Supporting Information

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SI Materials and Methods

Culture of Plant and Fungal Material. Ri T-DNA–transformed carrot (*Daucus carota* clone DCI) roots colonized by *Glomus intraradices* Schenck & Smith (DAOM 197198; Biosystematics Research Center) were grown in two-compartment Petri dishes with one root compartment (RC) and one fungal compartment (FC) as described by Bücking and Shachar-Hill (1). The FC was filled with modified M medium (2) without sucrose and KNO_3 and in which $Ca(NO_3)_2 \times 4H_2O$ was replaced with an equimolar concentration of $CaCl_2 \times 2H_2O$, so that the supplied N compound (see below) was the sole N source (pH 5.3). To facilitate the harvest of extraradical mycelium (ERM) for enzymatic assays and gene expression studies, only 175 mg L^{-1} Gel-Gro (ICN Biochemicals) was used to weakly solidify the medium of the FC (other experiments used 3.5 g L^{-1} Gel-Gro). At 3 w after the plates were split, the development of the ERM in the FC was examined stereomicroscopically. The plates were distributed equally among the different experimental treatments, so that each treatment had the same variability in amount of ERM in the FC.

Arginase and Urease Assays. The arginase activity in mycorrhizal root and ERM samples was evaluated spectrophotometrically following the protocol of Cruz et al. (3) with minor modifications. The frozen root or ERM sample was ground in liquid nitrogen and homogenized with 20% polyvinylpyrrolidone (PVP; wt/wt) and 200 μL of the extraction buffer [100 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1 mM phenylmethylsulphonylfluoride, and 1% 2-mercaptoethanol, vol/vol]. The sample was centrifuged for 15 min at 20,000 × *g* and 4 °C and kept refrigerated until further analysis. The sample was activated with 1 mM $MnCl_2$ at 37 °C for 40 min. The reaction buffer contained 50 mM Tris-HCl (pH 9.5), 2 mM $MnCl_2$, 33 μM urease inhibitor, and 250 mM L-arginine. The reaction was started by adding 45 μL of the sample to 155 μL of reaction buffer and stopped after 20 min at 37 °C by adding 20 μL of 70% (vol/vol) perchloric acid. The reaction mixture was thoroughly mixed with 1.5 mL of an acid mixture containing 9% phosphoric acid (vol/vol), 27% sulfuric acid (vol/vol), and 50 μL of 3% a-isonitrosopropiophenone (wt/vol) (Sigma-Aldrich) in 95% ethanol and heated to 95 °C in the dark for 60 min. After the mixture was allowed to cool for 10 min, absorbance was measured at 540 nm.

The urease assay was carried out according to the method described by Witte and Medina-Escobar (4). For this assay, 100 mg of root tissue was grounded in liquid nitrogen and homogenized with 20% PVP. The sample was extracted with 100 μL of phosphate buffer (pH 7.4) containing 50 mM $NaCl_2$, 1 mM EDTA, 20 mM DTT, and 0.1 mM phenylmethanesulfonylfluoride (solution stored in the dark) and centrifuged at 14,000 × *g* at 4 °C for 10 min. The supernatant was added to a spin column and centrifuged at 735 × *g* for 2 min at 4 °C. To 20 μL of eluate, 50 μL of 100 mM urea was added and incubated for 40 min at 37 °C. An aliquot of 20 μL obtained and diluted with 980 μL of sterile aqua bidest, 100 μL of phenol nitroprusside, and 200 μL of hypochloride solution. Urease activity was measured spectrophotometrically at 636 nm.

