

Supplemental Figure Legends

Figure S1. Time-course development of *in vivo* nitrogenase activity in *A. vinelandii* DJ estimated by the acetylene reduction assay. Data are the average of three biological replicates (\pm SE).

Figure S2. *A. vinelandii nif* regulon. (A) Distribution of putative binding motifs for elements involved in *nif* gene initiation of transcription. Red denotes the size of the intergenic region (non-coding DNA) between transcriptional units. (B) Number of motifs expected randomly in a 500-bp DNA fragment. (C) Consensus sequences logo and position weight matrix (PWM) of binding sites for σ^{54} (square), IHF (diamond), and NifA UAS (circle) in *A. vinelandii*. Logos and PWMs were created using Web-LOGO <http://weblogo.berkeley.edu/logo.cgi> and Unipro UGENE software <http://ugene.unipro.ru>, respectively.

Figure S3. (A) Immunoblots to detect cellular levels of NifDK, NifH, NifB, NifEN, NifU, NifQ, NifX and NifY in wild type and the $\Delta fdxN$ strains. Time elapsed after NH_4^+ removal from the medium is indicated at the top of the panels. (B) Immunoblots of known amounts of purified proteins used to generate quantification standard curves. Squared correlation coefficients R^2 are shown to the right of the panel.

Figure S4. Time-dependent profile of *nif* gene expression upon nitrogen step-down in wild-type *A. vinelandii* (closed symbols) and the $\Delta nifA$ mutant (open symbols). In each sample, cDNA was quantified by qRT-PCR using standard curves generated with known amounts of the corresponding synthetic DNA amplicon. Data was then normalized to 16 rRNA content of each sample to yield the normalized absolute signal (NAS) parameter. In the figure, genes are organized according to chromosomal clustering. Data are the average of three biological replicates (\pm SE).

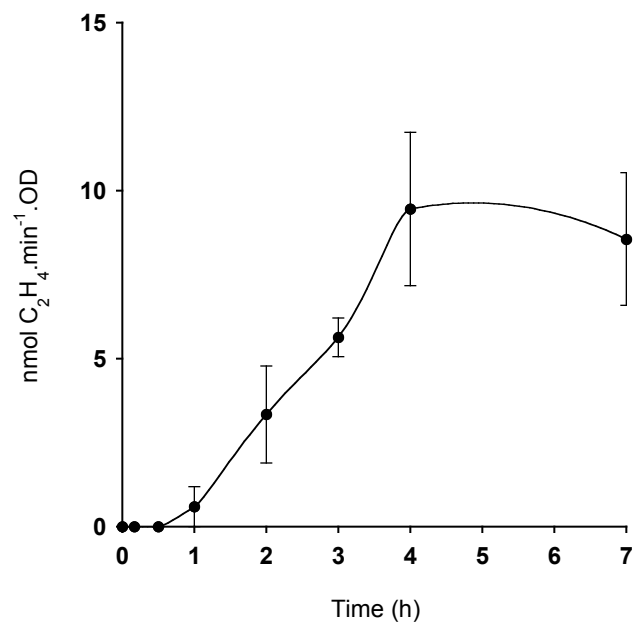
Figure S5. Time-dependent profile of *nif* gene expression upon nitrogen step-down in wild-type *A. vinelandii* (closed symbols) and the $\Delta fdxN$ mutant (open symbols). Experimental conditions are described in Figure S4. Data are the average of three biological replicates (\pm SE).

Figure S6. Time-dependent profile of *nif* gene expression upon nitrogen step-down in wild-type *A. vinelandii* (closed symbols) and the $\Delta nifDK$ mutant (open symbols). Experimental conditions are described in Figure S4. Data are the average of three biological replicates (\pm SE).

