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Supporting Information

Dual roles of glutathione in silver nanoparticle detoxification and enhancement of nitrogen assimilation in soybean (*Glycine max* L. [Merrill])

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Text S1. Substrate optimization

Figure S1A shows phenotypic images of soybean grown in the different substrates. Different percentages of vermiculite or potting mix did not cause overt phenotypic differences in terms of root length, shoot height (**Figure S1B**), and fresh weight (**Figure S1C**). Although the fresh biomass of seedlings grown in field soil amended with 25 or 50% potting mix was greater as compared to other treatments, the total number of nodules was higher in the substrate composed of 25% vermiculite and 75% field soil (**Figure S2**). Thus, field soil amend with 25% vermiculite was used as the substrate for the following pot experiment.

Text S2. Pigment content, net photosynthesis rate, transpiration rate, stomatal conductance

Chlorophyll content was measured as described in Lichtenthaler (1987).[1] Briefly, 50 mg fresh leaf tissue were cut into pieces (< 1 cm), and added to 15 mL centrifuge tubes containing 10 mL 95% ethanol. The tested tubes were kept in dark for 3–5 days and the chlorophyll content was measured by a UV-Vis spectrophotometer (Agilent 8453). Chlorophyll a, chlorophyll b and total chlorophyll were calculated by the following equations: $Chla = 13.36A_{664.2} – 5.19A_{648.6}$, $Chlb = 27.43A_{648.6} – 8.12A_{664.2}$ and Total chlorophyll = Chla + Chlb.

A LI–6400XT Portable Photosynthesis System (LI-COR Biosciences) was used to measure the photosynthesis rate (Pn), stomatal conductance (Sc) and transpiration rate (Tr) across all the treatments once the first trifoliate leaves were fully developed. The instrument conditions were as follows: CO_2 in reference chamber was 400 μmoles; relative humidity was between 50–65%; light intensity was 750 μmoles; and the flow was 200 μmoles. The instrument was calibrated every ten samples in order to obtain stable readings.

Text S3. Inhibition curve and dehydrogenase activity of Ag NP treated *Bradyrhizobium*

It was previously reported that the abundance of *Bradyrhizobium* in the collected soil was greater than that of *Frankia* and *Rhizobium*.[5] Thus, *Bradyrhizobium japonicum* (USDA 110) was used to test the effects of Ag NPs on rhizobium growth in HEPES–MES (HM) medium with or without the addition of GSH. The compositions of HM medium are provided in **Table S1**. *Bradyrhizobium* was inoculated in HM medium and the culture was shaken at 30 °C with a speed of 200 rpm. When OD_{600nm} reached to approximately 1.0, different amounts of Ag NPs were added into the culture to make the final concentrations of 50 and 75 mg/L. Five mM GSH was used in this experiment to investigate whether GSH could reduce NP–toxicity to *Bradyrhizobium*. The culture in each treatment was sampled at Day 1, 2, 3, 5 and 7. A fixed amount of Ag NPs and GSH treated culture was spread on petri dishes containing growth media and the total number of colonies was counted across all the treatment at each time point.

Dehydrogenase activity was used to evaluate *Bradyrhizobium* activities in response to Ag NPs exposure with or without the GSH addition. At Day 7, a volume of 2 mL culture in each treatment was transferred into an Eppendorf tube and centrifuged at 4000 rpm at 4 °C for 15 min. The cell pellet was re–suspended in 2 mL deionized H_2O, and 2mL 0.2% triphenyl tetrazolium chloride (TTC) was then added into the suspension. The mixture was inoculated at 37 °C overnight. The red formazan was extracted by 4 mL acetone, and then the OD value was measured at 484 nm using UV-Vis spectrophotometer (Agilent 8453).[6]

Text S4. Gene expression analysis by quantitative PCR

Soybean shoots or roots were homogenized in liquid nitrogen prior to RNA isolation. Procedures for total RNA isolation, cDNA synthesis, and gene expression using qRT–PCR were described in Ma *et al*. (2013).[7] Briefly, RNeasy plant mini kits were used to isolate total RNA, with the concentration being quantified by NanoDrop spectrophotometry (ThermoScientific, West Palm Beach, FL). A Verso cDNA synthesis kit was used to synthesize cDNA and the

gene-specific primer was designed using Primer Quest (Integrated DNA Technologies, Coralville, IA). A complete list of primer sequences is provided in **Table S2**. The qRT-PCR amplification program was 95 °C for 15 min; 95 °C for 45 s, 57 °C for 45 s, 72 °C for 1 min, repeating 40 cycles; 95 °C for 15 s, 57 °C for 15 s, melting curve for 20 min; 95 °C for 15 s. The total volume of each reaction was 20 μL and ELF was used as a housekeeping gene for normalization. Relative quantities ($2^{-\Delta\Delta Ct}$ method) were used to calculate the transcription level of each gene.

Text S5. Physiological responses of soybean upon Ag NP exposure

Low exposure doses (3.9–15.6 mg/kg) of Ag NPs had no significant impact on soybean growth in terms of phenotypic appearance of aboveground tissues, fresh biomass, and total number of nodules (**Figure S3A–C**). Although exposure to 31.2 mg/kg Ag NPs did not decrease the total fresh biomass, the number of nodules was decreased by 80.7% relative to the control (**Figure S3C**). Similarly, exposure to a mixture of metal**–**based NPs (Ag, ZnO and TiO_2) decreased the total number of nodules in alfalfa more than 13–fold as compared to the control and bulk-sized particle treated one.[2] However, Judy *et al.* (2018) reported that upon exposure to 100 mg/kg Ag and Ag_2S NMs had no impact on nitrogen–fixing bacteria in alfalfa as determined by the total number of nodules per plant.[3] With increasing concentration to 62.5 mg/kg, Ag NPs decreased the seedling biomass by more than 50% and completely inhibited the nodule formation. It is worth noting that the equivalent amount of Ag in forms of $AgNO_3$ and bulk-sized Ag particles had no negative impact or slightly enhanced soybean growth (**Figure S4**).

The low doses of Ag NPs did not alter the total N level in soybean seedlings; however, with increasing Ag NP concentration to 31.2 and 62.5 mg/kg, a decrease of 26.4 and 46.1% in the shoot total N were observed, respectively (**Figure S3D**). In addition, the total N level in the nodules decreased by 32.3–74.4% upon exposure to 7.8–31.2 mg/kg Ag NPs (**Figure S3E**); as

a result, the total N content in the root system treated with 62.5 mg/kg Ag NPs was approximately 40% less than the control (**Figure S3F**). Similarly, Wang *et al*. (2018) also reported that carbonaceous NMs reduced the total plant nitrogen fixation potential by over 90%.[4] In addition, the negative impact on net photosynthetic rate, stomatal conductance and transpiration rate suggest that exposure to Ag NPs above 31.2 mg/kg compromised the photosynthesis system in soybean; notably, bulk-sized Ag particles had no impact (**Figure S5A–C**). The observed decreases in chlorophyll content were consistent with low photosynthetic efficiency upon exposure to higher doses of Ag NPs (**Figure S5D**).

Test S6. Physiological responses of soybean as affected by GSH

The addition of 0.8 mM GSH increased the total fresh weight of soybean by more than 50% over the control (**Figure S8**); with increasing the concentration to 1.6 and 3.2 mM, GSH caused abiotic stresses as evident by the phenotypic appearance of the aboveground tissues (**Figure S8**) and the decreased photosynthetic efficiency (**Figure S9**). Thus, 0.8 mM GSH was chosen for the soil pot experiment to investigate whether the addition of GSH in soil could significantly alleviate Ag NP–induced phytotoxicity to soybean.

Text S7. Amino acid profile in soybean treated with different concentrations of GSH

For the concentration selection of GSH, we also measured the content of essential amino acids in soybean shoots and roots (**Table S3**–**S4**). A common finding was that a dose–dependent response was evident for each amino acid content with increasing the GSH concentrations. For example, the glycine content in 20 mM GSH-treated soybean shoots was almost 10–fold greater than controls (**Table S3**). Similar results were also evident in soybean roots (**Table S4**).