NMR Analysis of the Total ^{15}N Content in Root Tissues. Lyophilized roots (20–30 mg) were digested in 0.5 mL of concentrated H_2SO_4 and heated for 2 h at 225 °C, followed by the addition of 36 drops of 30% H_2O_2 (three drops every 30 s). The solution was then heated for an additional 3 h at 225 °C to remove any traces of water and allowed to cool. Then 34 μL of the resulting clear solution of $(NH_4)_2SO_4$ in H_2SO_4 was dissolved in 600 μL of 99.9% $DMSOd_6$ (solvent used to provide a frequency lock signal to stabilize the spectrometer field) containing 0.05% (vol/vol) TMS reference. The ^1H spectrum was obtained in a 5-mm *z*-axis PFG dual broadband probe on a 9.2-T Varian INOVA spectrometer operating at 400 MHz. The spectra were acquired using ~1,400 transients with a 90° (10.8 μs) pulse width, a spectral width of 5,042 Hz, a pulse delay of 1.0 s, and an acquisition time of 1.6 s at 25 °C. The T_1 relaxation time of the NH_4 protons was 0.4 s. The triplet resonance of the ^1H-^{14}N and doublet resonance of the ^1H-^{15}N were observed centered at 7.2 ppm relative to the TMS resonance 0.0 ppm, with observed ^1H-^{15}N couplings of 53 Hz and 74 Hz, respectively. The integrated area of the ^1H-^{15}N doublet resonances divided by the sum of the doublet and triplet resonance area yielded the percentage labeling with ^{15}N of the total N in the tissue.

Liquid Scintillation Counting. The ERM was harvested from the medium in the FC after several washing and centrifugation steps in Na citrate buffer (10 mM; pH 6.0). An aliquot of the medium from the FC and RC was obtained to measure radioisotope residues in the medium and to verify the absence of cross-contamination between compartments in the plates. Root and ERM samples were dried in an oven at 70 °C, weighed, and digested with a tissue solubilizer (TS-2; RPI). Radioactivity was measured with a Wallac liquid scintillation counter (Perkin-Elmer). The accuracy of all measurements was corrected using an internal standard.

GC-MS Analysis of Free Amino Acids. Freeze-dried and weighed roots were homogenized by grinding with preground, acid-washed sand in a glass mortar. Then 2.5 mL of methanol/0.01 N HCl (70:30 vol/vol) was added, the sample was ground again, and the resulting sample was transferred into a tube and centrifuged at 1,487 × *g* for 10 min. The supernatant was loaded on a cation exchange column filled with 0.5 mL of DOWEX (Acros Organics) 50 × 8 (100–200 mesh). Before use, the DOWEX had been sequentially washed in 3 N NH_4OH, deionized water, 2 N HCl, and deionized water until the pH was neutral. Neutral compounds, especially carbohydrates, were washed off in two washes with 0.5 mL deionized water. The free amino acids were then eluted by two additions 0.5 mL 3 N NH_4OH and collected in a glass vial. The eluate was frozen, lyophilized, resuspended in 0.3 mL 0.01 N HCl, and again frozen and lyophilized. The amino acids were derivatized by adding first 10–20 μL of dry N, *N*-dimethylformamide and then 30–50 μL of *N*-methyl-*N*-tert-butyldimethylsilyl-trifluoroacetamide to the sample, depending on sample size. The sample was heated on a hot plate for 30 min at 110 °C in sealed glass vials with a rubber septum and then assessed by GC-MS according to the method described by Mawhinney et al. (5). The silylated extracts were injected on a Finnigan TRACE MS 2000 equipped with a splitless injector and a fused 0.25-μm-thick silica capillary column (0.25 mm i.d., 30 mm long, RTX-5MS; Restek), and interfaced to a Finnigan quadrupole mass detector (Thermo Scientific). Helium was used as a carrier gas with a flow rate of 1 mL min^{-1}. The oven temperature was maintained at 110 °C for 2 min after injection, then ramped up to 260 °C at 10 °C/min and held there for 5 min. The injection temperature was 250 °C. Mass spectra were acquired with an electron energy of 70 eV, and the mass range was scanned at a mass-to-charge ratio of 150–600 with a scan rate of 2 Hz. The identities of amino acids were confirmed by comparison of mass

fragments of authentic standards. Except for arginine (Arg), amino acid labeling was determined by evaluation of the M-57 fragments of the *N*-methyl-*N*-tert-butyldimethylsilyl-trifluoroacetamide derivatives. The 57 fragment is a t-butyl group lost in the fragmentation. The measured fragment of Arg had a *m/z* ratio of 442 (M-188), because one guanido N was lost from the tetra-substituted tert-butyldimethylsilyl(tBDMS)-derivatized Arg (6). The isotopic contents given by the M, M+1, M+2, M+3, and M+4 were used to calculate the extent of isotopic enrichment in each amino acid compared with the isotopic ratios of the nonlabeled derivatized amino acids.

