Supplementary Information for:

Alleopathic effects of exogenous phenylalanine:

A comparison of four monocot species

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This SI document in the following pages (S2 – S26) includes text and figures with detail on:

(1) Effects of phenylalanine and phenylalanine-d_8 on growth of grasses

(2) Spectrophotometric analysis of growth solutions from annual ryegrass inhibition experiment

(3) Analysis of deuterium incorporation by FTIR and NMR

(4) Toxicity studies with *Lemna minor*

(1) Effects of phenylalanine and phenylalanine-*d8* on growth of grasses

Growth media for all of the plants was Schenk and Hildebrandt's basal salts for plants (Phytotechnology Laboratories, Shawnee Mission, Kansas, U. S. A.) reconstituted at 3.20 g L^{-1} with distilled water purified with an E-pure water purification system (Barnstead Thermolyne, Dubuque, Iowa, U. S. A.). Composition is given by the manufacturer as: 300 mg L^{-1} ammonium phosphate monobasic, 5 mg L^{-1} boric acid, 151 mg L^{-1} anhydrous calcium chloride, 0.1 mg L^{-1} cobalt chloride hexahydrate, 0.2 mg L^{-1}cupric sulfate pentahydrate, 20 mg L^{-1} Na_2EDTA dihydrate, 15 mg L^{-1} ferrous sulfate heptahydrate, 195.4 mg L^{-1} anhydrous magnesium sulfate, 10 mg L^{-1} manganese sulfate monohydrate, 0.1 mg L^{-1} sodium molybdate dehydrate, 1 mg L^{-1} potassium iodide, 2500 mg L^{-1} potassium nitrate, 1 mg L^{-1} zinc sulfate heptahydrate, pH 4.2. Cinnamic acid stock solution was titrated with sodium hydroxide to pH 6.76. Phenylalanine stock solution was pH 5.98 without adjustment. Phenylalanine, deuterated phenylalanine, and cinnamate solutions were filter-sterilized by passage through 0.2 µm pore size sterile filters. All other solutions, equipment, and soil were steam-sterilized in an autoclave before use.

(1a) Comparison of effects of phenylalanine and phenylalanine-d_8 on growth of annual ryegrass (*Lolium multiflorum*)

Annual ryegrass seeds were obtained from Mayo Garden Center (Knoxville, Tennessee, U. S. A.). Species was identified as *Lolium multiflorum* based on morphological characteristics of seeds and mature soil-grown and hydroponic plants (Evans et al. 2014). Seeds were surface-sterilized with 70% ethanol before use. For each sample, a sterilized 47-mm glass fiber filter (Gelman Sciences P/N 66078) was placed in a 6-cm sterile polystyrene culture dish and wetted with 5 ml of nutrient solution. The nutrient solutions for each set of four replicates were Schenk and Hildebrandt's basal salts in distilled water: without additives (control set C1 – C4), with 2 mM phenylalanine (phenylalanine set F1-F4), and 2 mM deuterated phenylalanine-d_8 (Cambridge Isotope Laboratories, Cambridge, Massachusetts, U. S. A.) (deuterated phenylalanine set DF1 – DF4). Each dish was seeded with 30 ryegrass seeds placed on top of the glass filter (Fig. S-1). The culture dishes were incubated in the dark at 20 degrees C for 18 h, then under 7 µmol m^{-2} s^{-1} white fluorescent light for 2 days, and finally under a metal halide growth lamp with illumination at 131 µmol m^{-2} s^{-1} and 27 °C, diurnal cycle 12 h light/12 h dark. Seeds began to sprout on day 6. The lids were removed from the culture dishes and each dish was placed into a 946 mL (32 oz) sterile plant jar (Phytotechnology Laboratories), except for C4, F4, and DF4 which were placed into 473 mL (16-oz) plant jars (Fig. S2). Germination and growth were recorded periodically (Fig. S-1 and Table S-1, and Fig. S-3 and Table S-2, respectively). Each plant sample was watered by pipetting 2 ml of the corresponding solution into the culture dish inside the plant jar on days 14, 21, 25, 27, and with 3 ml on day 29. When the plants in the 473-mL jars reached the tops of the jars, the lids were removed and small plastic bags were fastened over each jar using rubber bands to allow these plants comparable space to that of the 946-mL jars. After one month, the tallest plants in each jar had reached heights greater than 15 cm. The plants were harvested after 33 days (Fig. 2). The stems and leaves were cut above the roots and removed from the glass filters. The glass filters, which were extensively colonized by the roots, were transferred to fresh sterile 6-cm culture dishes and stored at 4 °C. The residual nutrient solutions in the F and DF sets were observed to turn brown, while the C set did not. The absorbance of the solutions was scanned and compared to those of phenylalanine and cinnamic acid (Fig. S-7). Harvested plants were dried for 3 days at 20 °C under vacuum in a desiccator containing a jar of silica gel desiccant.

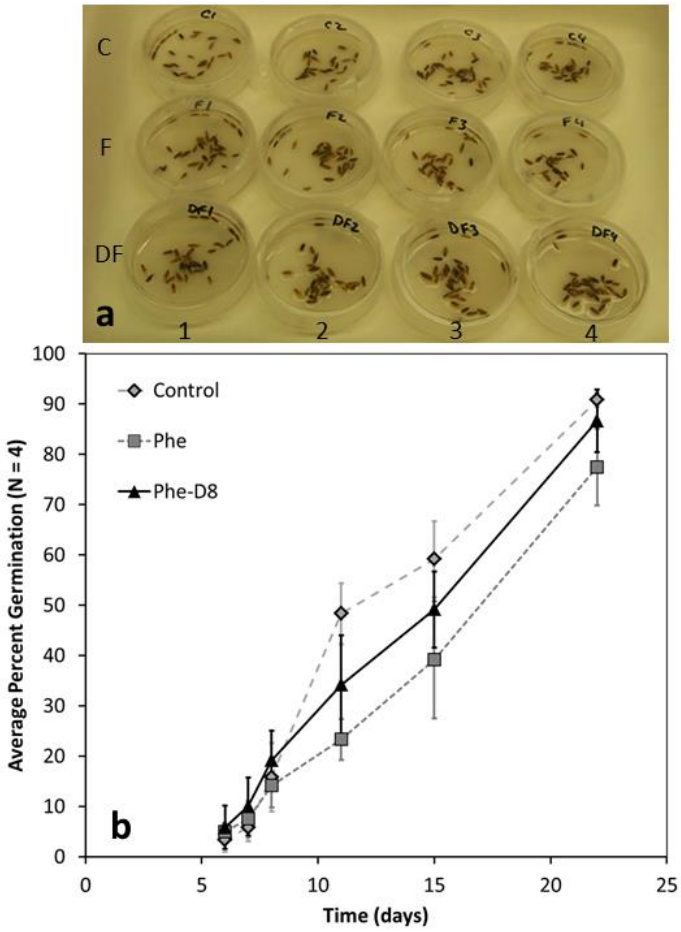

Figure S1. Percent germination averaged for the four replicates, each comprised of 30 seeds, was compared for annual ryegrass (*Lolium multiflorum*) ed for the experimental conditions: set C, control; set F, 2 mM phenylalanine; set DF, 2 mM deuterated phenylalanine-*d8* (a). The number of germinated seeds in each dish was counted from day 6 to day 23, then averages were calculated and graphed for each set (b). Error bars are standard deviations calculated from the set averages at each time point taken.

Table S-1. The *Lolium multiflorum* seeds in the four replicate sets of 20 seeds without inhibitors (C1, C2, C3, C4), with 2 mM phenylalanine (F1, F2, F3, F4), and with 2 mM deuterated phenylalanine-d8 were periodically examined and the number of germinated seeds recorded. The analysis of the data is presented in Fig. S-1.

