63% (79%)

Supplemental Material

GpAMT2

GpAMT3

GiAMT1

GiAMT2

60% (74%)

59% (76%)

57% (74%)

61% (74%)

Amino acid identity (similarity)				
	GpAMT1	GpAMT2	GpAMT3	GiAMT1

63% (78%)

61% (77%)

58% (74%)

65% (81%)

65% (81%)

Fig. S1 Comparison of glomeromycotan AMT amino acid sequences. GpAMT1, GpAMT2, GpAMT3, GiAMT1 and GiAMT2 amino acid sequences were analyzed pairwise with the BLAST algorithm and Blosum62 (1), and percentages of identical (similar) amino acids are listed. The comparison of *Geosiphon pyriformis* ammonium transporters is marked in dark grey.

GpAMT1 GpAMT2 GpAMT3 GiAMT1 GiAMT2 ScMep1 ScMep2 ScMep3 TbAmt1 HcAmt1	1	54 49 66 81 55 59 72 58 79 69
GpAMT1 GpAMT2 GpAMT3 GiAMT1 GiAMT2 ScMep1 ScMep2 ScMep3 TbAmt1 HcAmt1	III III III III III III III III III II	133 128 145 160 134 139 152 138 158 148
GpAMT1 GpAMT2 GpAMT3 GiAMT1 GiAMT2 ScMep1 ScMep2 ScMep3 TbAmt1 HcAmt1	V V V V V V V V V V V V V V V V V V V	211 205 222 237 211 216 233 215 237 227
GpAMT1 GpAMT2 GpAMT3 GiAMT1 GiAMT2 ScMep1 ScMep2 ScMep3 TbAmt1 HcAmt1	VII VII VII VII VII 212 GT I ML WF GWF GF NG GS AL GA NP RA T NA I I VT N L SA SI GG I TWMLWDY- R L E GK F SALG FC SGA I SG L V A I TP A SG YV SP SF 223 GT V MMWF GWF GF NG GS AL AA GP O A VN AY YT VT N L SA SV GG L TWMAMD F- RV E RK L ST L SF C SG I LAG L VA I TP G SG YV SP SF 223 GT V LLWF GWF GF NG GS AL SAN L R AA NAMY VT N L SA SV GG L TWMAMD F- RV E RK L ST L SF C SG I LAG L VA I TP G SG YV SP SF 223 GT V LLWF GWF GF NG GS AL SAN L R AA NAMY VT N L SA SV GG L TWMFLDY- R L E RK L ST L SF C SG I LAG L VA I TP G SG YV SP SF 224 GT V LLWF GWF GF NG GS AF CART R AMM I AV TN LSA SV GG L TWMLMDY- R L E RK L ST L SF C SG I LAG L VA I TP G SG YV SP SF 225 GT V LLWF GWF GF NG GS AF CART R AMM I MV TN TA AS FG GL AWMUMDY- R L E RK L SA L AF C SG AV AG L VA I TP G SG YV SP PA 226 GT SI LWF GWLL FN SA SS L SP NL R SV YA FMNT C L SA I T GG MTWC L L DY- R L E RK K ST V GL C SG I L AG L VA I TP A SG YV F PI WS 226 GT V FLWF GWF GF NG GS AF CART R AMM I NY TN TA AS VG GL TWC L L DY- R SE K KWST V GL C SG I L AG L VA I TP A SG YV F PI WS 226 GT V FLW GWF GF NG GS AL SG NNR AVMA C V VT N LAA SV GG I TWC L L DY- R SE K KWST V GL C SG I L AG L VA I TP A SG YV PS PA 228 GT V FLW GWF GF NG GS AL SG NNR AVMA C V VT N LAA SV GG I TWC L L DY- R SE K KWST V GL C SG I L AG L VA I TP G SG YV PS PA 228 GT V FLW GWF GF NG GS AL SG NNR AVMA C V VT N LAA SV GG I TWC L L DY- R L E C KWST V GL C SG I L AG L VA I TP G SG YV PS WA 228 GT V FLW GWF GF NG GS AL SG NNR AVMA C V VT N LAA SV GG I TWC L L DY- R L E C KWST V GL C SG V AG L VA I TP G SG YV PS WA 228 GT V FLW GWF GF NG GS AL SG NNR AVMA C V VT N LAA SV GG I TWC L L DY- R L E C KWST V GL C SG V AG L VA I TP G SG YV PS WA 228 GT V FLW GWF GF NG GS AL SG NNR AVMA C V VT N LAA SV GG I TWC L L DY- R L E C KWST V GL C SG V GL VA I TP G SG YV PS WA 228 GT V FLW GWF GF NG GS AL SG NNR AVMA C V T N L AA SV GG I TWC L L DY- R L E C KWST V GL C SG AV SG L VA I TP G SG YV PS WA 329 GT V FLW GWF GF NG GS AL SG NNR AVMA C V T N L AA SV GG I TWC L L DY- R L E C KWST V GL C SG AV SG	291 285 302 317 291 296 314 295 317 307