P Content and P Pool Distribution. Root material was ground in liquid nitrogen, weighed, dried at 90 °C, and digested by the addition of 1 M HCl for 2 h at 95 °C. P content was analyzed spectrophotometrically at 436 nm after the addition of ammonium-molybdate vanadate solution (Ricca Chemical). The P pools in mycorrhizal roots were analyzed according to a protocol of Aitchison and Butt (7). The following P pools were extracted: (*i*) orthophosphate and short-chain polyP (acid-soluble, chain length $\leq$20 P_i) after extraction with 10% trichloroacetic acid (wt/vol) at 4 °C; (*ii*) phospholipids after extraction with first 100% ethanol and then ethanol:ether (3:1, vol/vol); (*iii*) long-chain polyP (acid-insoluble, chain length $\geq$21 P_i) after extraction with 1 M KOH at room temperature, and (*iv*) DNA, RNA, and protein phosphates (residue). The polyP within the supernatants were precipitated by $BaCl_2$ (saturated) twice overnight at 4 °C. The orthophosphate and phospholipid pool aliquots were measured directly, and the polyP and DNA, RNA, and protein phosphates were measured after acid hydrolysis with 1 M HCl for 2 h at 95 °C. The P content was determined spectrophotometrically at 436 nm after the addition of ammonium-vanadate-molybdate solution.

Quantitative PCR of Genes Involved in Fungal N Metabolism. Using quantitative real-time PCR, we analyzed the effect of different C supply conditions on the expression of several fungal genes putatively involved in nitrogen uptake and assimilation and Arg biosynthesis and breakdown (Table S2). RNA was extracted from homogenized root and ERM samples with TRIzol Reagent (Invitrogen). The supernatant after separation was treated with an RNeasy MinElute Cleanup Kit (Qiagen) according to the manufacturer's instructions with an additional RPE buffer wash step. The extracted RNA was treated with DNase (RQ1 RNase-Free Dnase; Promega), and cDNA was synthesized using M-MLV Reverse-Transcriptase (Promega), Random Primer 6 (New England Biolabs), and dNTPs (Qiagen). The QuantiTect SYBR Green PCR Kit (Qiagen) was used to amplify 0.4 μg of cDNA (total volume 20 μL) on an ABI 7900HT thermal cycler (Applied Biosystems). All gene-specific primers were as described in ref. (8). The PCR conditions were as follows: 50 °C for 2 min; 95 °C for 15 min; 40 cycles of 95 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s; 95 °C for 15 s; 60 °C for 15 s; and 95 °C for 15 s. Changes in fungal gene expression compared with untreated controls were quantified using the comparative $\Delta\Delta C_T$ method (9) and the reference gene SR4 ribosomal protein (10).

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2. Chabot S, Bel-Rhlid R, Chenevert R, Piché Y (1992) Hyphal growth promotion in vitro of the VA mycorrhizal fungus *Gigaspora margarita* Becker and Hall by the activity of structurally specific flavonoid compounds under CO_2-enriched conditions. *New Phytol* 122:461–467.
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8. Tian C, et al. (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: Gene characterization and the coordination of expression with nitrogen flux. *Plant Physiol* 153:1175–1187.
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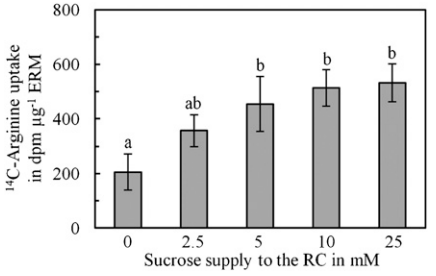

Fig. S1. Effect of sucrose addition to the RC on uptake of ^{14}C-Arg by the ERM from the FC (mean ± SEM of n = 6–7). Different letters on the bars indicate statistically significant differences according to ANOVA and the LSD test ($P \leq 0.05$).

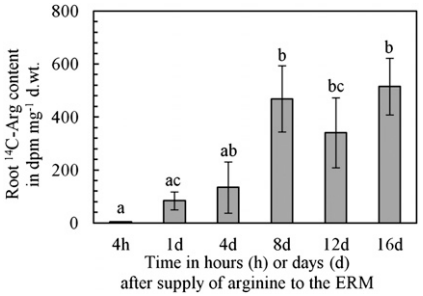

Fig. S2. Time course of Arg transport to mycorrhizal roots after addition of 50 μM ^{14}C-Arg to the FC. Three root aliquots were analyzed per biological replicate (mean ± SEM of n = 7). Different letters on the bars indicate statistically significant differences according to ANOVA and the LSD test ($P \leq 0.05$).