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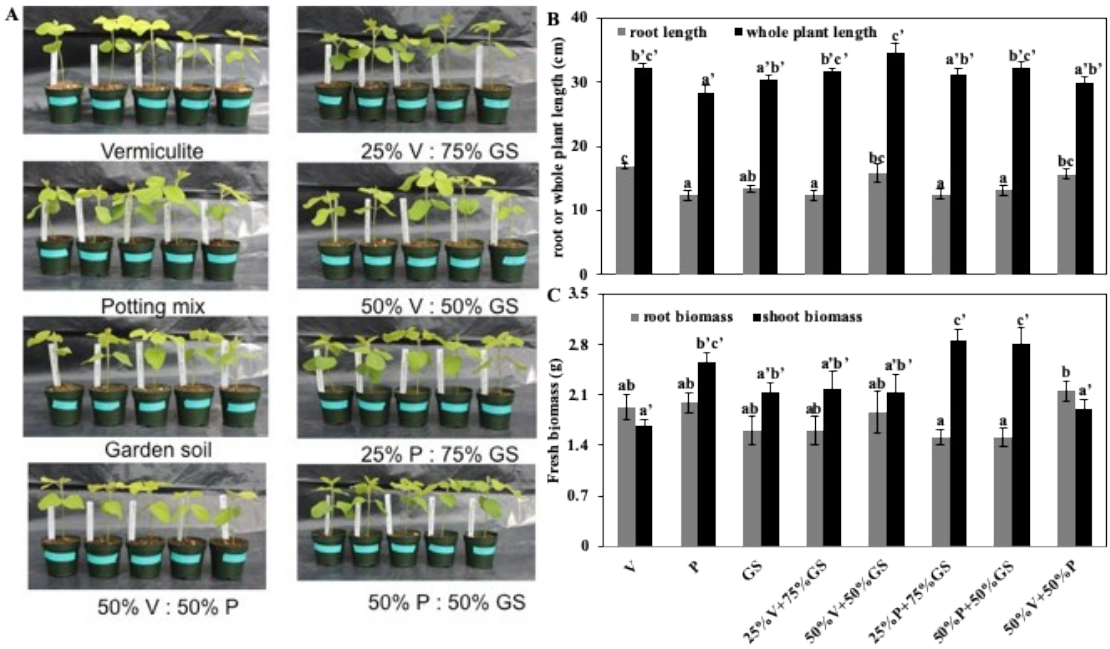

Figure S1. Effect of different types of amendments on soybean growth. (**A**) phenotypic images of soybean grown in soil amended with different percentages of vermiculite and potting mix; (**B**) root length and whole plant length; (**C**) fresh biomass of root and shoot tissues. V: vermiculite; P: potting mix; GS: garden soil. Error bars correspond to standard error of mean. Values followed by different letters with signal quotation mark indicate the significant difference of root length or biomass at $p < 0.05$; values followed by different letters without signal quotation mark indicate the significant difference of shoot length or biomass at $p < 0.05$.

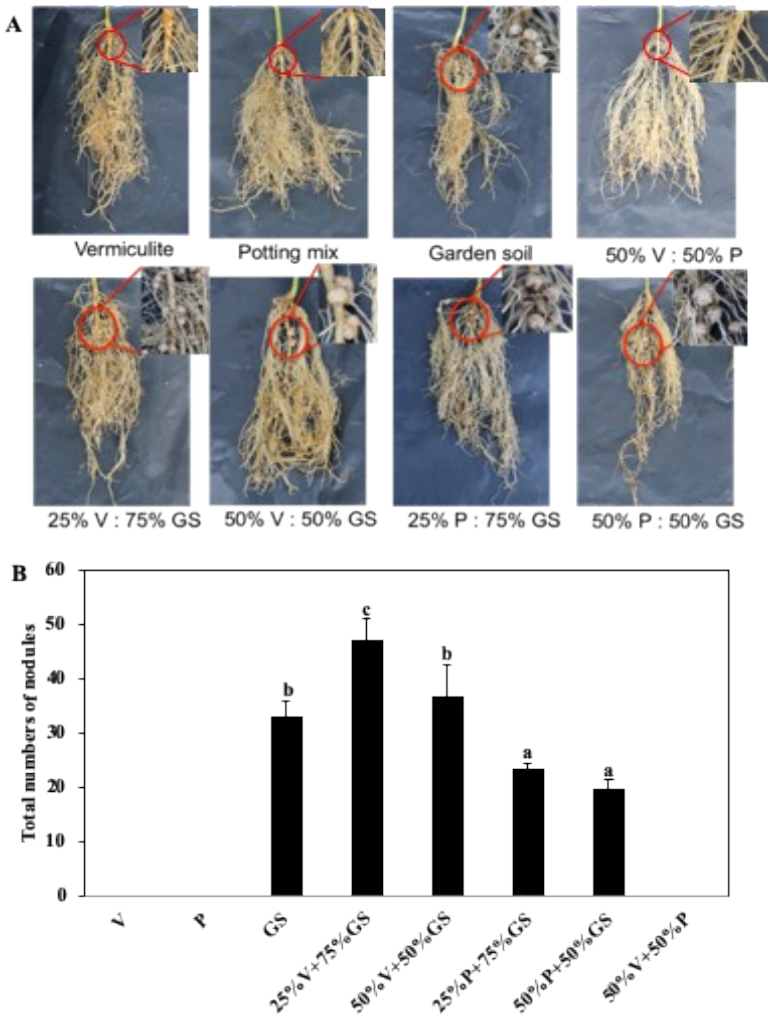

Figure S2. Effects of different types of amendments on total numbers of nodules. (**A**) phenotypic images of soybean nodules grown in soil amended with different percentages of vermiculite and potting mix; (**B**) total numbers of nodules. V: vermiculite; P: potting mix; GS: garden soil. Error bars correspond to standard error of mean. Values followed by different letters are significantly different at $p < 0.05$.

