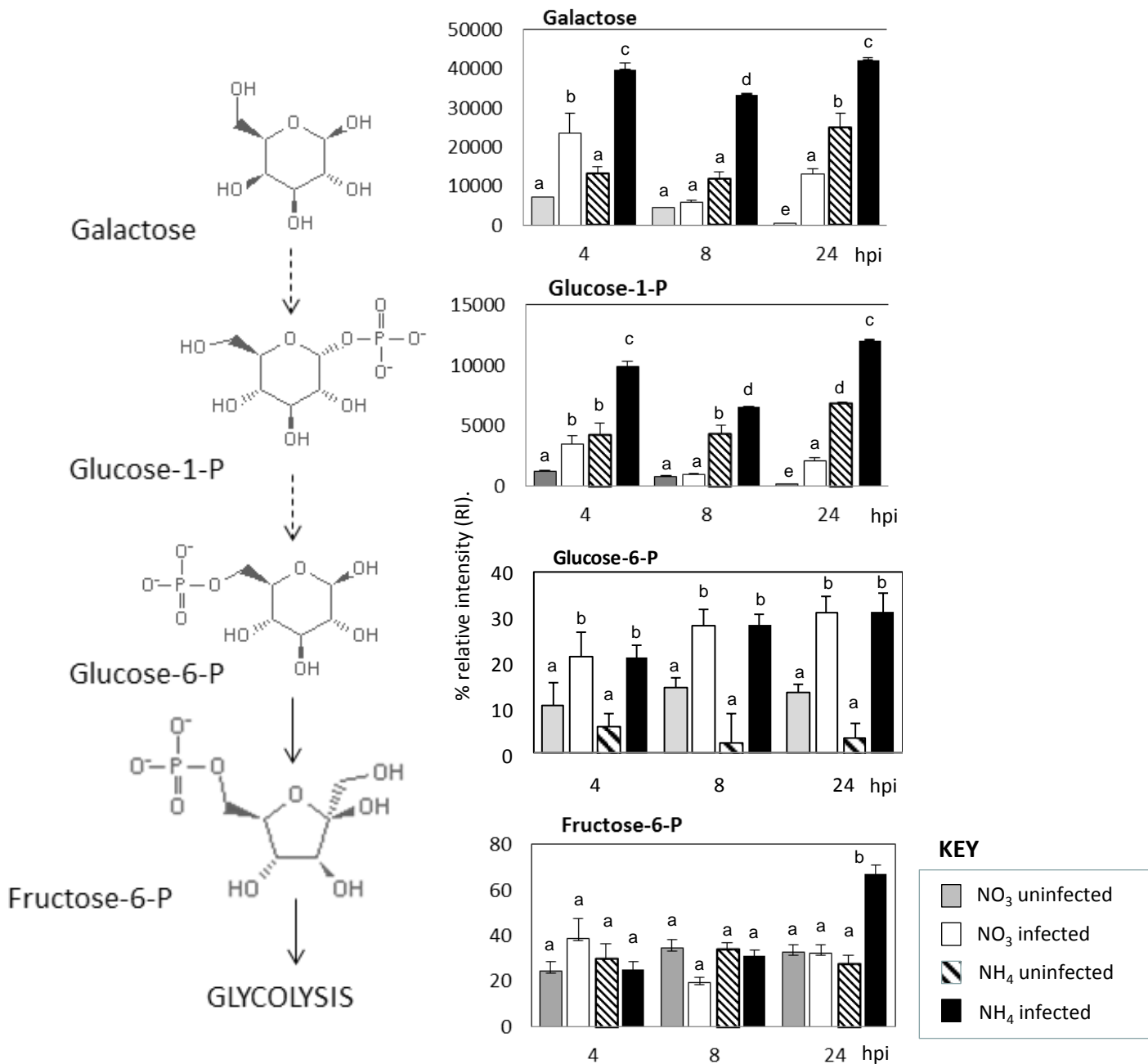
**Supplementary Figure 1: Nitrate reductase dependent effects on the hypersensitive response HR elicited by *Pseudomonas syringae* pv. *phaseolicola* in tobacco**