GpAMT1 GpAMT2 GpAMT3 GiAMT1 GiAMT2 ScMep1 ScMep2 ScMep3 TbAmt1 HcAmt1	X 292 AIAFGUVSGFLCNLATKLKNIFNVDDACDVFAVHFVGGFIGNILTAIFAQNSIVQLDGH-SDPIDGGFLDHNWIDIVVQLA 295 AIAFGFIAGLICNLATKLKHVFDFDDALWVFQVGGILGNLLTGIFAQKSIAALDGR-LE-IDGGLLDGNWKQIVVQLA 303 ALLFGFLAGLICNLSVKLKHVFEFDDALDVFAVHGVGGILGNLLTGIFAQKSIAALDGR-LE-IDGGLLDGNWKQIVVQLA 303 ALLFGFLAGLICNLSVKLKHVFEFDDALDVFAVHGVGGILGNLLTGIFAQKSIAALDGR-LE-IDGGLLDGNWKQIVAQLA 303 SLUFGFLAGLICNLSVKLKHVFEFDDALDVFAVHGVGGVIGNILTAIFAEQKIVALDETVLPGGWLDHHWEQVAYQLA 305 SLIGGIVAGVVCNFATKLKYVAKVDDAMDILAEHGVAGVIGUIFNALFGADVVIGMDGTTEHEGGWVTHNVKOWVGQLA 305 SLIQGIVAGVVCNFATKLKYVAKVDDAMDILAEHGVAGVIGLIFNALFGADVVIGMDGTTHEGGWVTHNVKOWVGQLA 306 SAVAFGVUGGAACNVATKLKFVIGVDALDIFAEHGVGGIVGNILTAFFAADVIAHDG-STEINGGWLNGHVKQGVQLA 308 AVAFGVUGGAACNVATKLKFVIGVDALDIFATHALGGVVGLIFAALFAADWVIGMDGTTEHEGGWLDHHVKOVGQLA 308 AVAFGVUGGAACNVATKLKFVIGVDALDIFATHALGGVVGLIFNALFAADVVIADDG-STEINGGWLNGHYKQUGAVA	371 364 381 395 369 375 395 374 396 386
GpAMT1 GpAMT2 GpAMT3 GiAMT1 GiAMT2 ScMep1 ScMep2 ScMep3 TbAmt1 HcAmt1	XI 372 NS LA GG LY SF FAT LMIVLIM NE IP GL SL RA SP D SET LG I DQ SE LG E SA YY FLD E LIMANVQT G EY HQMP R - GRVQT NG NI R 385 G SC AG LA Y SF SI TY I I LF IM DR V P G F SL RV KK EN T SG LD KT LIG E SA YD FV D EV I LLNT RTOR RER VQ P EP SNQET SI NE R 382 T SM AG LF Y SF AV TY I I LF IM DR V P G F SL RV KK EN T SG LD KT LIG E SA YD FV D EV I LLNT RTOR RER VQ P EP SNQET SI NE R 382 T SM AG LF Y SF AV TY I I LF IM DK P G LS LR A SP E A EV KG I D E SE I G ENA YY HV AR LMA AN AG TG ELRT I R ET SN FQ NNVG 396 D SV TGV A YS FV II Y LI LF IM DK P G LS LR AD P E SE A KG LD E FE LG E LA YY HV DR LVA AN NT RT GE TK TV KE ET I HQ 376 YI AA SI GY TA AV TA I LC FV LGY I P GMB LR I SE E LA E E AG MD E DO I G E FA YD Y FU RW VA SI QV LI AS XT MS SPQ TQ LQ 376 YI AA SI GY TA AV TA I LC FV LGY I P GMB LR I SE E A E AG MD E DO I G E FA YD Y E VR DY Y LWG VD E D SOR SO VN - HR VNNAH 386 GI C AA LAWTY VT YI SI LLITM NAI P FLK KL SAD E E LGT D AA CI G FFT YE ESTA XT J PE P I RS XT SAOM PP PH EN I DDK I VG 377 YI G A SA GY CA V YT A I I C FV LGK I P GYH LR YT E EA E ALG LD E DO I G E FA YD Y V EVR DY Y LWG VD TD A LHTT CNG AN SA SE T 397 D SV AG FA YS FG GI C I I LE IM N LI P GL SLRAT E EA E ILG I DDA E LG E FA YD Y V ELTR EV I SDDT V PK MS S E NNA SV E 387 D SV AG FA YS FG GI C I I LE IM N LI P GL SLRAT E EA E ILG I DDA E LG E FA YD Y V E LTR EV I SDDT V PK MS S E NNA SV E 387 D SV AG FA YS FG GI C I I LE IM N LI P GL SLRAT E EA E ILG I DDA E LG E FA YD Y V E LTR EV I SDDT V PK MS S E NNA SV E 387 D SV AG FA YS FY MTT I LLWWMH I P GL T LR TT E EA E I LG V DDA EM G FA YD Y W G I DQ E I G HT LDT G LTAT G G R PD HAKAV	451 445 460 470 446 1 455 i 476 455 473 467
GpAMT1 GpAMT2 GpAMT3 GiAMT1 GiAMT2 ScMep1 ScMep2 ScMep3 TbAmt1 HcAmt1	452 Q N D V E D G R L Q Q P S	464 459 472 479 454 491 499 489 483 477