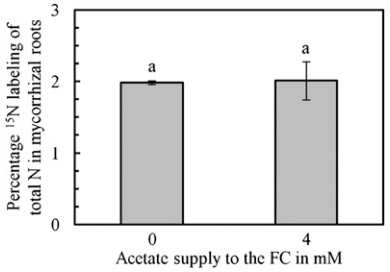

Fig. S3. Transport of ^{15}N from the fungal ERM to the roots without or after addition of 4 mM acetate to the FC. The ^{15}N labeling of total N in mycorrhizal roots is shown (mean ± SEM of n = 3).

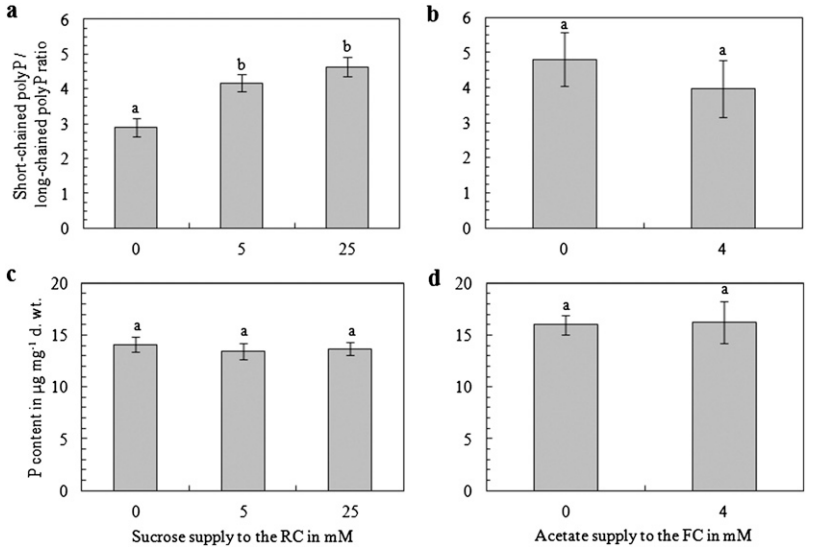

Fig. S4. Ratio of short-chain to long-chain polyP (*A* and *B*) and P content (*C* and *D*) in mycorrhizal roots after the addition of sucrose to the RC (*A* and *C*) and addition of acetate to the FC (*B* and *D*). Data are mean ± SEM of n = 4–5. Different letters on the bars indicate statistically significant differences according to ANOVA and the LSD test ($P \leq 0.05$).

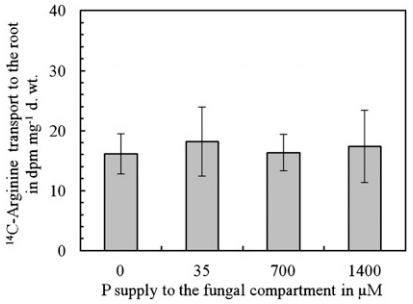

Fig. S5. Arginine transport from the ERM to the root dependent on the P supply to the FC. Three root aliquots were analyzed per biological replicate (mean ± SEM of n = 6). There were no statistically significant differences between treatments according to ANOVA and the LSD test ($P \leq 0.05$).