Day	C1	C2	C3	C4	F1	F2	F3	F4	DF1	DF2	DF3	DF4
6	0	1	1	2	2	1	1	2	3	0	1	3
7	1	1	3	2	2	2	1	4	4	0	4	4
8	3	5	8	3	3	6	3	5	6	3	6	8
11	12	15	17	14	5	8	8	7	7	10	15	9
15	18	14	20	19	6	12	14	15	11	15	16	17
22	27	28	27	27	21	25	21	26	27	23	26	28

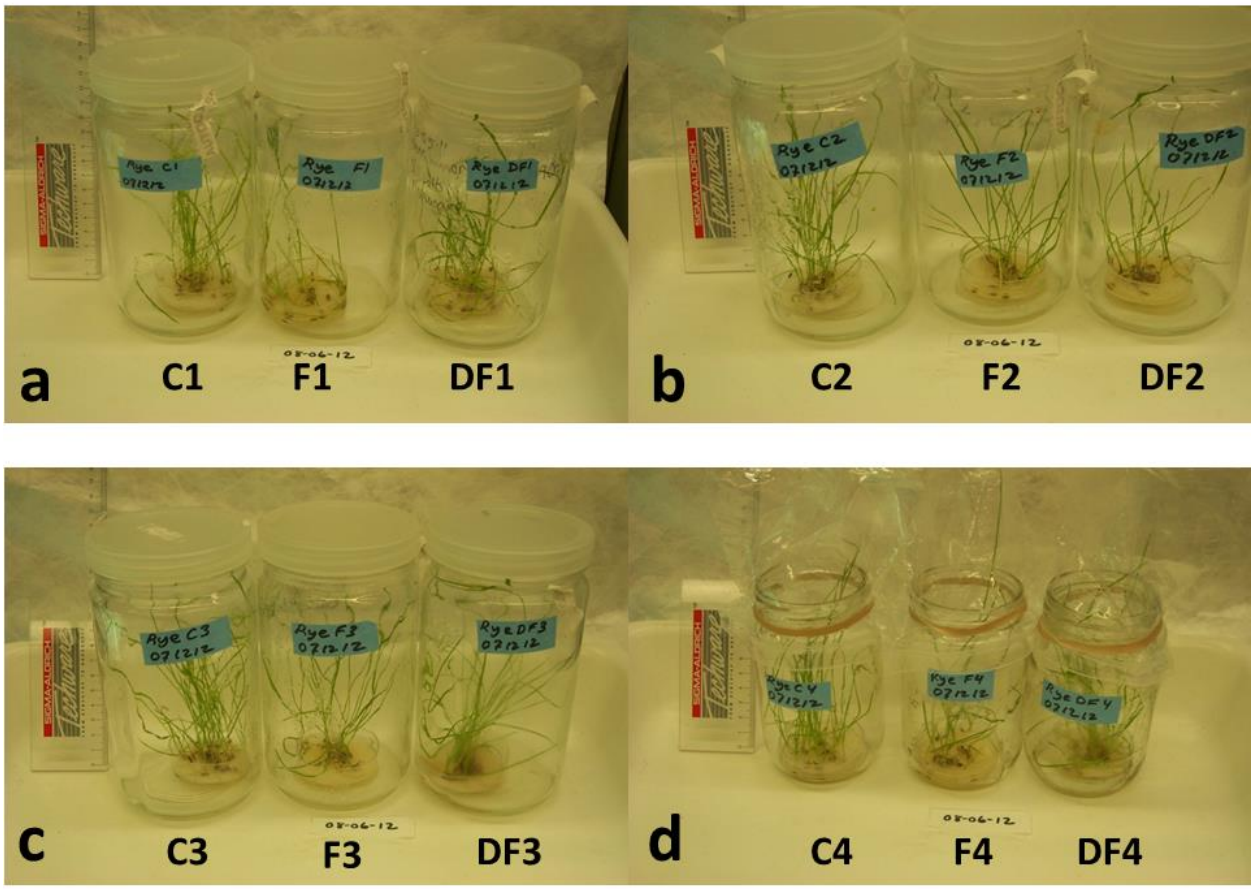

Figure S-2. The appearance of the annual ryegrass (*L. multiflorum*) seedlings is shown after 25 days of growth: (a) replicate 1 for control (C), 2 mM phenylalanine (F), and 2 mM deuterated phenylalanine-d_8 (DF); (b) replicate 2; (c) replicate 3; (d) replicate 4.

Table S-2 Heights of *Lolium multiflorum* seedlings sprouted from four replicate sets of 20 seeds without inhibitors (C1, C2, C3, C4), with 2 mM phenylalanine (F1, F2, F3, F4), and with 2 mM deuterated phenylalanine-d8 were measured during the first 15 days of growth to determine growth rates (Fig. S-3).

Time (d)	**Leaf shoot height (cm)**											
	C1	C2	C3	C4	F1	F2	F3	F4	DF1	DF2	DF3	DF4
6		0.5	0.5	0.5	2	0.5	2	0.5	0.5		2	0.5
				0.5	0.5			0.5	0.5			0.5
									2			2
8	0.5	0.5	0.5	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	1	0.5	1	1	1	1	2	2	0.5	0.5	0.5	0.5
	2	1.5	1	3	2	1	2	2	2	1	1	1
		1	2		2		3	3	3		2	2
		2	2		2		3	3	3		2	2
			1.5		2				3		3	3
			1.5						3			3
												3
11	7	0.5	1	7	7	0.5	0.5	7	7	0.5	0.5	0.5
	6	0.5	1	7	3	0.5	0.5	7	6	0.5	0.5	0.5
	5	0.5	5	3	2	5	2	3	5	0.5	0.5	1
	3	0.5	5	2	2	6	2	3	4	2	1	1
	3	1	5	2	1	6	2	2	2	2	2	2
	2	1	5	1		6	4	0.5	0.5	2	3	5
	2	1	5	1		4	5	0.5	0.5	2	3	7
	2	4	6	1		4	6			3	3	7
	2	4	7	0.5						5	5	7
	2	4	7	0.5						5	6	7
	1	4	4	0.5							6	
	1	6	4	0.5							6	
		6	2	0.5							7	
		6	2	0.5							8	
15	10	9	0.5	7	8	8	8	8	11	8	12	10
	10	9	0.5	7	8	8	8	8	9	7	10	10
	8	8	0.5	7	5	7	7	7	8	6	10	10
	8	8	7	7	5	7	7	6	6	5	10	10
	7	7	7	7	2	6	7	6	6	5	8	10
	6	7	7	7	3	6	7	5	5	4	8	1
	6	6	12	7		6	5	3	4	4	9	1
	6	6	10	5		5	5	3	4	3	7	1
	6	6	9	5		5	5	2	3	2	7	3
	4	5	6	5		3	5	2	3	2	5	6
	4	5	5	5		3	4	2	2	1	4	5
	4	5	5	5		3	2	7	0.5	1	3	0.5
	4	3	7	5			1.5	1		1	2	2
	4	2	7	5			1	1		1	2	2
	4		7	5						1	1	2
	4		5	3							1	8
	3		5	3								8
	3		5	2								
	3			1.5								

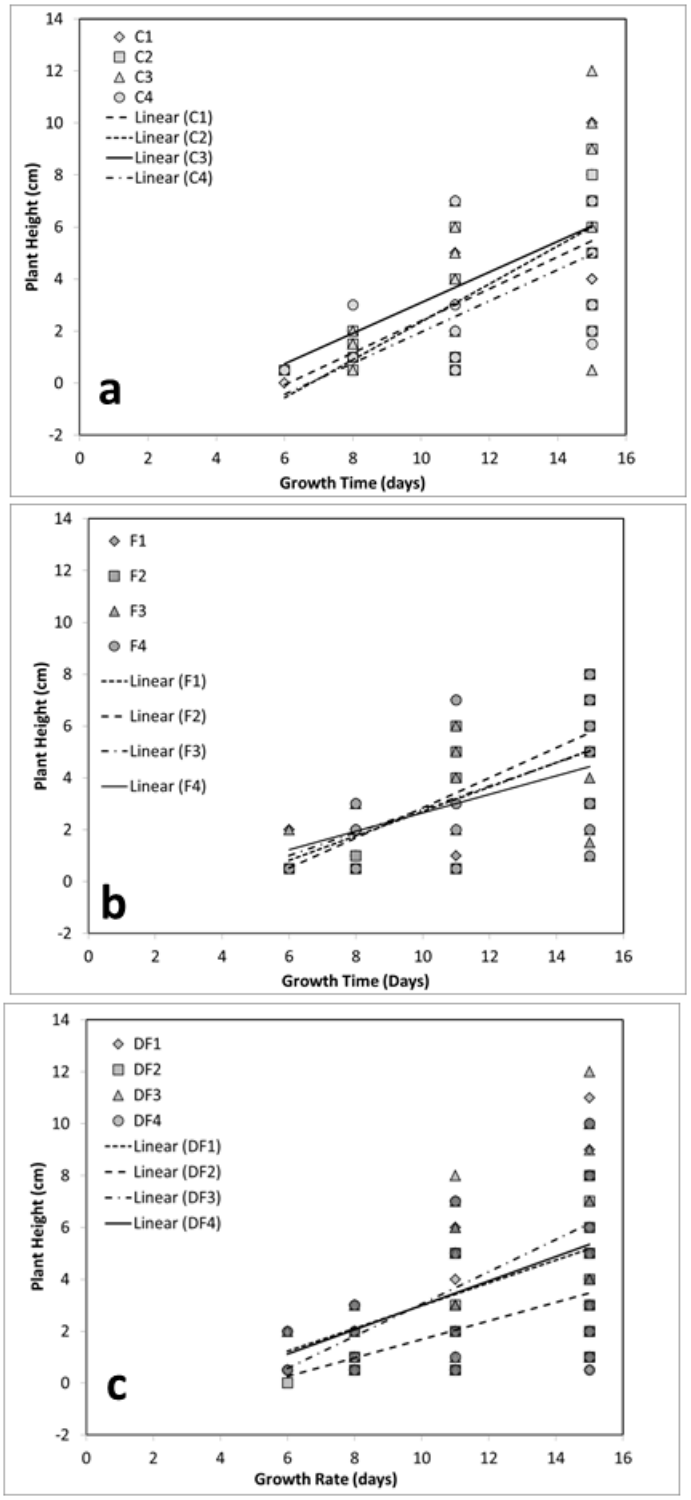

Figure S3. Effects of phenylalanine and deuterated phenylalanine-d_8 on annual ryegrass (*Lolium multiflorum*) were analyzed by linear regression to obtain averaged growth rates during days 6-15 after

seed imbibation of each of the four 30-seed sets: (a) control; (b) 2 mM phenylalanine; (c) 2 mM phenylalanine-*d8*.