**(A)** Nitric oxide production in detached wild type tobacco leaves or *Nia30* nitrate reductase suppressed lines following inoculation with avirulent *Pseudomonas syringae* pv. (*P. s. pv.*) *phaseolicola* (*Psph*) using a Quantum Cascade Laser (QCL). For comparative purposes NO production from mock-inoculated (10 mM MgCl<sub>2</sub>) detached leaves of wild type tobacco **(B)** Electrolyte leakage from leaf discs sampled from areas of either NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> fed wild type plants or the *nia30* line and inoculated with *Psph*, **(C)** *Psph* populations 1 cm<sup>2</sup> diameter explants from in NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> -fed tobacco and *nia30* plants at 3 (grey boxes) and 7 (black boxes) days with *Psph* (n = 6 ± SD).



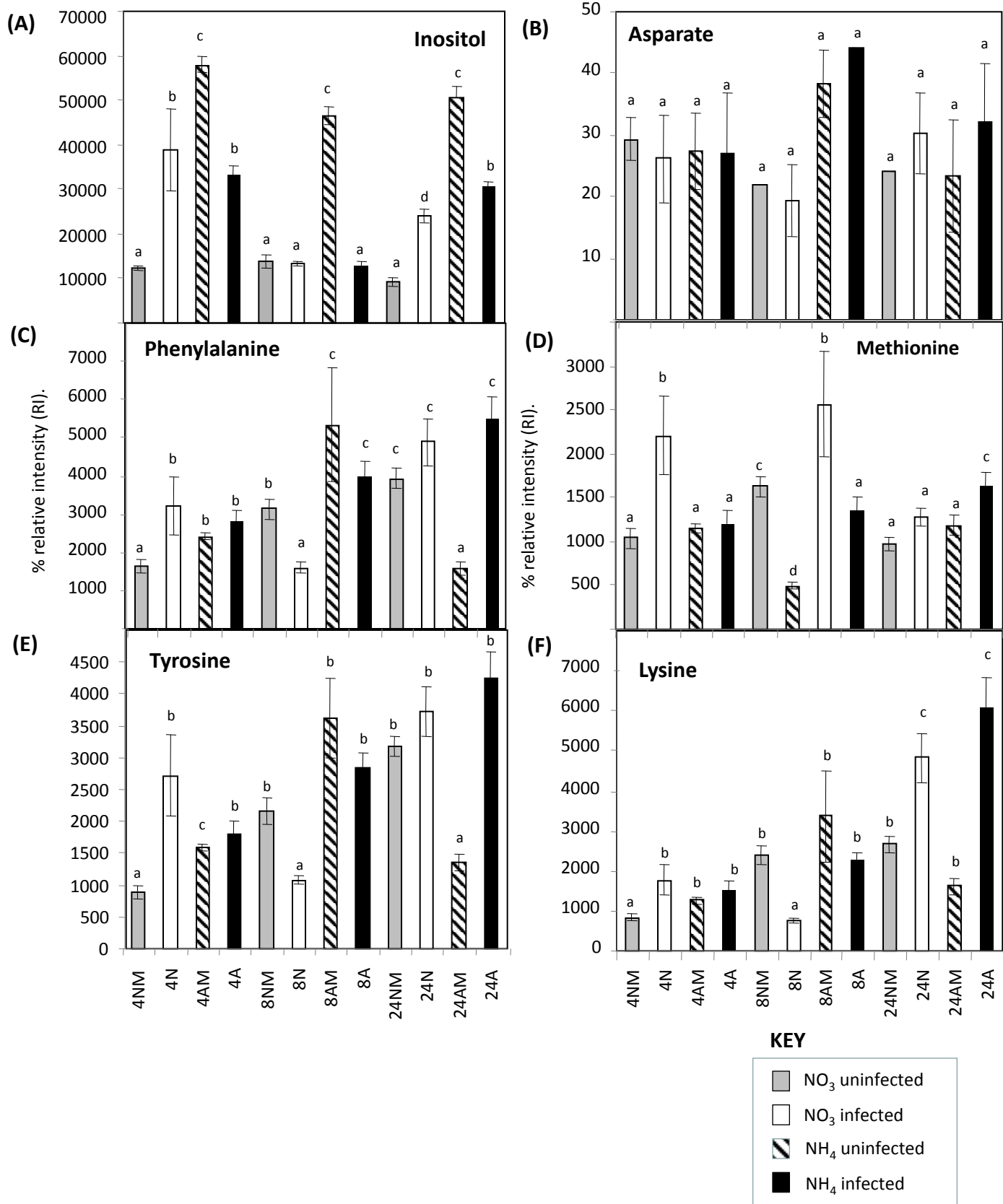
**Supplementary Figure 2:** Multivariate analyses of metabolite profiles of in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>-fed tobacco leaves following inoculation with *Pseudomonas syringae* pathovar *phaseolicola*