Fig. S2 Alignment of 10 fungal AMT amino acid sequences. The 14 conserved residues reported to be of functional significance for conducting ammonium through the pore region (2, 3) are marked with red stars. Sequences were obtained from the GenBank database with the following accession numbers: *Geosiphon pyriformis* (GpAMT1, JX535577; GpAMT2, JX535578; GpAMT3, JX535579), *Rhizophagus irregularis* (GiAMT1, CAI54276; GiAMT2, CAX32490), *Saccharomyces cerevisiae* (ScMep1, P40260; ScMep2, P41948; ScMep3, P53390), *Tuber borchii* (TbAmt1, AAL11032), *Hebeloma cylindrosporum* (HcAmt1, AAM21926). Residues shaded in light, medium, and dark blue indicate at least 50%, 70%, and 90% conservation of the 10 sequences, respectively. Red thick lines indicate positions of the 11 transmembrane domains predicted for GpAMT1. Green box and star mark the unusual cysteine residue in GpAMT2 and GpAMT3 located in close proximity to the histidine dyad (see Fig. S5) that plays an important role in ammonium transduction (4).

	1 mM NH ₄ +	75 mM NH ₄ +
control		به 😵 🌒
GpAMT1		🕘 🔮 👾 🎌
GpAMT2		0 3 4 ·
ScMEP1	• * * •	
GFP		***
GpAMT1-GFP		• • •
GpAMT2-GFP		* * * *
ScMEP1-GFP	• * * •	• # f •
	6 d at 30°0	C 3 d at 30°C

Fig. S3 Complementation assay of yeast strain MLY131a/a (*mep1-3* Δ). The strain was transformed with the empty vector or *GFP* as negative controls, *ScMEP1* or *ScMEP1-GFP* as positive controls, and the annotated AMT genes or genes encoding AMT-GFP fusion proteins. Strains were cultivated on minimal medium containing 1 mM NH₄⁺ as sole nitrogen source or on HC-U medium containing 75 mM NH₄⁺. Note that transformation with GFP-tagged AMTs resulted in the same functional complementation as transformation with untagged versions.