Table S-3. Calculation and statistical analysis of growth rates of annual ryegrass (*Lolium multiflorum*) grown in 2 mM phenylalanine and in 2 mM deuterated phenylalanine-d8 (data presented in Table S-2 and linear regression plots in Fig. S-3).

Set	Growth Rate (cm d^{-1})	Average	Standard Deviation	R^2	P*
C1	0.6146	0.6324	0.0559	0.4098	
C2	0.728			0.5152	
C3	0.5891			0.3342	
C4	0.5981			0.4347	
F1	0.4711	0.4640	0.0791	0.4414	(F vs C) 0.02694
F2	0.5796			0.4521	
F3	0.4478			0.3142	
F4	0.3575			0.2061	
DF1	0.4392	0.4723	0.0958	0.2900	(DF vs C) 0.05613
DF2	0.3572			0.2091	
DF3	0.6218			0.2741	
DF4	0.4711			0.2074	

*P values were calculated using Student's T-test to compare the growth rates for the four replicates in the phenylalanine (F) or deuterated phenylalanine-*d8* (DF) sets to the four replicates in the control set (C).

(1b) Effects of phenylalanine, phenylalanine-d_8, and cinnamate on winter grain rye (*Secale cereale*)

Three separate experiments were carried out with this species.

Winter grain rye (*Secale cereale*) seeds were obtained from W. Atlee Burpee Company. Seeds were surface-sterilized by soaking in two changes of 5% double-strength Clorox (final concentration 0.4% sodium hypochlorite in distilled water), 20 min per change, rinsed twice with sterile distilled water, and air-dried before seeding. Glass fiber filter disks (diameter 47 mm) were used as supports. The growth solution was Schenk and Hildebrandt's basal salts in distilled water, pH 4.2. Growth temperature was 25 °C and the plants were illuminated with Sylvania Gro-Lux lamps at1400 lux (24 µmol m^{-2} s^{-1}) with a 12 h light/ 12 h dark diurnal cycle. Seedlings that withered and turned brown were counted as dead.

The effect of deuterated phenylalanine-d_8 on germination and growth was initially investigated for sets of 20 seeds placed on two glass fiber filters inside two 946 mL glass jars. Ten mL of growth solution were added to the control jar while ten mL of 2 mM phenylalanine-d8 in growth solution were added to the other jar. Seedlings were harvested after a total of 12 days after imbibition. Roots and leaf shoots were separated and fresh weights determined (Fig. S-4 and S-5; Table S-4).

The effect of deuterated phenylalanine-d_8 on growth of sprouted rye seedlings was also examined. Ten four-day old rye seedlings (average height 3 cm) were transferred to a 6-cm culture dish inside a 946-mL

plastic growth container and watered with 10 mL of growth solution supplemented with deuterated phenylalanine-d_8. Ten control seedlings were transferred to a 6-cm dish inside another 946-mL growth container and watered with 10 mL of growth solution. The containers were incubated under Sylvania GRO-LUX lamps. Seedlings were harvested after 4 days for a total growth time of 8 days after seed imbibition (Table S-4).

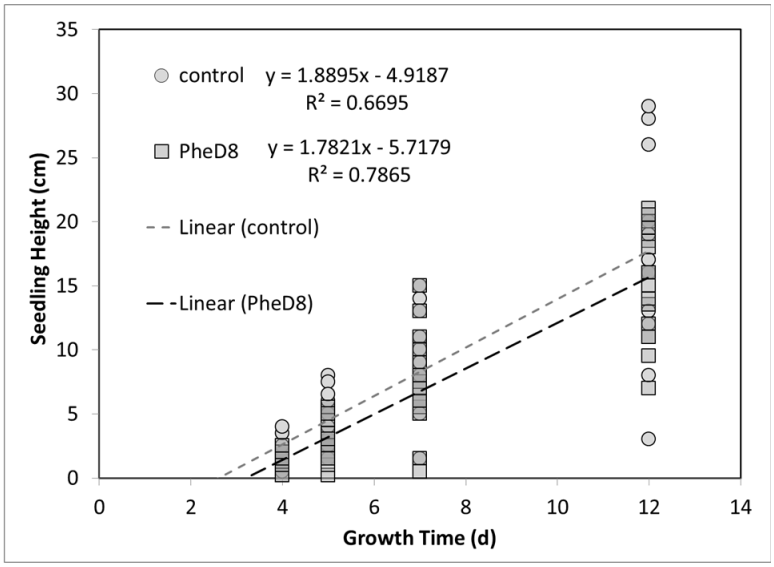

Figure S4. Effects of deuterated phenylalanine-d_8 on germination and growth of winter grain rye (*Secale cereale*) were analyzed by linear regression of seedling heights during days 4 – 12 after seed imbibition of sets of twenty seeds. Circles, control plants; squares, plants germinated and grown in 2 mM phenylalanine-d_8.

Figure S5. Gross morphology after removal of seedlings from plant jars after 12 d growth showed increase in length and secondary lateral roots for winter grain rye (*S. cereale*) control plants (a) and plants germinated and grown in 2 mM phenylalanine-d_8 (b).

Table S-4. Stimulation of root growth during germination of winter grain rye (*Secale cereale*) in deuterated phenylalanine-d8 resulted in increased root biomass compared to control seedlings, which was not observed in seedlings transferred four days after germination.

Parameter	Germination and 12 d growth in PheD8 (N = 1)		Transfer on Day 4 to PheD8 and 4 d growth (N =1)	
Number of control seedlings	15		7	
Number of seedlings in phenylalanine-*d8*	19		9	
Biomass type	Leaf shoots	Roots	Leaf shoots	Roots
Total PheD8 biomass yield (g)	2.1448	1.2201	0.5182	0.4478
PheD8 biomass yield (g seedling^{-1})	0.1129	0.06412	0.05758	0.05353
PheD8 biomass yield (g d^{-1} seedling^{-1})	0.009903	0.005351	0.007197	0.006667
Total control biomass yield (g)	1.8727	0.5279	0.3331	0.4800
Control biomass yield (g seedling^{-1})	0.1248	0.03519	0.04759	0.06397
Control biomass yield (g d^{-1} seedling^{-1})	0.01040	0.002933	0.005948	0.007996
PheD8/control (%)	95.22	182.4	121.00	83.34

The response of winter grain rye (*Secale cereale*) to 2 phenylalanine and 2 mM cinnamate was examined for sets of twenty seeds. For each set, four 47- mm glass fiber disks were placed in a 473-mLglass plant jar and moistened with 10 ml of the appropriate growth solution. Twenty surface-sterilized seeds were placed in each jar (control, phenylalanine, cinnamate). After seven days, the seedlings were harvested, the maximum heights for each set measured, and fresh weights determined. Roots were not separated from the leaf shoots. They were dried under vacuum for 1 day before determination of dry weights (Table S-5). Average weights per seedling were calculated by dividing the weights of each set of seedlings (control, phenylalanine, and cinnamate) by the number of surviving seedlings.

Table S-5. The effects of 2 mM phenylalanine and 2 mM cinnamate on survival rate, tallest heights, and biomass yields were examined for winter grain rye (*Secale cereale*), 20 seeds per sample set, harvested after 7 days growth.

Inhibitor	Seedlings surviving at harvest	Survival (%)	Tallest height (cm)	Fresh weight (g)	Seedling average fresh weight (g)	Dry weight (g)	Seedling average dry weight (g)
None	20	100	19.8	4.5677	0.2284	2.5509	0.1275
Phenylalanine	17	85	17.4	3.4632	0.2136	2.2483	0.1322
Cinnamate	14	70	15.5	1.868	0.1334	1.3256	0.0947

(1c) Growth of switchgrass (*Panicum virgatum)* in phenylalanine-d_8 solution

A hydroponic switchgrass plant was started from a young tiller cut from an established plant grown in soil. After rooting, the plant was grown in 80 mL of Schenk and Hildebrandt's basal salts (Phytotechnology Laboratories, Shawnee Mission, Kansas) in distilled water in a 1-L glass chamber under continuous perfusion with ambient air at 80 mL min^{-1} as described previously (Evans et al. 2013; Evans et al. 2014; Evans & Shah 2015). Illumination was provided by a metal halide lamp in a SunSystem 2 fixture fitted with a glass screen (Future Garden, North Lindhurst, New York, U. S. A.) mounted in an in-house built stand. Light intensity was 10,660 lux, 138 µmol m^{-2} s^{-1} PAR (400 - 800 nm), 280 µW cm^{-2} long wavelength UV (300-400 nm), 220 µW cm^{-2} IR. Temperature increased from 23 °C to 28 °C during the light period. Tiller heights were measured periodically. The hydroponic plant was grown for 127 days, with new tillers starting around day 60 and day 100. The tillers were then cut at 10 - 20 cm above the root crown and stored frozen at -20 °C for later analysis. Phenylalanine-d_8 (20 mL of a 20 mM solution in distilled water) was injected into the chamber through a sterile syringe filter in the inflow perfusion port to bring the final concentration to 2 mM. Cultivation was continued, with injection of 10 – 20 mL of 2 mM phenylalanine-d_8 in Schenk and Hildebrandt's basal salts every one to two weeks to maintain volume and provide nutrients. Growth rates of tillers were determined graphically (Fig. S-6) from linear fitted lines using Microsoft Excel.