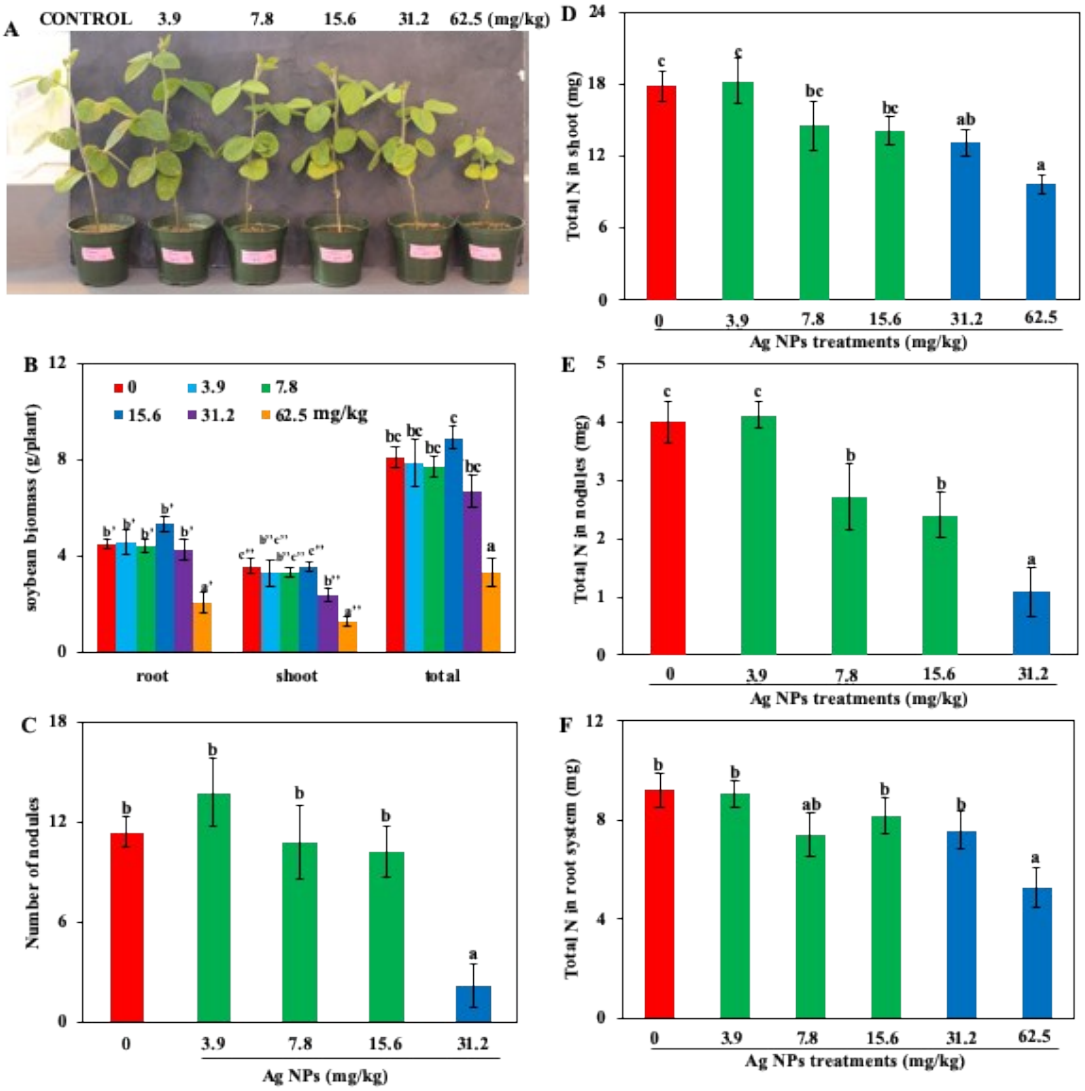

Figure S3. Physiological effects of Ag NPs on soybean growth and N_2 content. (**A**) phenotypic images of soybean grown in different concentrations of Ag NPs–amended soil; (**B**) fresh biomass of soybean; (**C**) total numbers of nodules; (**D–F**) represent total N content in the shoot, nodule, and root system of soybean, respectively. Error bars correspond to standard error of mean. Values of biomass or the N content followed by different letters are significantly different at $p < 0.05$. In Figure S3B, values followed by different letters with signal quotation mark indicate the significant difference of root biomass at $p < 0.05$; values followed by different letters without double quotation marks indicate the significant difference of shoot biomass at $p < 0.05$; values followed by different letters without any marks indicate the significant difference of total biomass at $p < 0.05$.

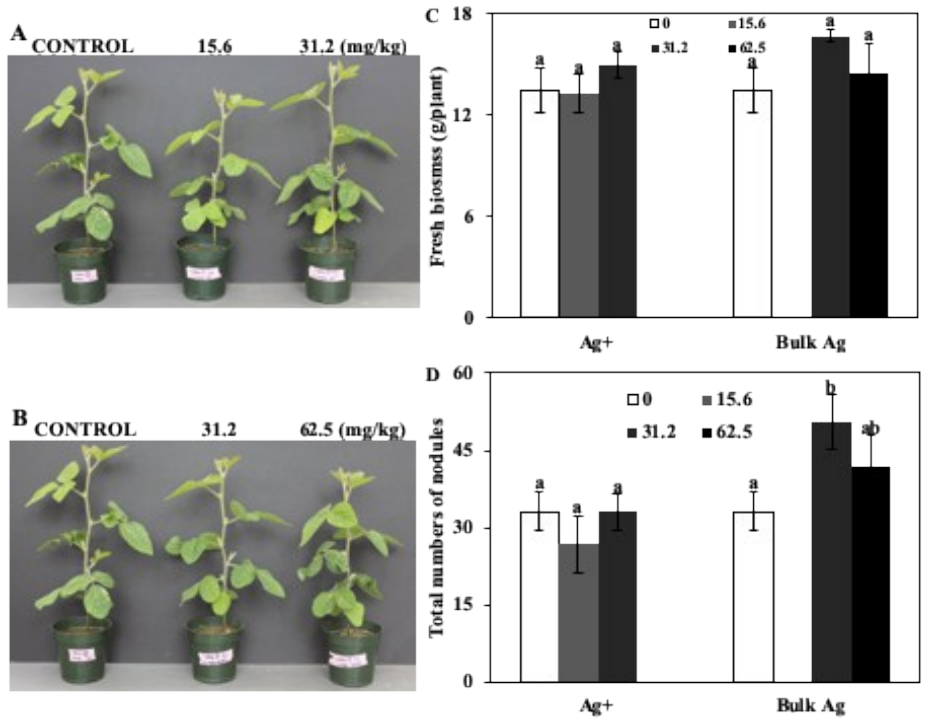

Figure S4. Physiological effects of Ag ions and bulk Ag on soybean growth. Phenotypic images of soybean grown in Ag ions (**A**) and Bulk Ag (**B**) amended soil, respectively; (**C**) fresh biomass of soybean; (**D**) total numbers of nodules. Error bars correspond to standard error of mean. Values of biomass or the nodule number followed by different letters are significantly different at $p < 0.05$.

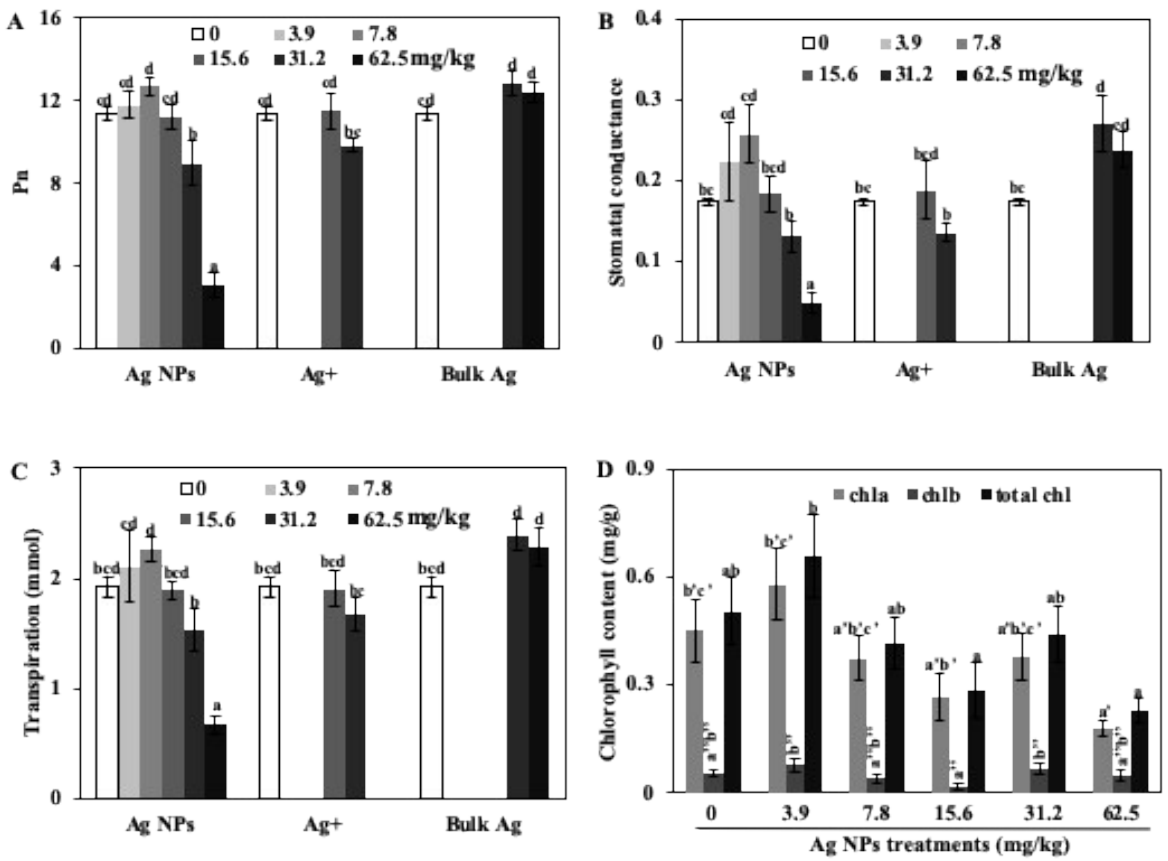

Figure S5. Effects of Ag NPs on the photosynthetic system of soybean. Figure **A**–**D** represent the net photosynthesis rate, stomatal conductance, transpiration and chlorophyll content, respectively. Error bars correspond to standard error of mean. Values of biomass or the N content followed by different letters are significantly different at $p < 0.05$. In Figure S5D, values followed by different letters with signal quotation mark indicate the significant difference of chla content at $p < 0.05$; values followed by different letters without double quotation marks indicate the significant difference of chlb content at $p < 0.05$; values followed by different letters without any marks indicate the significant difference of total chlorophyll at $p < 0.05$.