3



Fig. S4 Growth assays in the presence of cytotoxic methylammonium (MA). The yeast strain MLY131a/a (*mep1-3* Δ) was transformed with the empty vector (negative control), *ScMEP1* (positive control), *GpAMT1*, or *GpAMT2*. (A) pH dependence of MA uptake and cytotoxicity. Yeast cells were cultivated in buffered liquid medium containing 0.1% proline as nitrogen source and 100 mM MA. At pH values below 7, clear differences between *ScMEP1* expressing yeast and the other strains were observed. At pH 7 passive influx of MA seems to limit growth of all strains. Error bars, ±SD; n = 4 biological replicates. Different letters above bars indicate highly significant differences ($P \le 0.001$, ANOVA test) between pairwise comparisons. (B) Growth in buffered liquid medium with 0.1% proline as sole nitrogen source, at pH values of 3-7. Yeast growth was not affected by medium pH. Error bars, ±SD; n = 4 biological replicates. (C) Growth on solid mimimal medium containing 0.1% proline as nitrogen source, at pH values of 3-7. Yeast growth was not affected by medium pH. Error bars, ±SD; n = 4 biological replicates. (C) Growth on solid mimimal medium containing 0.1% proline as nitrogen source and 50 mM MA (left), or on HC-U medium containing 75 mM NH₄⁺ (right).



Fig. S5 Structural model of the hydrophilic pore in GpAMT2. (A,C) Representation of the conserved and functionally important residues inside the hydrophilic pore for ammonium transduction and of cysteine/serine 173. (B,D) Stereo images of the 3D structure of the pore region. Cysteine/serine 173 is located in close proximity to the histidine dyad. Structures of GpAMT2 and GpAMT2 C₁₇₃S were generated using SWISS-MODEL and the PDB template 2b2h of *Archaeoglobus fulgidus* amt-1.





Fig. S6 Test for invasive pseudohyphal growth on solid medium. The yeast strain MLY131a/a (*mep1-3* Δ) was transformed with the empty vector (negative control), and with vectors expressing wild type and mutant yeast and *Geosiphon pyriformis* AMTs. The respectice wild type constructs are indicated in bold. Yeast strains were grown on solid minimal medium with 100 μ M or 200 μ M NH₄⁺ as sole nitrogen source. After 14 days at 30°C colonies were documented and subsequently washed away with water, leaving only invasively growing cells. (A) Photographs of fivefold serial dilutions of the indicated strains. Representative examples from 4 biological replicates are shown. (B) Photographs of colony morphologies were taken directly from Petri plates with a Leica MZ 16 FA stereomicroscope with a 63x primary objective and a 0.8x camera adaptor (bar, 200 μ m). Representative examples from 4 biological replicates are shown.

Note that only ScMep2 constructs complement for pseudohyphal growth and that the $S_{197}C$ mutation does not interfere with pseudohyphal growth.

 Table S1
 Plasmids used in this study.

Plasmid	Backbone	Insert	Reference	
pDR196sfi	pDR196	Sfil restriction sites	(5)	
pRU4		EGFP	(6)	
pBL101	pDR196sfi	GpAMT1 with UTRs	This study	
pBL102	pDR196sfi	GpAMT2 with UTRs	This study	
pBL103	pDR196sfi	ScMEP1	This study	
pBL104	pDR196sfi	ScMEP2	This study	
pBL106	pDR196sfi	GFP	This study	
pBL107	pDR196sfi	GpAMT1-GFP	This study	
pBL108	pDR196sfi	GpAMT2-GFP	This study	
pBL109	pDR196sfi	ScMEP1-GFP	This study	
pBL110	pDR196sfi	<i>GpAMT1</i> ORF	This study	
pBL111	pDR196sfi	<i>GpAMT2</i> ORF	This study	
pBL130	pDR196sfi	GpAMT1_S ₁₇₈ C	This study	
pBL131	pDR196sfi	GpAMT2_C ₁₇₃ S	This study	
pBL134	pDR196sfi	$ScMEP2_S_{197}C$	This study	
pBL151	pDR196sfi	GiAMT1	This study	
pBL154	pDR196sfi	GiAMT1-GFP	This study	
pBL179	pDR196sfi	GpAMT3 with UTRs	This study	
pBL180	pDR196sfi	cGpAMT3	This study	
pBL181	pDR196sfi	cGpAMT3_C ₁₈₉ S	This study	
pBL183	pDR196sfi	gGpAMT3	This study	
pBL185	pDR196sfi	gGpAMT3_C ₁₈₉ S	This study	
pBL186	pDR196sfi	gGpAMT3-GFP	This study	

Table S2 Primers used in this study.