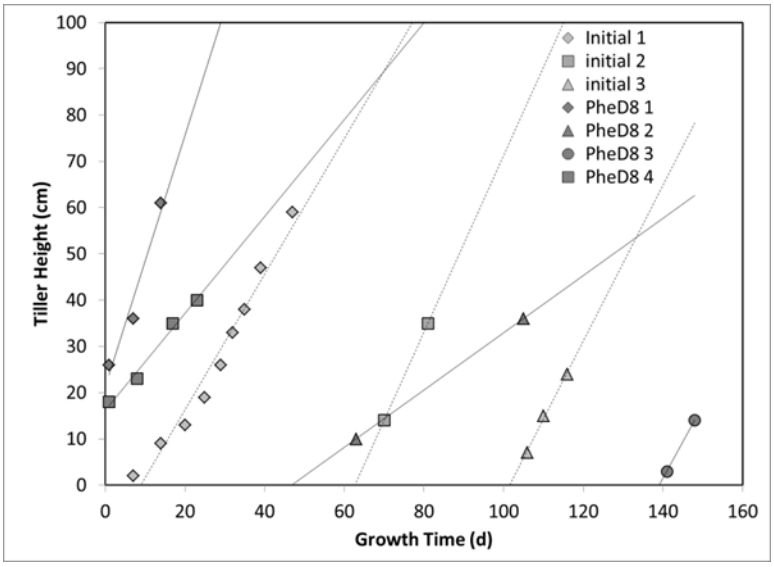

Figure S-6. Growth of tillers of a hydroponic switchgrass plant was measured before (Initial) and after (PheD8) addition of deuterated phenylalanine-d_8 to a final concentration of 2 mM. Rates were determined by linear fit to the data points.

(2) Spectrophotometric analysis of growth solutions from annual ryegrass (*Lolium multiflorum*).

Absorbance of growth solutions after 33 days growth was scanned from 200 to 800 nm in a Cary Bio50 UV-visible spectrophotometer (Varian Analytical Instruments, Walnut Creek, California). Solutions of sodium cinnamate, pH 6.7 (0.2 mM), phenylalanine (0.4 and 4 mM), and deuterated phenylalanine-d_8 (4 mM) were scanned for comparison (Fig. S-7).

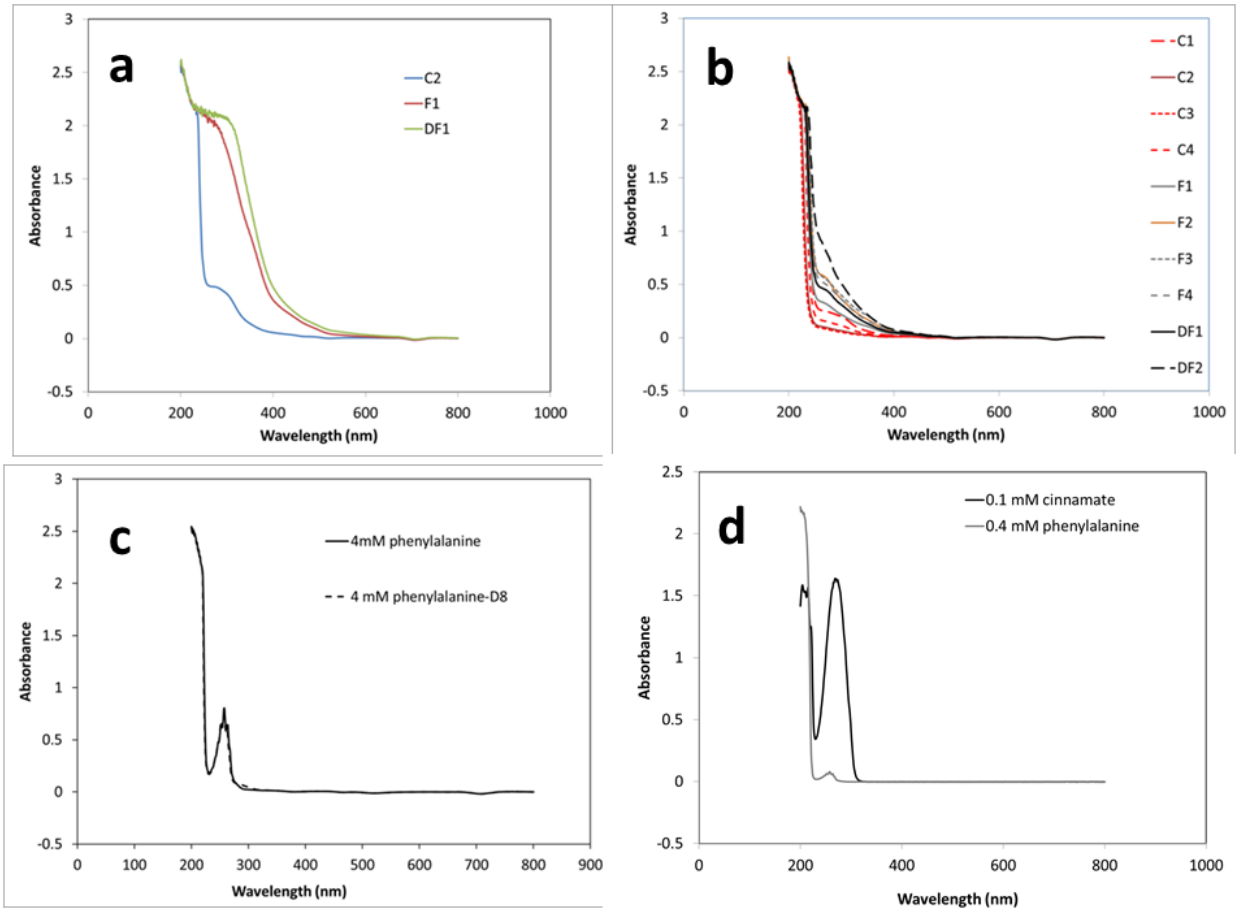

Figure S-7. Spectrophotometric analysis of annual ryegrass growth solutions (controls, C1-4; phenylalanine, F1-4; deuterated phenylalanine, DF1-4) detected the appearance of a large shoulder extending to 500 nm on the peak from the mineral salts in the far UV region in solutions from plants grown in phenylalanine that was not present in controls (a, undiluted; b, ten-fold diluted used growth solutions). The spectrum of deuterated phenylalanine-d_8 differs only by a 1-nm shift in peak maxium from that of its protiated counterpart (c). Comparison to spectra of phenylalanine and cinnamate indicates that production of cinnamate by deamination of phenylalanine, which results in a large increase in molar absorption as well as a peak shift from 258 to 269 nm (d), may be occurring, but that additional components that absorb in this region are produced.

(2) Analysis of deuterium incorporation by FTIR and NMR

Biomass samples were analyzed by FTIR for the appearance of deuterium-specific peaks as described previously (Foston et al. 2012; Bali et al. 2013; Evans et al. 2014, Evans et al. 2015; Evans and Shah 2015). A Perkin-Elmer 100 Series Fourier Transform Infrared Spectrometer in an attenuated total reflectance (ATR) mode was used to record spectra with accumulation of 128 scans and a resolution of 4 cm^{-1} in the range from 4000 cm^{-1} to 500 cm^{-1}. After the base-line correction, the spectra were scaled using the C–O stretching of the glucopyranose ring at ~1032 cm^{-1} (annual ryegrass, Fig. S-8; winter grain rye, S-11; switchgrass, S-13). Spectral band assignments: Aromatic C-H stretch 3030 cm^{-1}; Aromatic

C=C bending 1220 cm^{-1} to 1595 cm^{-1}; Aromatic C-H bending 680 cm^{-1} to 860 cm^{-1}; C-H 2900 cm^{-1}; C-D 2500 cm^{-1}; O-H 3300 cm^{-1}; O-D 2200 cm^{-1}.