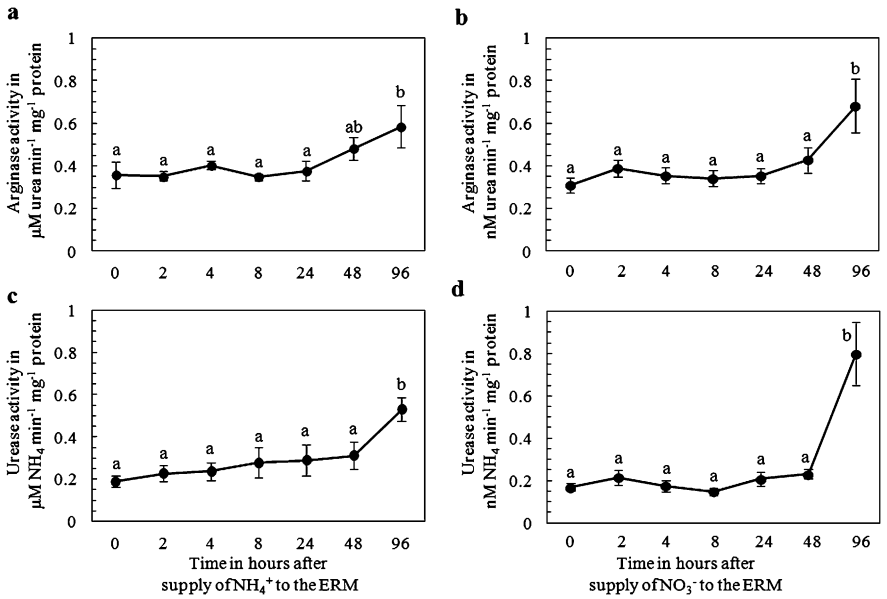

Fig. S6. Arginase (*A* and *B*) and urease (*C* and *D*) activities in mycorrhizal roots at different times points after the addition of 4 mM ^{15}N-NH_4Cl (A and C) or ^{15}N-KNO_3 to the FC. Data are given as µM urea (*A* and *B*) or NH_4 (*C* and *D*) min^{-1} mg^{-1} of protein (mean ± SEM of n = 6). Different letters indicate statistically significant differences according to ANOVA and the LSD test ($P \geq 0.05$).

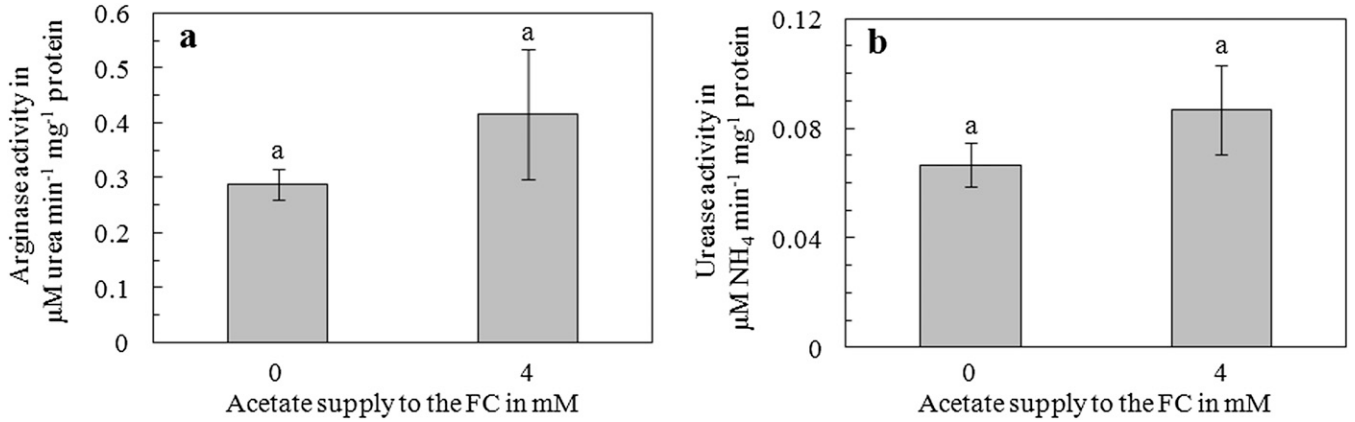

Fig. S7. Arginase (*A*) and urease (*B*) activities in mycorrhizal roots without or after the addition of 4 mM acetate to the FC. Data are µM urea (*A*) or NH_4 (*B*) min^{-1} mg^{-1} of protein (mean ± SEM of n = 5). The letters on the bars indicate no statistically significant differences between treatments according to ANOVA and the LSD test ($P \geq 0.05$).