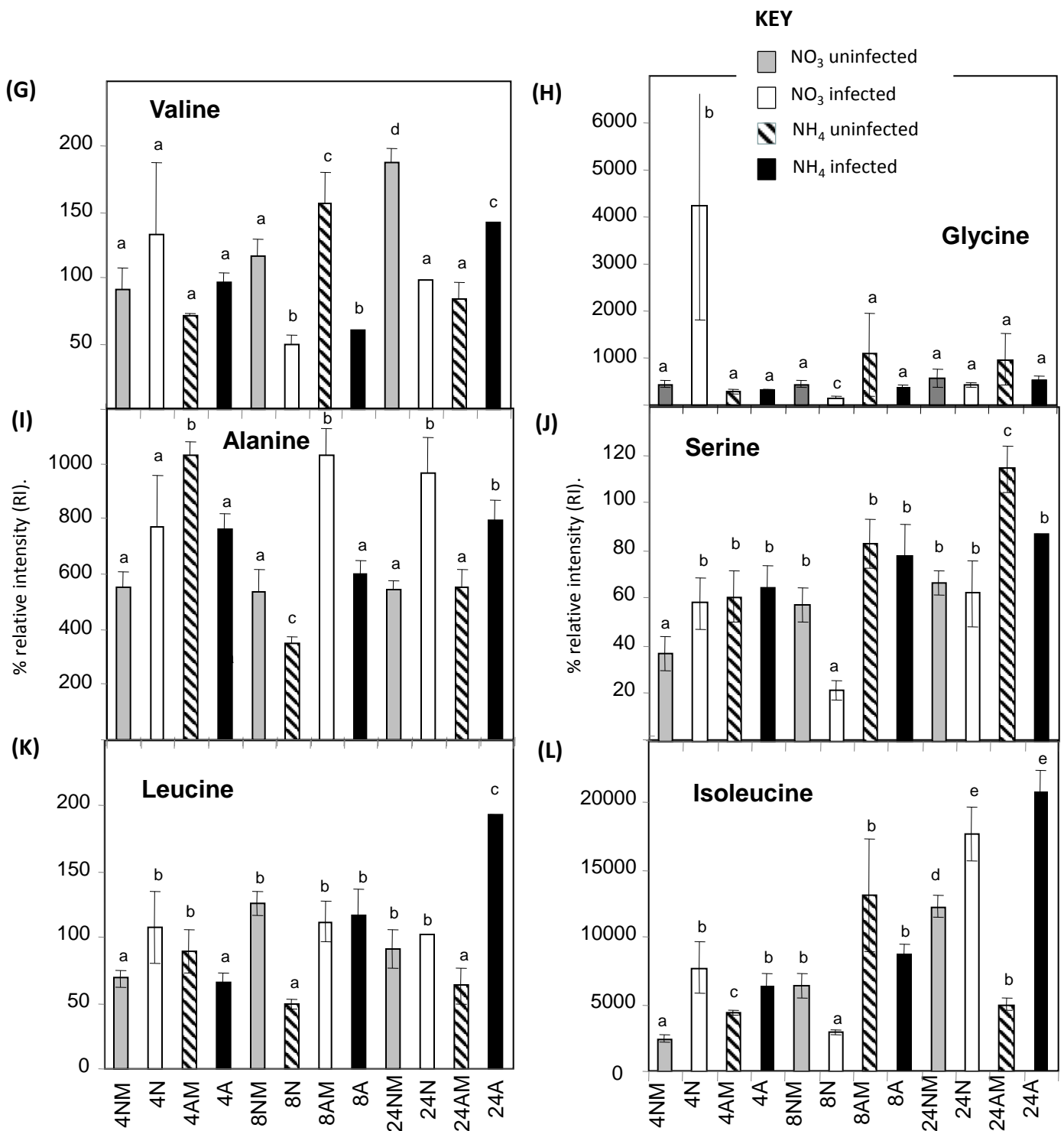
**(A)** Principal Component Analysis (PCA) of 150 metabolites detected by Gas Chromatography Mass Spectrometry (GC-MS) in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>-fed tobacco leaves following inoculation with *Pseudomonas syringae* pathovar *phaseolicola* (*Psp*) or mock inoculation with 10 mM MgCl<sub>2</sub>. **(B)** Discriminant Function Analyses (DFA) of the same data as analysed in A. Clustering of treatment and timepoint groups is seen in NO<sub>3</sub> and NH<sub>4</sub> fed 24 hpi samples as delineated in the plot. DFA was carried out on 5 PCs explaining 93.4% of the total variation. Discriminant Function (DF) 1 and 2 are plotted