For NMR analysis, air-dried biomass samples were dissolved in an ionic liquid solvent mixture prepared by mixing pyridinium chloride and dimethyl sulfoxide in 1:4 ratio under an intert atomosphere. Deuterated pyridinium chloride: methyl sulfoxide mixture was used for preparation of samples for ^{1}H-NMR, while protiated pyridimum chloride: dimethyl sulfoxide was used for ^{2}H-NMR samples.Deuterium dimethyl sulfoxide (anhydrous, 99.9%), pyridinium chloride (98%) and TFA (reagent grade, 99.0%) were purchased from Sigma Aldrich. Dimethyl sulfoxide (DMSO)-*d6* (99.9% D) and trifluoroacetic acid (TFA)-*d* (99.5% D) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MS). Deuterated pyridinium chloride was synthesized as described previously (Jiang et al. 2009). The NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer operated at 61.4 MHz for deuterium and 400.1 MHz for proton. ^{1}H NMR spectra were measured at 55 °C with 64 k data points, a 10 s recycle delay, and 16 scans. Deuterium (^{2}H) NMR spectra were recorded at 55 °C with 32 k data points, 10 s recycle delay, and 4000 scans (annual ryegrass, Fig. S-9 and S-10; winter grain rye, Fig. S-12;switchgrass, Fig. S-14).

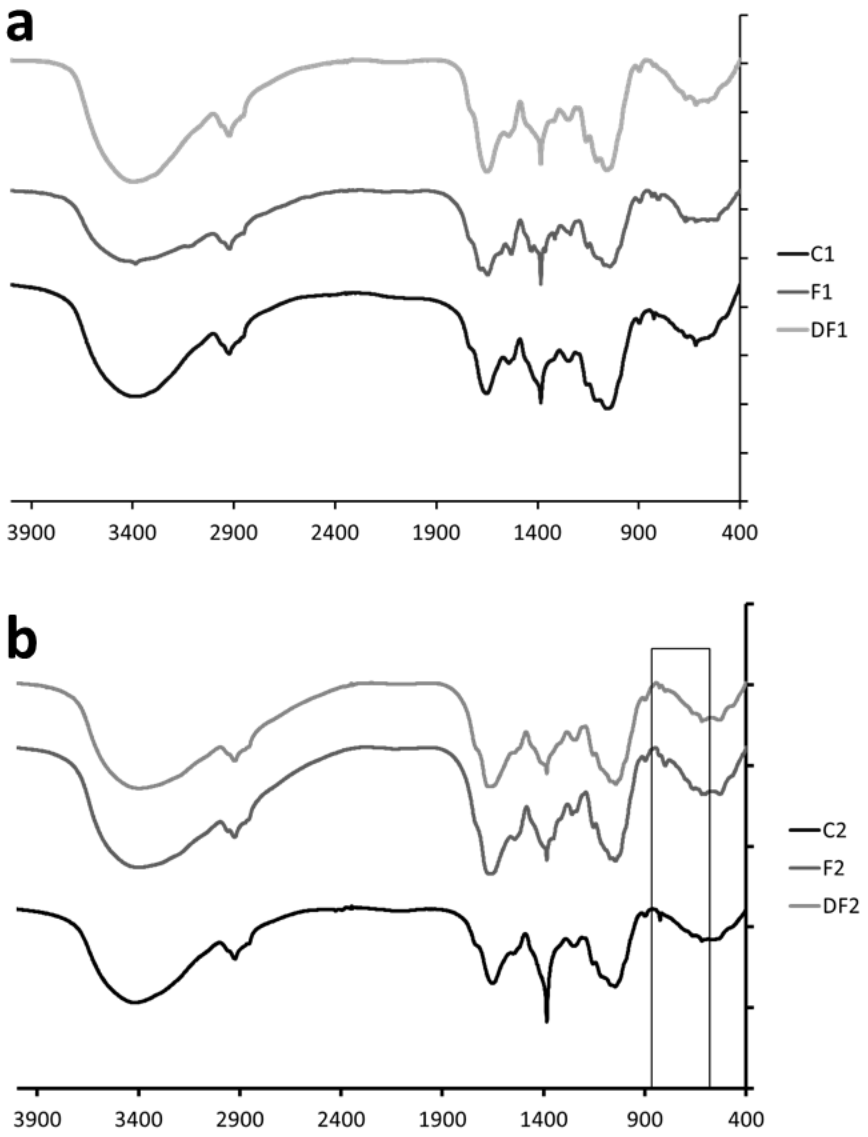

Figure S-8. Leaf shoots from two sets (1 and 2) of annual rye grass grown in 2 mM deuterated phenylalanine-d_8 (DF), 2 mM phenylalanine (F), and control solution (C) were analyzed by FTIR. No changes in chemical composition due to phenylalanine supplementation were apparent and no deuterium-specific peaks were detected.

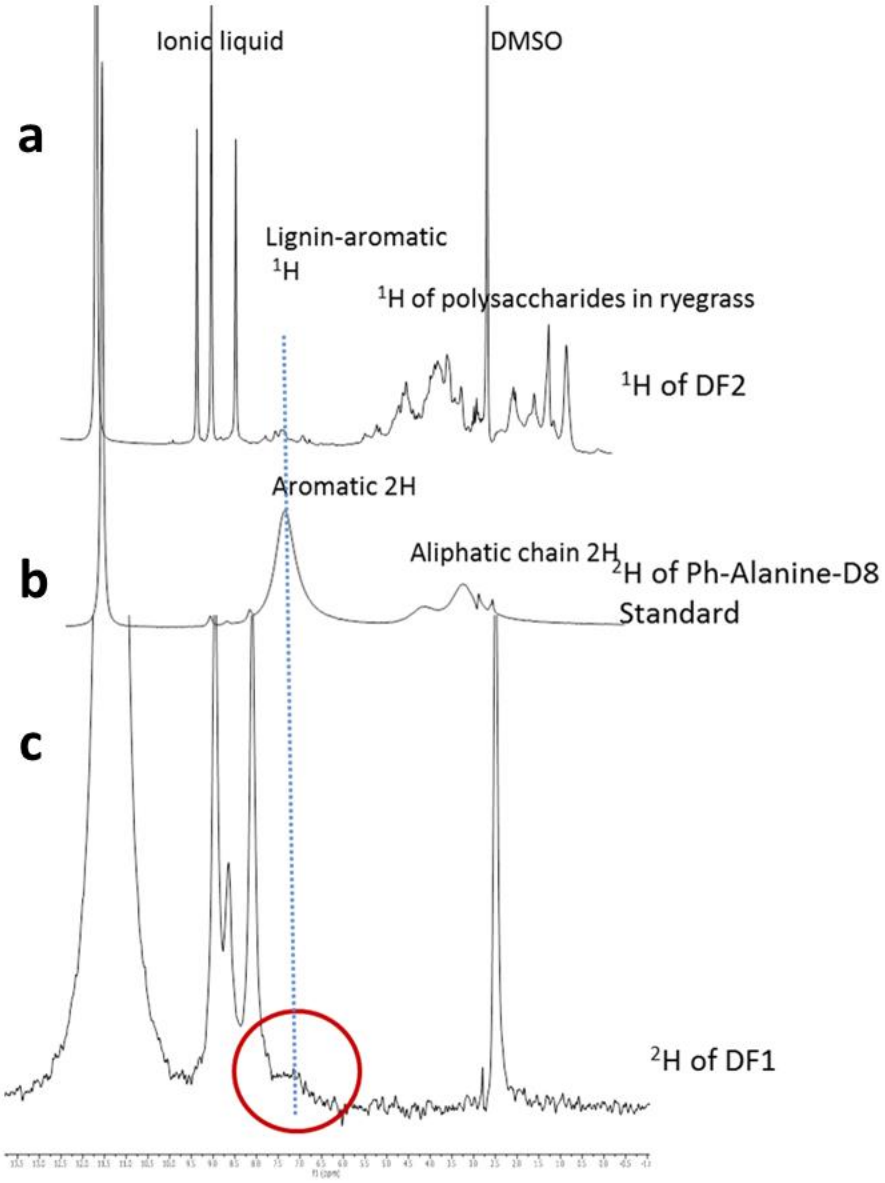

Figure S-9. Annual ryegrass (*Lolium multiflorum*) grown in 2 mM deuterated phenylalanine-d8 was further analyzed for deuterium incorporation by whole cell wall dissolution and ^{2}H-NMR analysis:(a): sample DF1; (b) deuterated phenylalanine-*d8* standard; (c) sample DF2. Incorporation was estimated to be 2-3%.