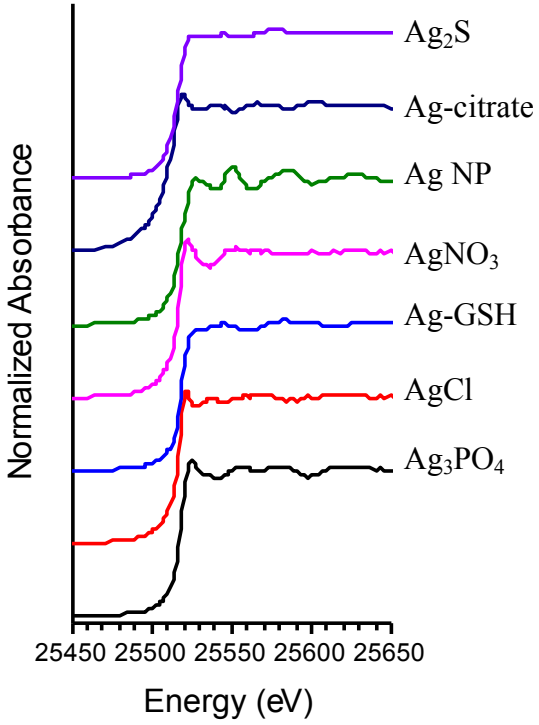

Figure S6. Ag K–edge XANES spectra of reference compounds used for linear combination fitting.

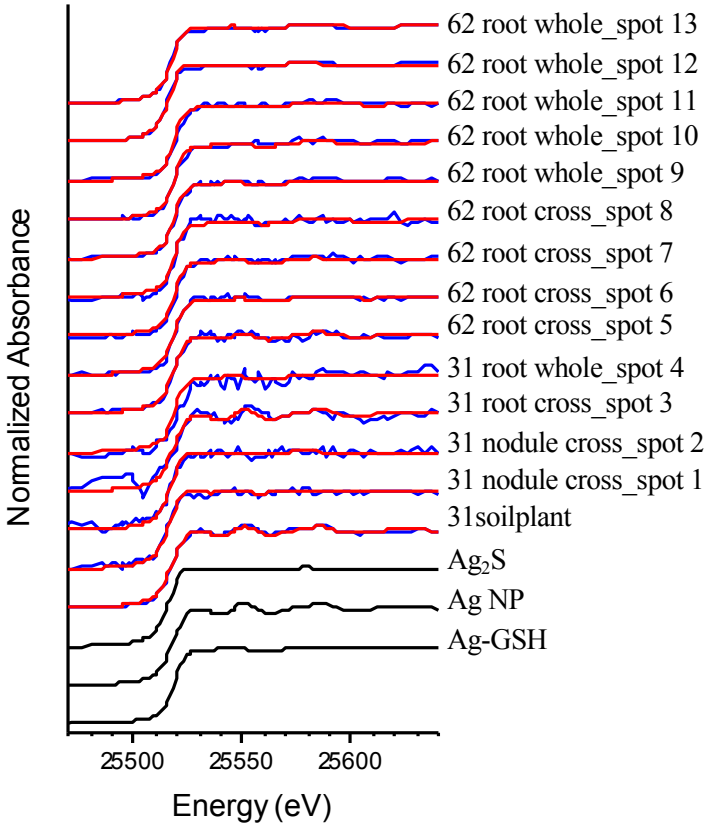

Figure S7. Ag K–edge XANES spectra of reference compounds Ag_2S, Ag NP, and Ag–GSH (black lines), as well as plant tissues (blue lines) and corresponding linear combination fits (red lines).

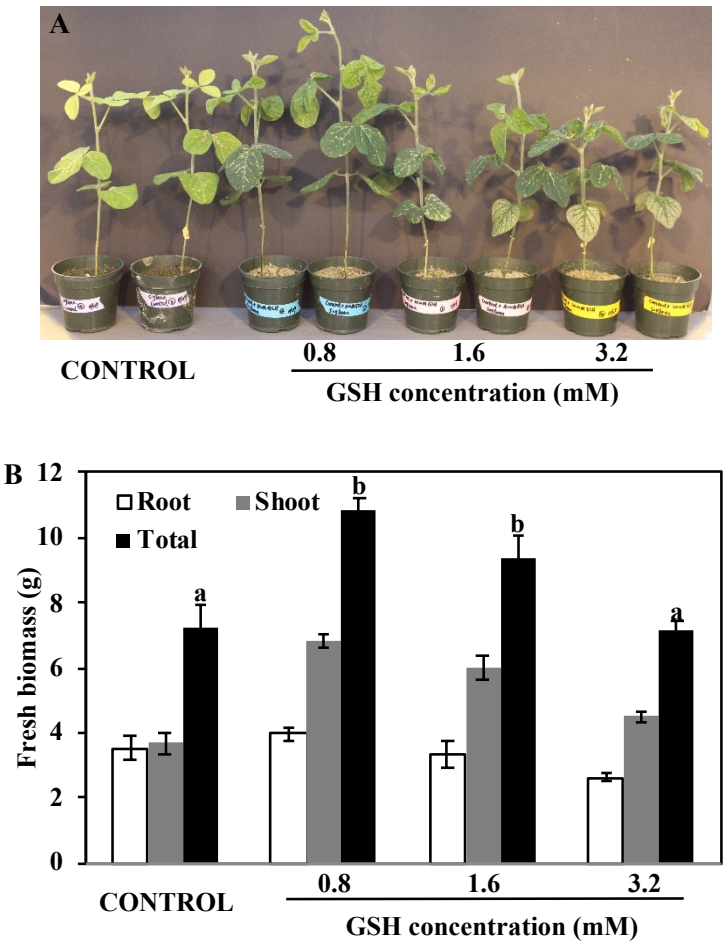

Figure S8. Effects of different concentrations of GSH on soybean growth. (**A**) Image of soybean grown in the presence of different concentrations of GSH; (**B**) Fresh biomass of soybean. Error bars correspond to standard error of mean. Values of biomass followed by different letters are significantly different at $p < 0.05$.