Primer name	Sequence (5'-3') ^a	Application
Primers in vector backbon	e	
ADHclose	AATACGACTCACTATAGG	pDR196sfi backbone, 3' of insertion site
ADH109-REV	CGACTCACTATAGGGCG	Nested to ADHclose
PMA5	CTCTCTTTTATACACACATTC	pDR196sfi backbone, 5' of insertion site
PMA16	CGGGCTGCAGGAATTCGG	Nested to PMA5
Cloning of GpyrAMT genes	8	
MEP1-50-FWD	GGTATGTATGATGGCTATTTGTGTTGTTATNYTNCARTGGT	Degenerate PCR
MEP1-53-FWD	ATGGCTATTTGTGTTGTTATTTTTCARTGGTWYTT	Degenerate PCR
MEP1-178-REV	AATTTCAACAGGACCACCACCNGCVHARTC	Degenerate PCR
GpAMT2-FWD1	CATATATTGGCAATTTAAGGAATG	Cloning of GpAMT2 3' end
GpAMT2-FWD2	GGCAATTTAAGGAATGCTTTCC	Nested to GpAMT2-FWD1
GpAMT2-REV3	CCAAATGCAATAGAAGATGAGG	Cloning of GpAMT2 5' end
GpAMT2-REV5	GGTCTTCTTGATGTCGCTTTCC	Nested to GpAMT2-REV3
GpAMT3-clone-FWD	CGGTTGGTTTGGATTTAACG	Cloning of GpAMT3
GpAMT3-clone-REV	CCCAGCAAGGAAACCAAATA	Cloning of GpAMT3
Cloning of yeast expressio	on constructs	
GpAMT1-sfi-FWD	CAAGGCCATTACGGCCAACATGGCTTCGATAAATTCGATATATTTCG	Cloning of GpAMT1 CDS
GpAMT1-sfi-REV	GATGGCCGAGGCGGCCTTAAAGAGACGGTTGCTGAAGTCT	Cloning of GpAMT1 CDS
GpAMT2-sfi-FWD	CCAGGCCATTACGGCCTACAAAATGGGCCGATTATAATTC	Cloning of GpAMT2 CDS
GpAMT2-sfi-REV	GATGGCCGAGGCGGCCTTAATTTTCCTTGAAAGCACTCGA	Cloning of <i>GpAMT2</i> CDS
GpAMT3-sfi-FWD	CAAGGCCATTACGGCCAACATGGATTCTGTGGAAGATACGAATG	Cloning of <i>GpAMT3</i> CDS
GpAMT3-sfi-REV	GATGGCCGAGGCGGCCTTAAAAATGAGCAAATGTTCTTTCGG	Cloning of <i>GpAMT3</i> CDS
GpAMT3-gen-FWD	CACAATGGATTCTGTGGAAGATACG	Constructing gDNA variant of GpAMT3
GpAMT3-gen-REV	CAGAGCACAAAAATATTTGCATTATTGCC	Constructing gDNA variant of GpAMT3
GiAMT1-sfi-FWD	CCAGGCCATTACGGCCAACATGTCTGCTCCCGCTGCTGC	Cloning of GiAMT1
GiAMT1-sfi-REV	CCTAGGCCGAGGCGGCCTTATACAATTGTTGCATTTGCGTCATTTTG	Cloning of GiAMT1
ScMEP1-sfi-FWD	CAAGGCCATTACGGCCTACAAAATGGAGAGTCGAACTAC	Cloning of <i>ScMEP1</i> CDS
ScMEP1-sfi-REV	CCAGGCCGAGGCGGCCTTACCTATTGGCAGGATCTTCTTGATG	Cloning of <i>ScMEP1</i> CDS
ScMEP2-sfi-FWD	CCAGGCCATTACGGCCAACATGTCTTACAATTTTACAGGTACG	Cloning of ScMEP2 cDNA
ScMEP2-sfi-REV	CCAGGCCGAGGCGGCCTTATACTATATGGTCAGTGTTCTTA	Cloning of ScMEP2 cDNA
Cloning of GFP-tags		
X-S-EGFP-FWD	GGACTCGAGTAACACGGCCGCCTCGGCCATGGTGAGCAAGGGCGAGG	Cloning of GFP into pDR196sfi
EGFP-A-REV	CATGGGCCCTTACTTGTACAGCTCGTCCATGCC	Cloning of <i>GFP</i> into pDR196sfi