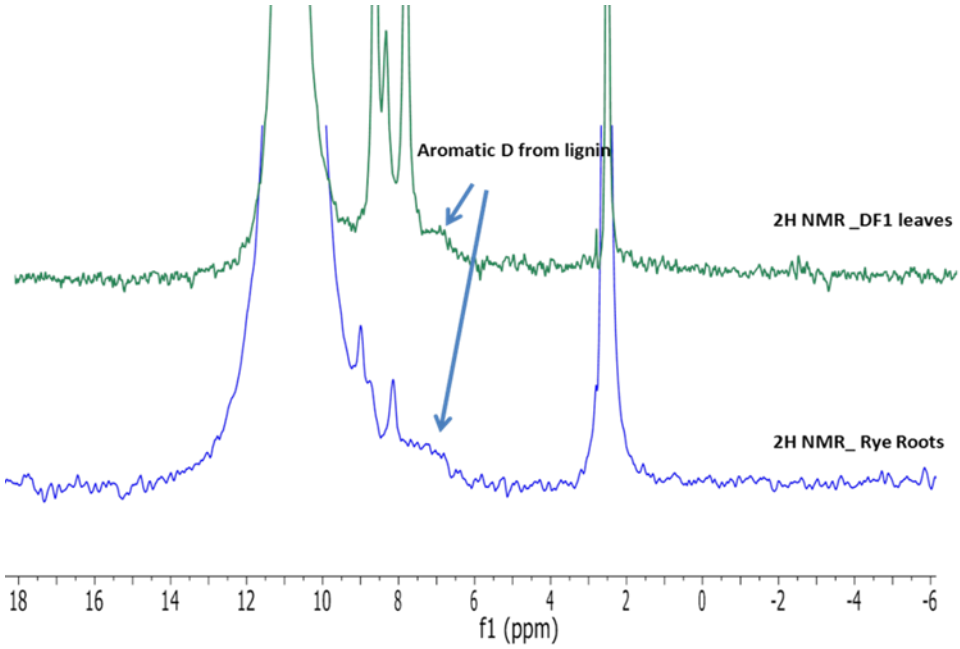

Figure S-10. Deuterium incorporation of annual ryegrass (*Lolium multiflorum*) grown in 2 mM deuterated phenylalanine-d_8 was compared for leaves and roots by ^{2}H-NMR analysis of whole cell wall samples dissolved in ionic liquid. A similarly low incorporation of 2-3% was estimated for both leaf and root samples.

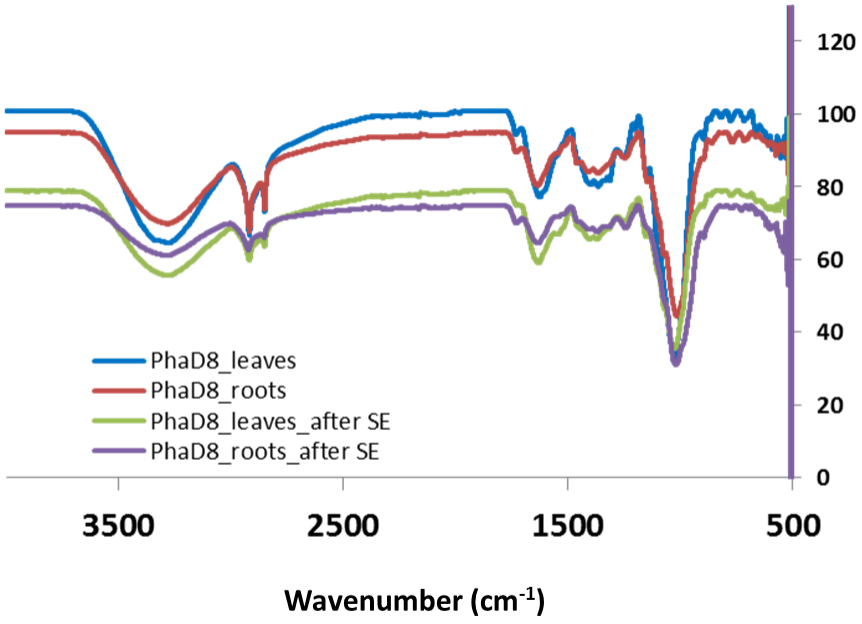

Figure S-11. No deuterium incorporation was detected by FTIR analysis of leaves and roots of winter grain rye (*Secale cereale*) germinated and grown in 2 mM phenylalanine-d_8.

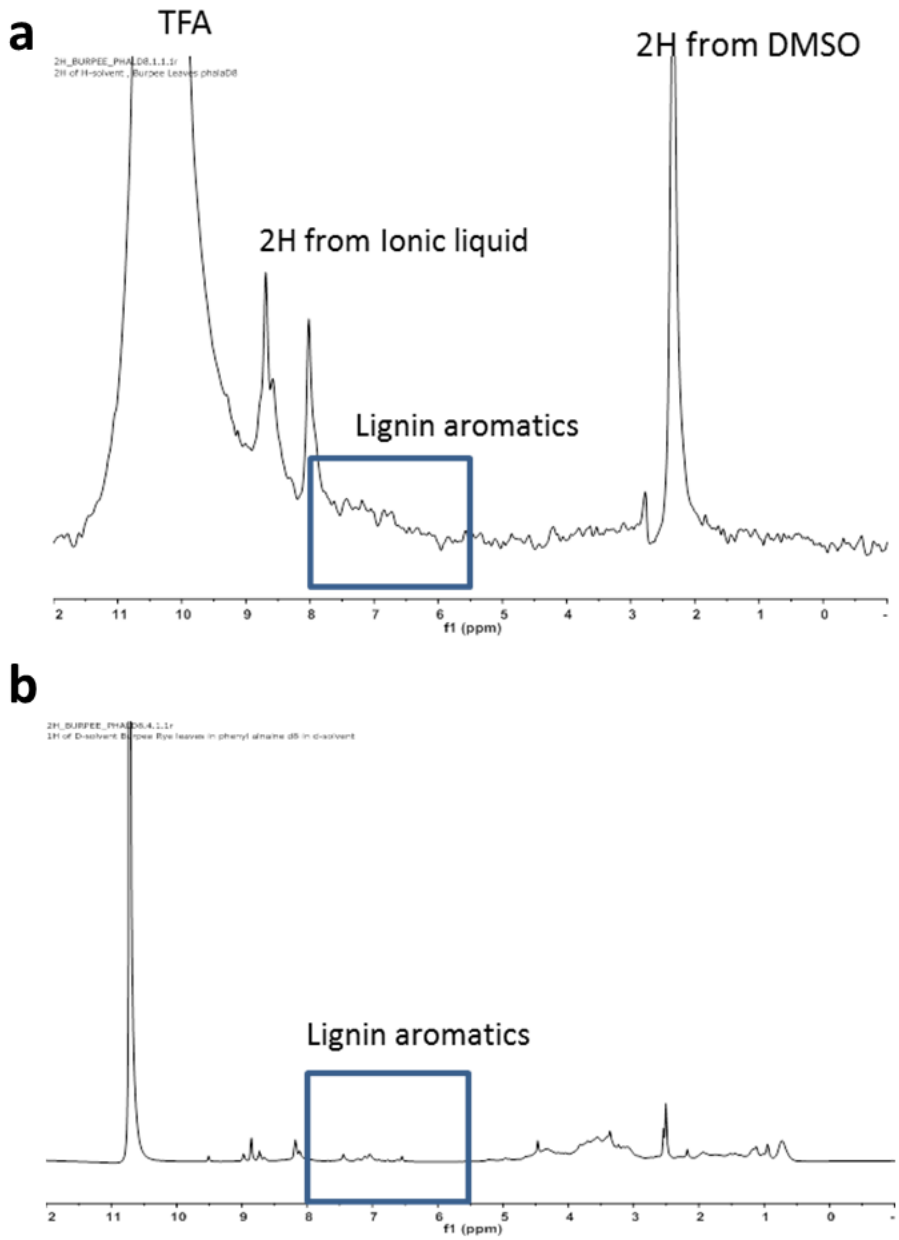

Figure S-12. Deuterium incorporation in leaves of winter grain rye (*Secale cereale*) germinated and grown in 2 mM phenylalanine-d_8 was determined to be 0.5% by ^{1}H ^{2}H-NMR analysis of whole cell wall samples dissolved in ionic liquid (a) ^{2}H-NMR analysis; (b) ^{1}H-NMR analysis.

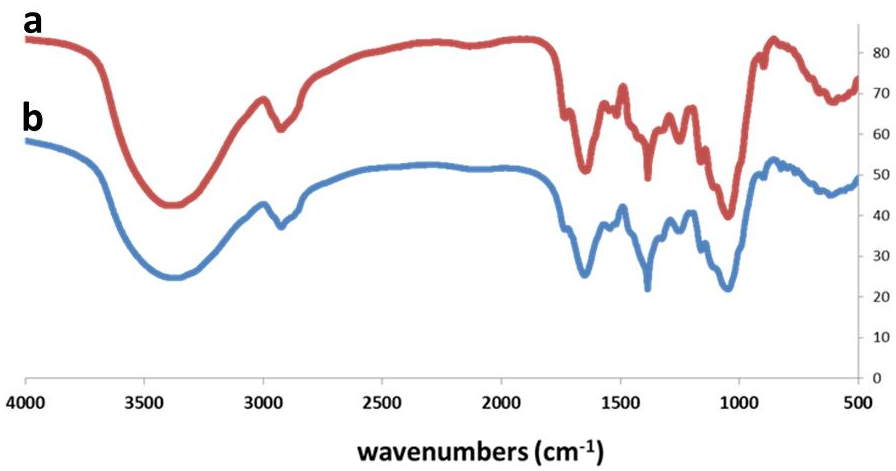

Figure S-13. Switchgrass (*Panicum virgatum*) leaf and stem biomass samples were analyzed by FTIR before (a) and after growth for 36 days in 2 mM deuterated phenylalanine-d_8 (b). No deuterium incorporation was detected.