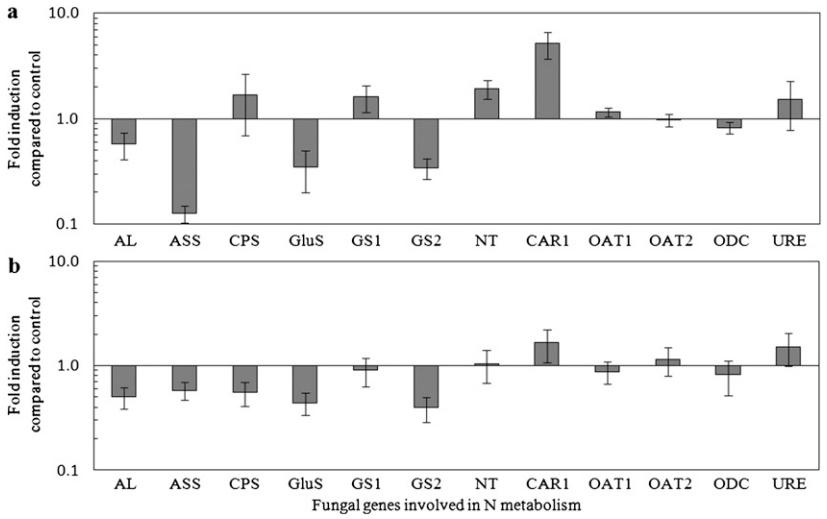

Fig. S8. Fungal gene expression in the ERM (*A*) and IRM (*B*) after addition of 4 mM NH_4Cl and 4 mM acetate to the FC. The results are expressed in fold induction compared with control, to which only 4 mM NH_4Cl was added (mean ± SEM of $n = 4$). Values <1 represent a decrease and values >1 an increase in transcript levels relative to control.

Table S1. ^{15}N labeling of free amino acids in mycorrhizal roots at 4 d after the addition of 4 mM $^{15}NH_4Cl$ to the ERM dependent on the sucrose supply to the RC or the acetate supply to the FC

	Ala	Asp	Asn	Arg	Gln	Glu	Gly	Ser	Val
Free amino acids in mycorrhizal roots after addition of sucrose to the RC									
0 mM	21.9 ± 10.2a	14.1 ± 6.5ab	41.7 ± 20.3	69.5 ± 26.6	54.7 ± 20.5a	14.5 ± 10.4a	10.6 ± 4.7a	14.7 ± 7.0ab	10.6 ± 7.8a
5 mM	9.0 ± 3.1a	2.8 ± 1.8a	ND	32.6 ± 16.3	19.5 ± 8.1a	7.2 ± 6.7a	3.2 ± 1.5a	4.7 ± 1.1a	5.1 ± 1.5a
25 mM	14.0 ± 4.6a	8.3 ± 4.0ab	2.86	60.4 ± 13.2	32.0 ± 9.5a	10.8 ± 2.8a	6.9 ± 3.0a	11.0 ± 2.2b	6.9 ± 3.2a
Free amino acids in mycorrhizal roots after addition of acetate to the FC									
0 mM	9.2 ± 2.0a	17.3 ± 10.0ab	5.1 ± 1.6	91.6	26.1 ± 6.7a	7.5 ± 2.7a	4.1 ± 2.0a	5.4 ± 1.2a	4.3 ± 1.7a
4 mM	22.3 ± 9.7a	8.3 ± 4.0b	10.3	76.6 ± 21.5	43.9 ± 10.8a	20.8 ± 10.2a	11.7 ± 5.8a	14.9 ± 9.2ab	13.5 ± 5.7a

Data are mean ± SEM. The number of biological replicates varied depending on the number of samples in which a particular amino acid was detectable. Different letters indicate statistically significant differences according to ANOVA and the LSD test ($P \leq 0.05$). ND, not determined.

Table S2. Fungal genes and their putative role in fungal nitrogen metabolism, according to Tian et al. (1)

Function	Gene name and abbreviation	GenBank accession no.
Nitrogen uptake	Nitrate transporter (*NT*)	GU111919
Nitrogen assimilation	Glutamine synthetase 1 (*GS1*)	GU111909
	Glutamine synthetase II (*GS2*)	GU111910
	Glutamate synthase (*GluS*)	GU111916
Arginine biosynthesis	Carbamoyl-phosphate synthase glutamine chain (*CPS*)	GU111917
	Argininosuccinate lyase (*AL*)	GU111918
	Argininosuccinate synthase (*ASS*)	GU111911
Arginine catabolism	Arginase (*CAR1*, E.C. 3.5.3.1)	GU111913
	Urease (*URE*)	GU111915
	Ornithine aminotransferase I (*OAT1*)	GU111912
	Ornithine aminotransferase 2 (*OAT2*)	
	Ornithine decarboxylase (*ODC*)	GU111914

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