**Supplementary Figure 3:** Galactose and glycolytic metabolite accumulation in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> -fed tobacco leaves following inoculation with *Pseudomonas syringae* pathovar *phaseolicola* or 10 mM MgCl<sub>2</sub>.

**(A)** Galactose contributes to a number of biochemical pathways but a link to glycolytic metabolism is shown. Broken arrows indicate multiple biochemical steps. Metabolites were detected by Gas Chromatography Mass Spectrometry (GC-MS) in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>-fed tobacco leaves following inoculation with *Pseudomonas syringae* pathovar *phaseolicola* (*Psph*) or mock inoculation with 10 mM MgCl<sub>2</sub>. hpi = hours following inoculation. Shown are the results for, **(B)** glucose-1-phosphate (P); **(C)** glucose-6-phosphate (P) and **(D)** fructose – 6-phosphate (P). Data are displayed as % relative intensity (RI). Data sets marked with a, b or c within each graph, indicate groupings within which non-significant differences were observed but were significantly different (*adjusted P* < 0.05) from all other groups





**Supplementary Figure 4:** Amino acid accumulation in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>-fed tobacco leaves following inoculation with *Pseudomonas syringae* pathovar *phaseolicola* or 10 mM MgCl<sub>2</sub>.

Amino acids were detected by Gas Chromatography Mass Spectrometry (GC-MS) in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>-fed tobacco leaves following inoculation with *Pseudomonas syringae* pathovar *phaseolicola* (*Psph*) or mock inoculation with 10 mM MgCl<sub>2</sub>. Samples designated with either 4, 8 and 24 refer to hours following inoculation; N = NO<sub>3</sub><sup>-</sup> fed; A = NH<sub>4</sub><sup>+</sup> fed plants. M = Mock inoculated. Samples with no M, indicate samples inoculated with *Psph*. Data are displayed as % relative intensity (RI). Data sets marked with a, b or c within each graph, indicate groupings within which non-significant differences were observed but were significantly different (*adjusted P* < 0.05) from all other groups