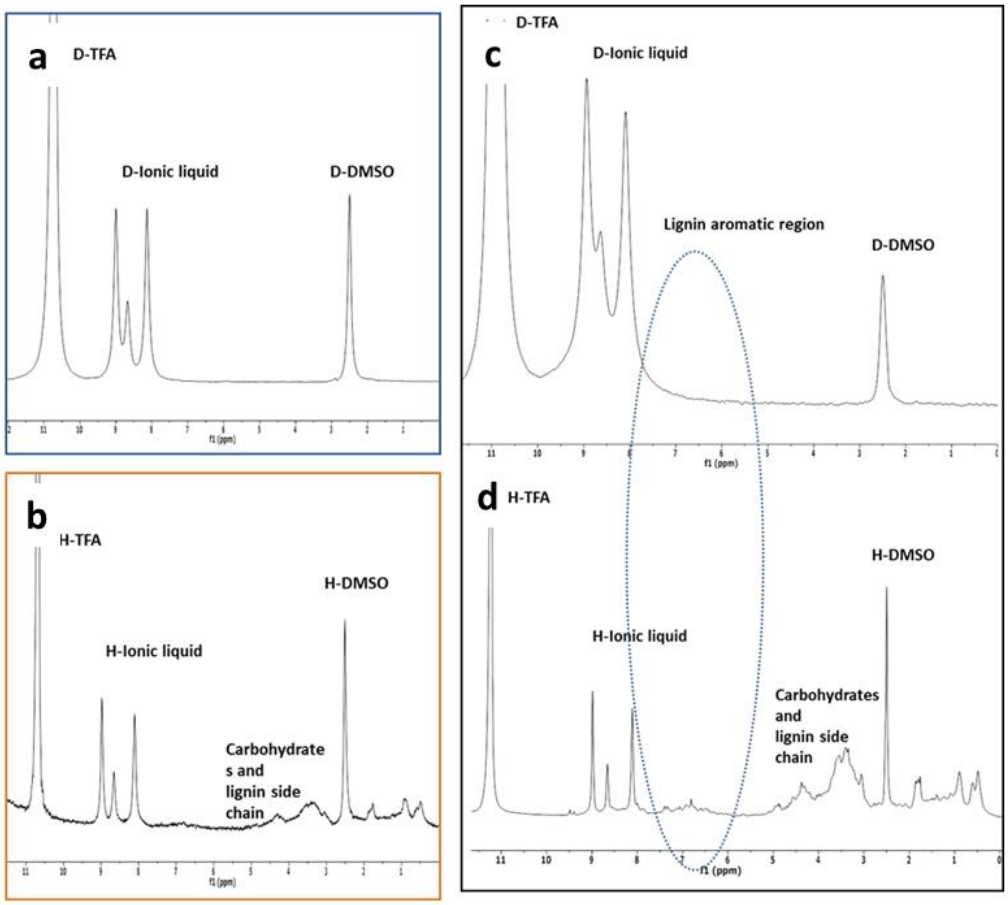

Figure S-14. Hydroponic switchgrass was analyzed before (a, ^{2}H-NMR; b, ^{1}H-NMR), and after 36 days growth in 2 mM deuterated phenylalanine-d_8 (c, ^{2}H-NMR; d, ^{1}H-NMR). For ^{2}H NMR of switchgrass before the experiment, 13.0 mg of leaf and stem biomass was dissolved in 550.7 mg of protio solvent system. For ^{1}H NMR, 2.4 mg of biomass dissolved in 534.8 mg of deuterated solvent system. For switchgrass growing in phenylalanine-d_8, 29.1 mg were dissolved in 716.7 mg of protio solvent system for ^{2}H-NMR and 10.0 mg Biomass were dissolved in 500 mg of deuterated solvent system for ^{1}H NMR. Based on these NMR results, ^{2}H incorporation was estimated to be 3.02%.

(3) Toxicity studies with *Lemna minor*

The duckweed strain *Lemna minor* UTCC 490 was used. Growth medium was Schenk and Hildebrandt's basal salts for plants (Phytotechnology Laboratories, Shawnee Mission, Kansas, U. S. A.) reconstituted at 3.20 g L^{-1} with distilled water purified with an E-pure water purification system (Barnstead Thermolyne, Dubuque, Iowa, U. S. A.). Medium composition is described above in section (1). Growth was quantified by counting of the fronds (leaves). The protocol for toxicity tests of phenylalanine and deuterated phenylalanine-d_8 was based on published methods (Ramirez et al. 1988; Moody and Miller 2005) with the following modifications. The growth medium was Schenk and Hildebrandt's basal salts instead of E medium or Hoagland's medium. For tests, cultures were grown in 10-mL aliquots of culture media in 6-cm diameter sterile polystyrene culture dishes sealed with Parafilm strips.

Two types of lamps were used for the *Lemna* toxicity studies. Illumination at1353 lux, 24 µmol m^{-2} s^{-1} PAR (400 – 800 nm), 0 µW cm^{-2} long wavelength UV (300-400 nm), 20 µW cm^{-2} IR, and temperature 25 °C was provided by double banks of Sylvania Gro-Lux lamps mounted on a plant cart (Carolina Biological Supply Company, Burlington, North Carolina, U. S. A.). A metal halide lamp in a SunSystem 2 fixture (Future Garden, North Lindhurst, New York, U. S. A.) mounted in an in-house built stand provided more intense illumination at 10,660 lux, 138 µmol m^{-2} s^{-1} PAR (400 -800 nm), 280 µW cm^{-2} long wavelength UV (300-400 nm), 220 µW cm^{-2} IR, and temperature increased to 28 °C during the light period. The diurnal cycle was 12 h light/12 h dark for both types of illumination.

Percent inhibition in the *Lemna* toxicity tests was calculated as %I = [(M-X)/M] X 100, where M is the average increase in frond number in the control culture during the test period, and X is the average increase in fronds in the test culture during the test period. All fronds in a culture are counted, both green living fronds and dead bleached ones.

Table S-6. Growth data and calculation of %I for first experiment examining growth inhibition of duckweed *Lemna minor* by deuterated phenylalanine-d_8 with and without glucose in the medium during illumination with Gro-Lux lamps at 1353 Lux (24 µmol $m^{-2}s^{-1}$ photons), diurnal cycle 12 h light/12 h dark. PheD8: deuterated phenylalanine-*d8*

Sample	control	glucose	1 mM PheD8	glucose + 1 mM PheD8	2 mM PheD8	glucose + 2 mM PheD8
Fronds on Day 1	32	26	34	30	30	25
Fronds on Day 8	110	140	85	97	55	78
Increase Day 8 –Day 1	78	114	51	67	25	53
% Inhibition			34.6	41.2	67.9	53.5

Table S-7. Growth data and calculation of %I for second experiment examining growth inhibition of duckweed *Lemna minor* by deuterated and protiated phenylalanine during illumination with Gro-Lux lamps at 1353 Lux (24 µmol $m^{-2}s^{-1}$ photons), diurnal cycle 12 h light/12 h dark. Phe: phenylalanine (protiated-no deuterium); PheD8: deuterated phenylalanine-d_8. For the calculation of %I, the values of controls 1 and 2 (no glucose), and of glucose 1 and 2 were averaged.

Sample	Fronds on Day 1	Fronds on Day 8	Increase Day 8 – Day 1	%I
Control 1	17	33	16	
Glucose 1	20	84	64	
Control 2	17	43	26	
Glucose 2	15	76	61	
1mM Phe	21	33	12	42.8
1mM Phe glucose	16	37	21	66.4
2 mM Phe	18	19	1	95.2
2 mM Phe glucose	19	36	17	72.8
1mM PheD8	19	34	15	28.6
1mM PheD8 glucose	19	52	33	47.2
2mM PheD8	16	17	1	95.2
2mM PheD8 glucose	17	24	7	88.8

Table S-8. Growth data and calculation of %I for growth inhibition of duckweed *Lemna minor* by phenylalanine and cinnamate without glucose in the growth medium during illumination with a metal halide lamp at 10,660 Lux (138 µmol $m^{-2}s^{-1}$ photons), diurnal cycle 12 h light/12 h dark. Cinnamate: sodium cinnamate; Phe: phenylalanine (protiated). Values for M and X are the averages of the two replicates (controls samples 1 and 2, etc.).