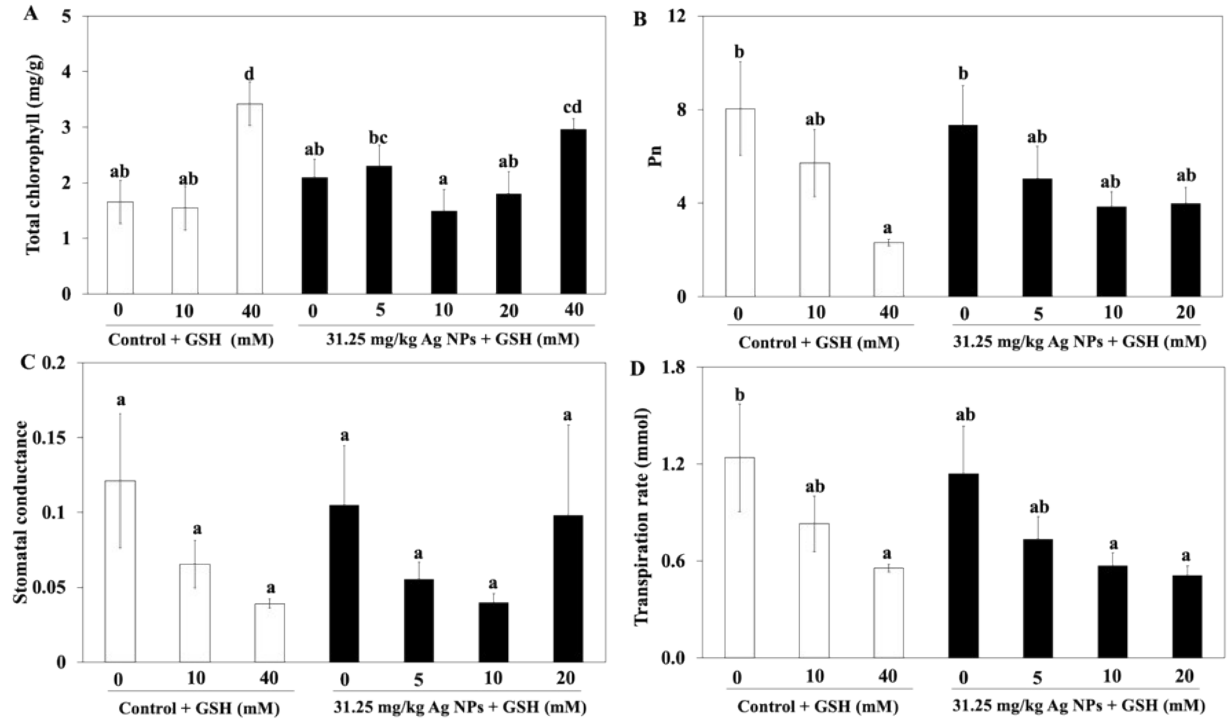

Figure S9. Photosynthetic efficiency in Ag NPs–treated soybean w/ or w/o the addition of GSH. Figure **A**–**D** represents the total chlorophyll content, net photosynthetic rate, stomatal conductance and transpiration rate, respectively. Error bars correspond to standard error of mean. Values of each parameter followed by different letters are significantly different at $p < 0.05$.

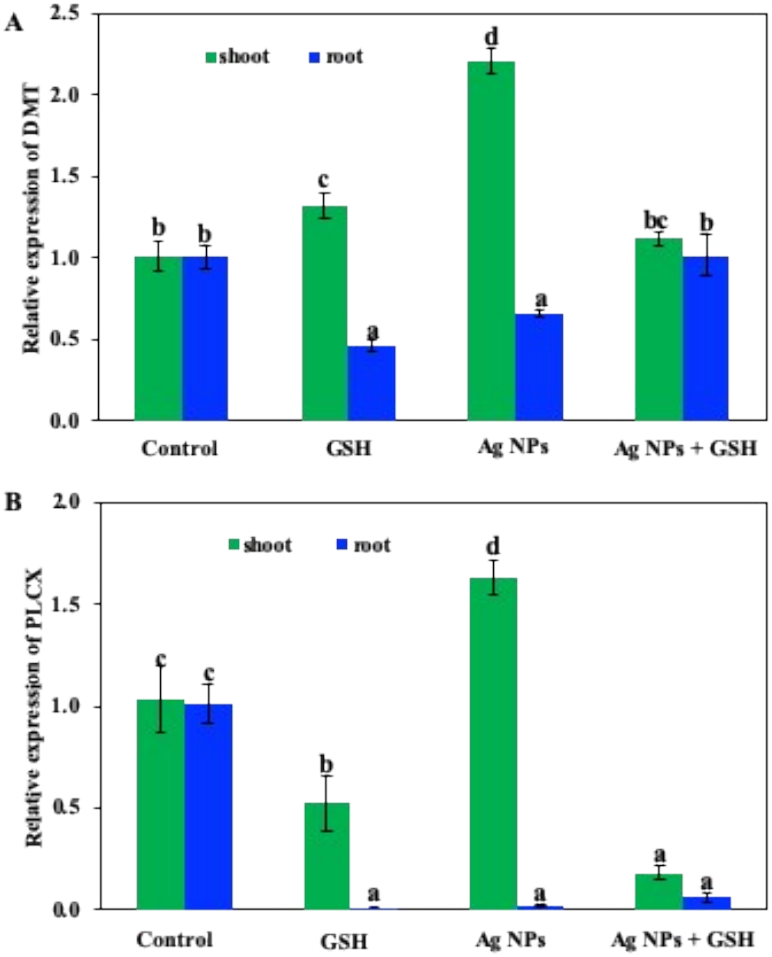

Figure S10. Relative expression of genes encoding divalent metal transporter (**A**) and nodule signaling (**B**) in Ag NP–treated shoots and roots w/ or w/o GSH addition. Error bars correspond to standard error of mean. Values of each gene followed by different letters are significantly different at $p < 0.05$.

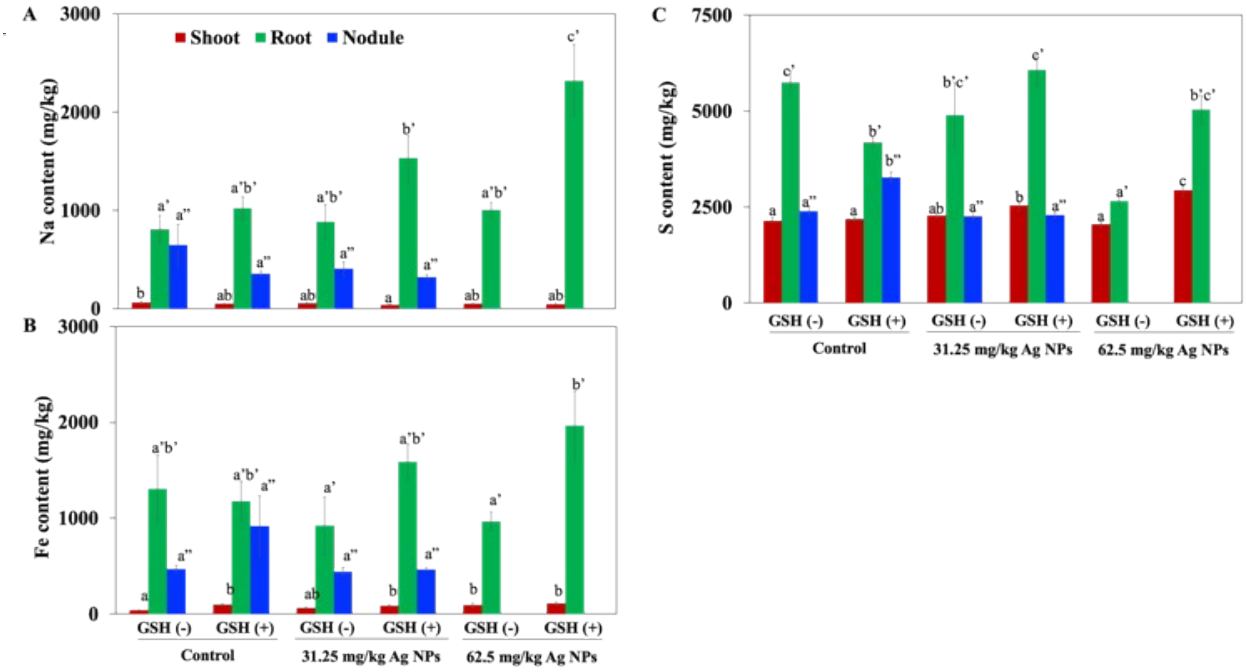

Figure S11. The content of other nutrients in Ag NPs treated soybean w/ or w/o the addition of GSH. Figure **A**–**C** represents the Na, Fe, and S content in soybean shoots, roots, and nodules, respectively. Error bars correspond to standard error of mean. Values of each nutrient element followed by different letters are significantly different at $p < 0.05$.