ORF-FWD	CGGCCTACAAAATGTCGGCCGCCT	Cloning of pBL106 (soluble GFP)
ORF-REV	CGGCCGACATTTTGTAGGCCGTAA	Cloning of pBL106 (soluble GFP)
GpAMT1-tag-sfi-REV	GATGGCCGAGGCGGCCGAAAGAGACGGTTGCTGAAGTCT	Reverse primer for tagging GpAMT1
GpAMT2-tag-sfi-REV	GATGGCCAGGCGGCCGAATTTTCCTTGAAAGCACTCGA	Reverse primer for tagging GpAMT2
GpAMT3-tag-sfi-REV	CCTAGGCCGAGGCGGCCAAAAAATGAGCAAATGTTCTTTCGG	Reverse primer for tagging GpAMT3
ScMEP1-tag-sfi-REV	GATGGCCGAGGCGGCCGACCTATTGGCAGGATCTTCTTG	Reverse primer for tagging ScMEP1
Cloning of CtoS and StoC	variants	
GpAMT1-StoC-FWD	GTTCATATGGCATGTGGAGCTGCTGCACTCG	Mutation S ₁₇₈ C in GpyrAMT1
GpAMT1-StoC-REV	CGAGTGCAGCAGCTCCACATGCCATATGAAC	Mutation S ₁₇₈ C in GpyrAMT1
GpAMT2-CtoS-FWD	GTACACATTTCTTCCGGAGCTGCTGTTTAG	Mutation C ₁₇₃ S in GpyrAMT2
GpAMT2-CtoS-REV	CTAAGCAGCAGCTCCGGAAGAAATGTGTAC	Mutation C ₁₇₃ S in GpyrAMT2
GpAMT3-CtoS-FWD	CTGTTCATATTTCTTCCGGAGCTTCTGCAC	Mutation C ₁₈₉ S in cGpyrAMT3
GpAMT3-CtoS-REV	GTGCAGAAGCTCCGGAAGAAATATGAACAG	Mutation C ₁₈₉ S in cGpyrAMT3
gGpAMT3-CtoS-FWD	CTGTTCATATTTCTTCCGGAGCTGCTGCAC	Mutation C ₁₈₉ S in gGpyrAMT3
gGpAMT3-CtoS-REV	GTGCAGCAGCTCCGGAAGAAATATGAACAG	Mutation C ₁₈₉ S in gGpyrAMT3
ScMEP2-StoC-FWD	GTGTCCATCTCACGTGTGGACATGGTGGTC	Mutation S ₁₉₇ C in ScMep2
ScMEP2-StoC-REV	GACCACCATGTCCACACGTGAGATGGACAC	Mutation S ₁₉₇ C in ScMep2

^a Overhangs are underlined

Supplemental References

- 1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.
- 2. Andrade S, Einsle O. 2007. The Amt/Mep/Rh family of ammonium transport proteins. Mol. Membr. Biol. 24:357-365.
- 3. **Lamoureux G, Javelle A, Baday S, Wang S, Bernèche S.** 2010. Transport mechanisms in the ammonium transporter family. Transfus. Clin. Biol. **17:**168-175.
- 4. **Wang S, Orabi E, Baday S, Bernèche S, Lamoureux G.** 2012. Ammonium transporters achieve charge transfer by fragmenting their substrate. J. Am. Chem. Soc. **134**:10419-10427.
- 5. **Schüßler A, Martin H, Cohen D, Fitz M, Wipf D.** 2006. Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. Nature **444**:933-936.
- 6. **Brachmann A, Weinzierl G, Kamper J, Kahmann R.** 2001. Identification of genes in the bW/bE regulatory cascade in *Ustilago maydis*. Mol. Microbiol. **42**:1047-1063.