Sample	Inhibitor	Fronds Day 1	Fronds Day 8	Increase	M	X	M-X	%I
1	Control	11	47	36	32.5			
2	Control	10	39	29				
3	0.5 mM cinnamate	11	17	6		5	27.5	84.6
4	0.5 mM cinnamate	12	16	4				
5	1 mM cinnamate	9	9	0		0	32.5	100.0
6	1mM cinnamate	11	11	0				
7	2 mM cinnamate	15	17	2		1.5	31	91.4
8	2 mM cinnamate	10	11	1				
9	0.5 mM Phe	11	60	49		30	2.5	7.69
10	0.5 mM Phe	11	22	11				
11	1 mM Phe	13	34	21		18	14.5	44.6
12	1 mM Phe	10	25	15				
13	2 mM Phe	14	29	15		11.2	21.3	65.5
14	2 mM Phe	12	20	8				

Table S-9. Growth data and calculation of %I for growth inhibition of duckweed *Lemna minor* by deuterated phenylalanine-d_8 with and without glucose in the growth medium during illumination with a metal halide lamp at 10,660 Lux (138 µmol $m^{-2}s^{-1}$ photons), diurnal cycle 12 h light/12 h dark. PheD8: deuterated phenylalanine-d_8

Sample	control	glucose	1 mM PheD8	glucose + 1 mM PheD8	2 mM PheD8	glucose + 2 mM PheD8
Fronds on Day 1	11	5	8	9	7	8
Fronds on Day 8	60	78	15	26	9	10
Increase Day 8 – Day 1	49	73	7	17	2	2
% Inhibition			85.7	76.7	95.9	97.3

Table S-10. Percent inhibition (%I) values of phenylalanine, deuterated phenylalanine-*d8*, and cinnamate (cinnamic acid sodium salt) were determined and the effect of illumination type compared for the duckweed *Lemna minor*. Data used for the calculations are presented in Tables S-6, S-7, S-8, and S-9, and depicted graphically in Figure S-15.

Inhibitor	**Illumination**	**%I at Concentration**		
		0.5 mM	**1mM**	**2mM**
Phenylalanine (N = 1)	Gro-Lux[a]	(nd)	42.8	95.2
Phenylalanine + glucose (N = 1)	Gro-Lux	(nd)	66.4	72.8
Phenylalanine-d_8 (N = 2)	Gro-Lux	(nd)	31.6 ± 3	81.55 ± 13.65
Phenylalanine-d_8 + glucose (N = 2)	Gro-Lux	(nd)	44.2 ± 3	71.15 ± 17.85
Cinnamate (N=2)	Metal Halide[b]	84.6	100	95.3
Phenylalanine (N =2)	Metal Halide	7.69	45.6	65.5
Phenylalanine (N = 2)	Metal Halide	66.1	49.2	64.6
Phenylalanine-d_8 (N = 1)	Metal Halide	(nd)	85.7	95.9
Phenylalanine-d_8 + glucose (N = 1)	Metal Halide	(nd)	76.7	96.3

[a] Illumination at1353 lux, 24 m^{-2} s^{-1} PAR (400 – 800 nm), 0 µW cm^{-2} long wavelength UV (300-400 nm), 20 µW cm^{-2} IR, temperature 25 °C.

[b] Illumination at 10,660 lux, 138 mol m^{-2} s^{-1} PAR (400 -800 nm), 280 µW cm^{-2} long wavelength UV (300-400 nm), 220 µW cm^{-2} IR, temperature 28 °C.

(nd): not done.

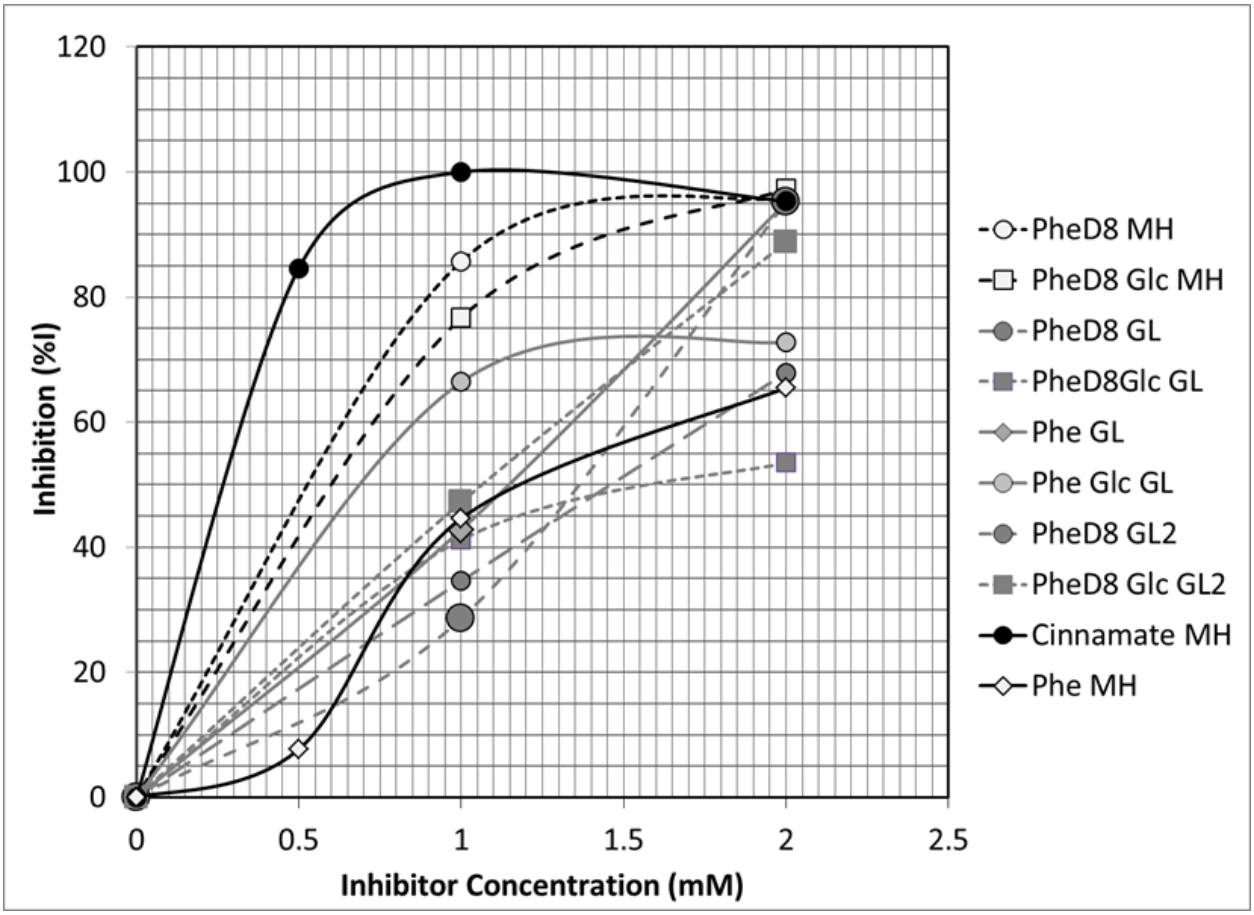

Figure S-15. Values of 25%I presented in Table 5 of the main manuscript were graphically determined for phenylalanine (Phe) , deuterated phenylalanine-d_8 (PheD8), and cinnamate under photoautotrophic and photoheterotrophic (with 50 mM glucose, Glc) conditions with illumination by two different light sources, a metal halide lamp (MH) or Sylvania extended range Grolux fluorescent lamps (GL). The data used for the graph are presented in Tables S-6, S-7, S-8, and S-9.

Table S-11. Cultures of *L. minor* grown under Gro-Lux lamps for ten days were transferred to illumination with a metal halide lamp to examine light induction of phenylalanine-d_8 toxicity while duplicate control cultures were left under Gro-Lux illumination.

Culture	Growth Medium	Illumination by MH Lamp	Growth rate (fronds/day)			Growth Stimulation by MH Lamp*
			A (days 1-10)	B (days 10-18)	Change (B/A)	
MH control	S&H	Days 1-18	2.24	5.50	2.45	
1	S&H	None	2.14	0.494	0.231	
2	S&H	Days 10-18	2.99	4.11	1.37	5.63
3	S&H, glucose	None	16.0	17.6	1.10	
4	S&H glucose	Days 10-18	16.3	32.7	2.01	1.83
5	1 mM PheD8, S&H	None	3.63	2.38	0.656	
6	1 mM PheD8, S&H	Days 10-18	3.16	1.36	0.430	0.655
7	1 mM PheD8, S&H, glucose	None	6.64	17.3	2.60	
8	1 mM PheD8, S&H, glucose	Days 10-18	4.21	27.0	6.41	2.46
9	2 mM PheD8, S&H	None	0.987	0.511	0.518	
10	2 mM PheD8, S&H	Days 10-18	2.26	1.89	0.836	1.61
11	2 mM PheD8, S&H, glucose	None	1.12	0	0	
12	2 mM PheD8, S&H, glucose	Days 10-18	0.766	0	0	0

*Calculated by dividing the change in growth rate (B/A) obtained with metal halide illumination during days 10 - 18 by the change in growth rate (B/A) for the control kept under Gro-Lux illumination with matched growth medium composition

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