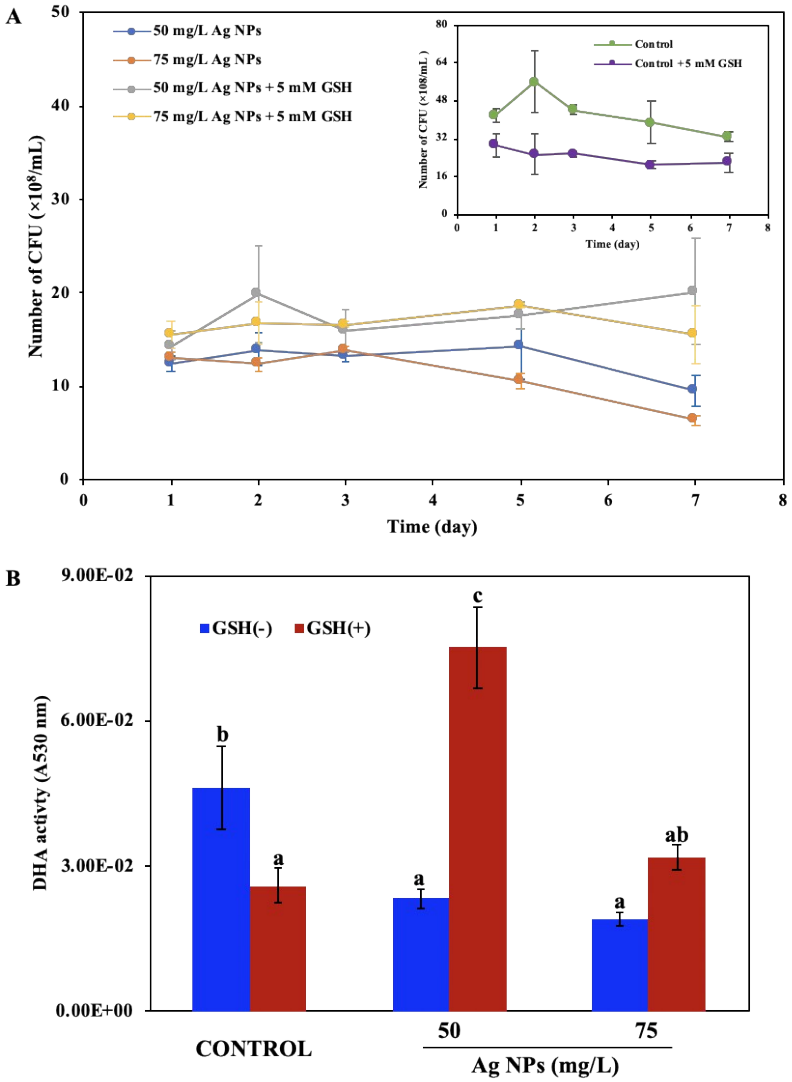

Figure S12. Growth curve of Ag NPs–treated *Bradyrhizobium* w/ or w/o the presences of GSH. (**A**) growth curve; (**B**) DHA activity in the Ag NPs treatment w/ or w/o the addition of GSH. Error bars correspond to standard error of mean. Values of DHA activity followed by different letters are significantly different at $p < 0.05$.

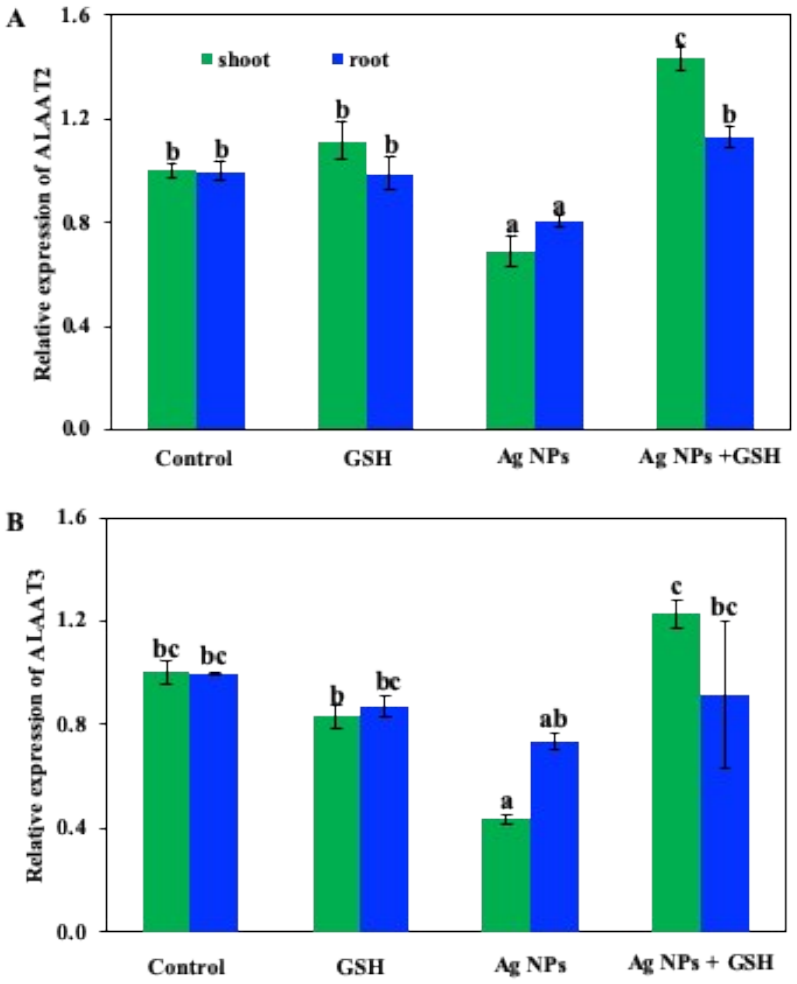

Figure S13. Relative expression of genes encoding alanine aminotransferase (ALAAT2 and ALAAT3) in Ag NPs–treated shoots and roots w/ or w/o the GSH addition. Error bars correspond to standard error of mean. Values of each gene followed by different letters are significantly different at $p < 0.05$.

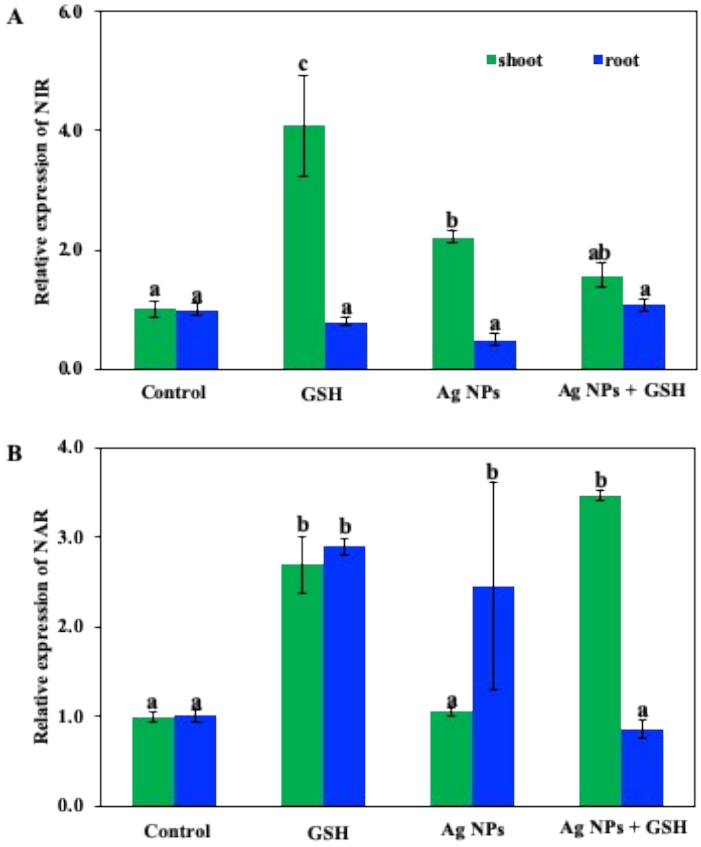

Figure S14. Relative expression of genes encoding nitrite reductase (**A**) and nitrate reductase (**B**) in Ag NPs–treated shoots and roots w/ or w/o the GSH addition, respectively. Error bars correspond to standard error of mean. Values of each gene followed by different letters are significantly different at $p < 0.05$.

Table S1. The composition of HM medium

HM medium (in 1L):	
Na_2HPO_4: 0.125 g	$FeCl_3$ (1 mM): 0.004 g
Na_2SO_4: 0.25 g	$CaCl_2 \cdot 2H_2O$: 0.013 g
NH_4Cl: 0.32 g	HEPES: 1.3 g
$MgSO_4 \cdot 7H_2O$: 0.18 g	MES: 1.1 g
Yeast extract: 0.25 g	Adjust pH 6.6 with NaOH. Autoclave for 30 minutes. Media can be stored at room temperature.

Table S2. A list of primers used for qPCR in this study

Name	Function	Sequence (5' – 3')
GmALAAT2-F	alanine aminotransferase 2	GGTTCAGGATTTGGTCAGAAAG
GmALAAT2-R		TCGTCTTCGTATTGCTCCATG
GmALAAT3-F	alanine aminotransferase 3	GTGCTTATAGTGACTCCCGTG
GmALAAT3-R		GTAGAGTGGGTATTGTGGGAC
GmNIR-F	nitrite reductase	TTCATGGAAGGTGGGATTGAG
GmNIR-R		GGAAACTTACGATGCTGCTTTC
GmNAR-F	inducible nitrate reductase	GGGTTCATCGGTGGAAGAAT
GmNAR-R		TACCACCAACCTTCGTCATTAG
GmPLCX-F	PI-PLCX domain-containing protein	GGATGAAGGGAGGTTCTTGTTC
GmPLCX-R		CGCAACCATGAAGCACATATTC
GmDMT-F	ferrous ion membrane transport protein	GCTGCTCTGGTGATAGTGATT
GmDMT-R		GGTGATGGCTTGCCAAATAAG
GmELF1B F	housekeeping gene	GTTGAAAAGCCAGGGGACA
GmELF1B R		TCTTACCCCTTGAGCGTGG

Table S3. Amino acid content in soybean shoots treated with concentrations of GSH

Treatment	Control	0.8 mM GSH	1.6 mM GSH	3.2 mM GSH
***Asp**	na	na	na	na
Glu	496.57±71.42	1231.69±162.93**	2456.74±241.26**	2814.42±570.20*
Gln	0.00	0.00	0.00	2848.27±1134.16*
Ser	85.73±11.18	255.71±22.61**	683.98±49.73**	2111.83±298.05**
***Arg+Thr**	0.00	177.46±61.19*	1772.74±1177.10	6228.17±1181.36**
Gly	26.60±1.48	55.86±6.35**	102.89±8.13**	221.13±34.39**
Ala	115.80±18.03	363.91±32.03**	618.82±79.62**	2330.51±449.18**
Pro	71.27±7.73	111.79±13.88*	257.68±58.31*	802.43±281.72*
GABA	192.99±34.81	971.47±102.13**	1018.92±144.13**	1737.98±348.47**
Val	65.67±6.55	160.89±18.54**	179.76±22.67**	486.07±119.91*
Met	16.03±1.10	19.18±2.22	31.61±2.75**	48.21±4.31**
***Ile**	nr	69.66±16.50	128.11±25.55	328.30±76.04
***Leu**	nr (114.91±4.45)	199.99±24.38	291.84±36.81	498.98±98.07
Trp	15.14±9.04	109.96±23.79*	199.05±40.82**	205.64±25.57**
Phe	72.11±4.03	185.99±22.05**	458.48±39.43**	1274.62±154.10**
Cys	365.64±27.20	805.46±60.92**	889.55±121.61*	945.99±121.62**
Orn	0.00	0.00	0.35±0.35	22.42±3.69
Lys	13.02±1.00	39.14±3.22**	52.72±2.75**	160.67±16.91**
His	73.23±12.50	130.29±18.95*	476.70±87.07**	1892.22±164.68**

Note: na=not available; nr=not resolved from adjacent peak(s);

*Arg+Thr do not separate in stds or samples. For quantitative comparison, the concentrations of the 2 are added together to create an Agr+Thr std curve;

*Ile indicates Ile at the low concentration does not separate from Leu;

*Leu: nr samples have tiny peaks of Ile included in quantitation, that data is provided in ( ), but not included in the calc of final data;

A student t-test is used to calculate the p value. Single asterisk "*" indicates a significant difference ($p<0.05$) between control and other treatments; double asterisks "**" indicate a significant difference ($p<0.01$) between control and other treatments.

Table S4. Amino acid content in soybean roots treated with concentrations of GSH

Treatment	Control	0.8 mM GSH	1.6 mM GSH	3.2 mM GSH
Asp	58.25±5.46	62.89±9.72	478.25±131.64*	1662.43±249.18**
Glu	99.00±10.92	173.57±29.72*	264.62±43.62*	1197.97±105.65**
Gln	674.21±55.50	731.35±86.19	959.17±129.47	5239.01±277.08**
Ser	96.38±14.17	77.74±8.29	383.44±56.91**	1451.58±271.08**
***Arg+Thr**	183.26±18.03	280.11±24.74*	393.37±81.98*	1623.02±236.56**
Gly	55.18±11.43	37.78±2.21	72.17±8.30	183.24±32.99*
Ala	61.36±5.99	85.40±8.31*	1120.09±298.20*	4376.58±966.88*
Pro	10.67±2.16	12.56±1.51	36.47±4.92**	37.32±16.63
GABA	54.55±4.32	186.91±17.54**	329.41±24.83**	859.77±130.89**
Val	24.92±3.35	33.78±3.02*	92.40±5.39**	239.31±30.42**
Met	18.30±1.54	21.87±3.44	20.23±8.51	22.86±1.47*
Ile	7.92±2.33	21.77±4.57*	53.02±1.72**	94.45±12.00**
Leu	24.12±3.30	40.00±5.68*	66.77±1.10**	63.23±7.46**
Trp	6.71±1.55	16.07±2.67*	46.22±4.50**	99.75±11.39**
Phe	74.69±4.42	129.18±11.45**	421.95±76.53**	1052.82±74.56**
Cys	248.06±30.42	382.01±25.79**	459.42±48.05**	658.97±77.20**
Orn+?	31.22±7.89	36.88±3.88	68.91±12.14*	63.37±4.58**
Lys	17.07±2.71	22.52±2.74	45.97±3.68**	82.93±8.96**
His	75.34±9.60	110.76±6.24*	633.89±127.26*	1290.61±74.81**

Note: *Arg+Thr do not separate in stds or samples. For quantitative comparison, the concentrations of the 2 are added together to create an Agr+Thr std curve; Orn+? Indicates Orn coelutes with another small unknown peak from sample; A student t-test is used to calculate the p value. Single asterisk "*" indicates a significant difference ($p<0.05$) between control and other treatments; double asterisks "**" indicate a significant difference ($p<0.01$) between control and other treatments.

Table S5. Amino acid content in Ag NPs treated soybean shoots w/ or w/o GSH addition

Treatment	Control		31.2 mg/kg Ag NPs		62.5 mg/kg Ag NPs	
	GSH(-)	GSH(+)	GSH(-)	GSH(+)	GSH(-)	GSH(+)
***Asp**	na	na	na	na	na	na
Glu	496.57±71.42	1231.69±162.93**	659.36±97.02	3047.20±917.80	1583.49±324.44*	1385.51±212.29**
Gln	0.00	0.00	306.55±102.49	582.17±336.89*	234.76±137.29	177.29±103.28
Ser	85.73±11.18	255.71±22.61**	86.42±9.40	557.71±138.28*	218.33±42.46*	227.59±44.00*
***Arg+Thr**	0.00	177.46±61.19*	0.00	298.02±194.46**	141.69±83.33	224.65±23.19
Gly	26.60±1.48	55.86±6.35**	38.57±3.60*	145.20±49.98**	64.75±8.99*	55.70±3.92*
Ala	115.80±18.03	363.91±32.03**	187.63±16.42*	1138.93±244.77*	259.78±54.52*	280.20±29.72**
Pro	71.27±7.73	111.79±13.88*	108.66±12.14*	391.91±100.76*	143.94±29.88*	121.09±12.99*
GABA	192.99±34.81	971.47±102.13**	383.73±28.74**	3906.81±948.68*	642.98±115.55*	847.31±122.17**
Val	65.67±6.55	160.89±18.54**	87.29±5.03	353.49±78.32*	132.64±14.30*	137.49±11.46
Met	16.03±1.10	19.18±2.22	18.93±1.92	49.46±11.97	23.44±3.19	20.73±2.04
***Ile**	nr	69.66±16.50	nr	124.77±28.56	45.12±9.39	54.01±6.01
***Leu**	nr (114.91±4.45)	199.99±24.38	nr (151.19±10.08)	490.19±109.80	252.47±41.34 (239.60±31.49)	210.98±9.93
Trp	15.14±9.04	109.96±23.79*	45.12±8.68*	193.48±60.41*	39.52±16.34	103.19±33.88*
Phe	72.11±4.03	185.99±22.05**	102.27±11.70*	465.59±108.56**	201.10±33.71*	184.81±24.73*
Cys	365.64±27.20	805.46±60.92**	665.13±45.13**	2037.83±424.27*	1106.27±103.04**	934.72±96.91**
Orn	0.00	0.00	0.00	0.00	0.00	0.00
Lys	13.02±1.00	39.14±3.22**	17.71±1.42*	119.42±28.47*	38.01±6.22*	42.02±2.87**
His	73.23±12.50	130.29±18.95*	95.12±15.58	312.12±93.15*	35.37±5.99*	32.81±3.83*

Note: na=not available; nr=not resolved from adjacent peak(s);

*Arg+Thr do not separate in stds or samples. For quantitative comparison, the concentrations of the 2 are added together to create an Agr+Thr std curve;

*Ile indicates Ile at the low concentration does not separate from Leu;

*Leu: nr samples have tiny peaks of Ile included in quantitation, that data is provided in ( ), but not included in the calc of final data;

A student t-test is used to calculate the p value. Single asterisk "*" indicates a significant difference ($p<0.05$) between control and other treatments; double asterisks "**" indicate a significant difference ($p<0.01$) between control and other treatments.

Table S6. Amino acid content in Ag NPs treated soybean roots w/ or w/o GSH addition

Treatment	Control		31.2 mg/kg Ag NPs		62.5 mg/kg Ag NPs	
	GSH(-)	GSH(+)	GSH(-)	GSH(+)	GSH(-)	GSH(+)
Asp	58.25±5.46	62.89±9.72	72.59±9.42	85.44±12.68	143.14±31.59*	78.67±10.23
Glu	99.00±10.92	173.57±29.72	110.00±10.00	234.75±39.64*	116.23±23.55	134.34±5.12*
Gln	674.21±55.50	731.35±86.19	911.07±92.70*	1026.48±93.30*	1095.12±92.50**	1031.26±100.85*
Ser	96.38±14.17	77.74±8.29	95.54±34.04	140.54±17.59	80.69±12.49	106.25±17.85
***Arg+Thr**	183.26±18.03	280.11±24.74	237.79±29.61	359.92±54.99*	210.36±26.10	286.60±23.63**
Gly	55.18±11.43	37.78±2.21	63.96±17.08	55.82±5.49	31.28±2.99	43.83±5.40
Ala	61.36±5.99	85.40±8.31	67.95±12.65	130.40±19.84*	73.86±28.08	207.63±42.91*
Pro	10.67±2.16	12.56±1.51	10.63±2.82	22.32±1.69	6.80±1.32	17.26±5.46
GABA	54.55±4.32	186.91±17.54	74.53±8.26*	287.03±35.32**	85.51±22.63	217.77±53.04*
Val	24.92±3.35	33.78±3.02	30.00±5.96	53.26±5.27**	39.16±5.24*	47.83±7.93*
Met	18.30±1.54	21.87±3.44	19.95±2.29	20.69±1.69	22.21±0.74*	21.07±3.29
Ile	7.92±2.33	21.77±4.57	13.24±3.26	36.15±5.29**	33.25±7.32*	37.95±7.45*
Leu	24.12±3.30	40.00±5.68	26.78±5.07	63.70±9.44**	41.93±8.53	61.32±12.05*
Trp	6.71±1.55	16.07±2.67	11.66±1.87*	19.57±3.91*	16.24±4.39	21.12±2.83**
Phe	74.69±4.42	129.18±11.45	131.86±28.86	182.27±24.11**	203.74±17.66**	159.09±5.57**
Cys	248.06±30.42	382.01±25.79	574.48±56.01**	790.35±85.69**	675.87±72.42**	510.79±18.28**
Orn+?	31.22±7.89	36.88±3.88	46.14±12.95	68.03±9.78*	52.19±2.91*	46.65±4.04
Lys	17.07±2.71	22.52±2.74	20.70±3.86	41.58±4.75**	25.19±2.89*	34.18±5.27*
His	75.34±9.60	110.76±6.24	106.14±19.99	136.64±12.80**	96.94±6.01	92.37±7.09

Note: *Arg+Thr do not separate in stds or samples. For quantitative comparison, the concentrations of the 2 are added together to create an Agr+Thr std curve;
Orn+? Indicates Orn coelutes with another small unknown peak from sample;
A student t-test is used to calculate the p value. Single asterisk "*" indicates a significant difference ($p<0.05$) between control and other treatments; double asterisks "**" indicate a significant difference ($p<0.01$) between control and other treatments.

Table S7. Amino acid content in Ag NPs treated soybean nodules w/ or w/o GSH

Treatment	Control		31.2 mg/kg Ag NPs	
	GSH(-)	GSH(+)	GSH(-)	GSH(+)
Asp	196.93±39.85	118.11±10.24	350.62±235.54	196.15±51.59
Glu	1108.60±61.31	1330.81±156.57	1061.72±122.88	1015.81±258.78
Gln	790.35±79.15	1034.48±167.23	1381.08±331.66	921.64±148.46
Ser	963.98±69.22	1548.07±208.91*	785.51±108.39	1164.98±258.63
***Arg+Thr**	247.78±22.56	358.10±71.59	252.70±25.97	245.34±50.20
Gly	176.40±43.94	307.08±186.75	142.91±18.96	158.96±34.47
Ala	651.03±22.72	1051.00±162.26*	636.92±79.82	369.75±50.38**
Pro	62.33±7.70	88.84±12.96	96.09±31.78	60.15±12.25
GABA	363.80±48.46	835.37±120.87*	535.81±79.38	515.87±109.80
Val	117.36±18.05	150.83±20.83	101.81±10.20	100.92±19.38
Met	85.14±4.36	99.79±11.00	71.05±9.73	77.97±17.08
Ile	44.37±6.93	68.33±12.97	36.89±4.95	45.07±9.93
Leu	114.03±13.23	151.12±21.93	154.50±37.56	114.93±26.58
Trp	87.77±15.19	203.73±45.61*	46.64±23.49	107.82±30.53
Phe	441.98±73.89	505.94±71.66	471.81±64.04	390.32±75.83
Cys	2245.77±197.84	3173.47±430.87	3072.09±304.04*	3768.22±827.35
Orn+?	72.06±16.72	49.21±8.81	48.36±12.00	37.59±8.67
Lys	69.52±6.14	94.17±14.35	82.15±9.08	74.65±17.49
His	582.10±99.20	1192.12±121.66**	556.95±101.64	975.11±257.51

Note: *Arg+Thr do not separate in stds or samples. For quantitative comparison, the concentrations of the 2 are added together to create an Agr+Thr std curve; A student t-test is used to calculate the p value. Single asterisk "*" indicates a significant difference ($p<0.05$) between control and other treatments; double asterisks "**" indicate a significant difference ($p<0.01$) between control and other treatments.