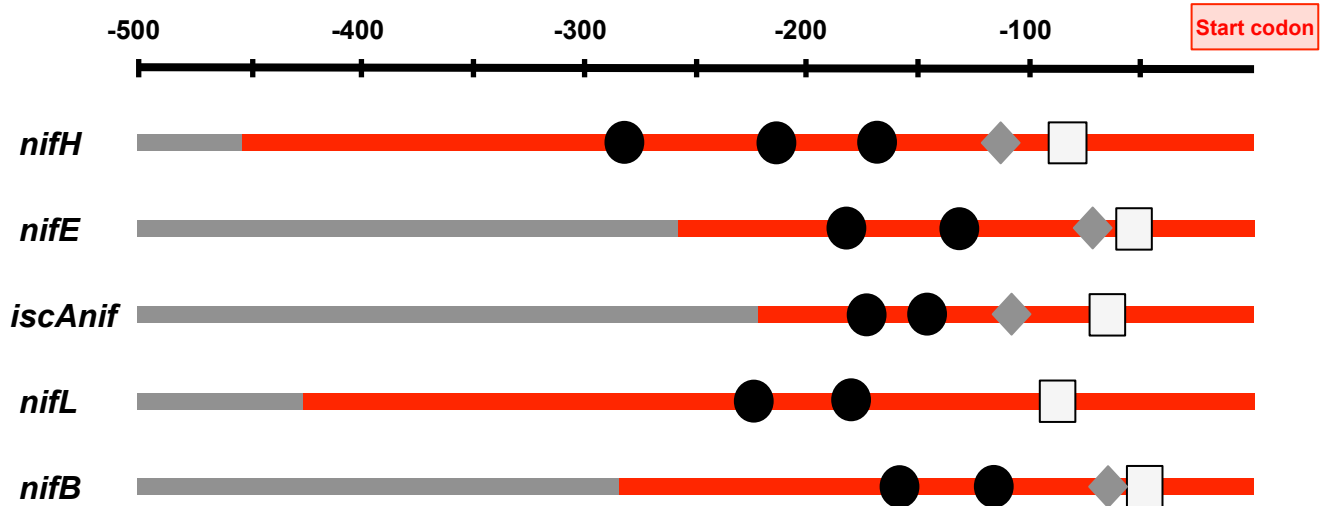
Figure S7. Time-dependent profile of *nif* gene expression upon nitrogen step-down in wild-type *A. vinelandii* (closed symbols) and the $\Delta nifB$ mutant (open symbols). Experimental conditions are described in Figure S4. Data are the average of three biological replicates (\pm SE).

Table S1. List of primers used for Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) experiments.

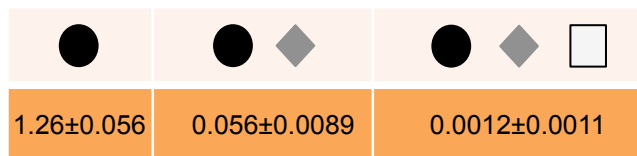
Table S2. *nif* mRNA levels in cultures of *A. vinelandii* growing with NH_4^+ as nitrogen source.



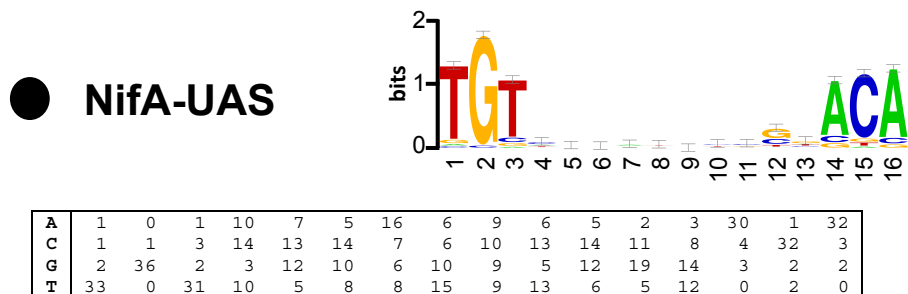
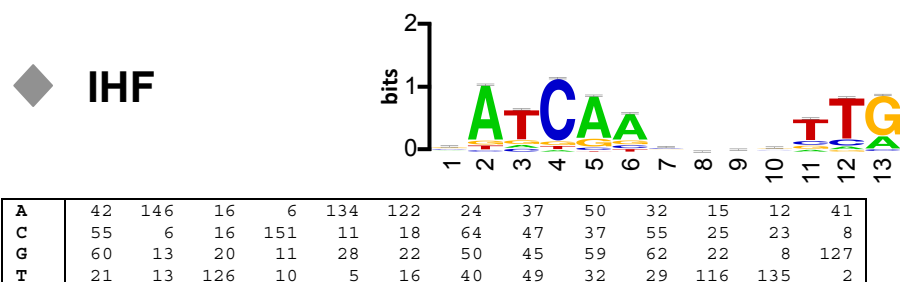
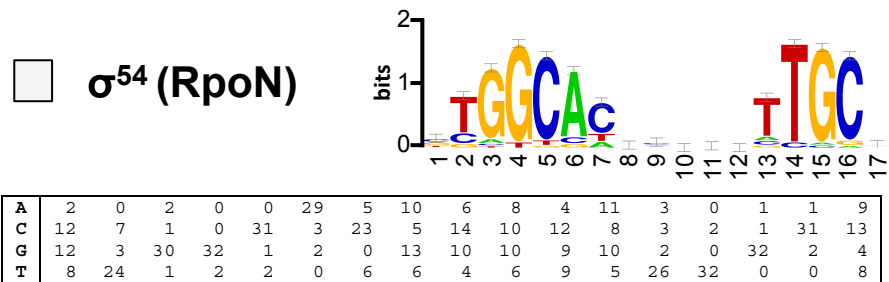
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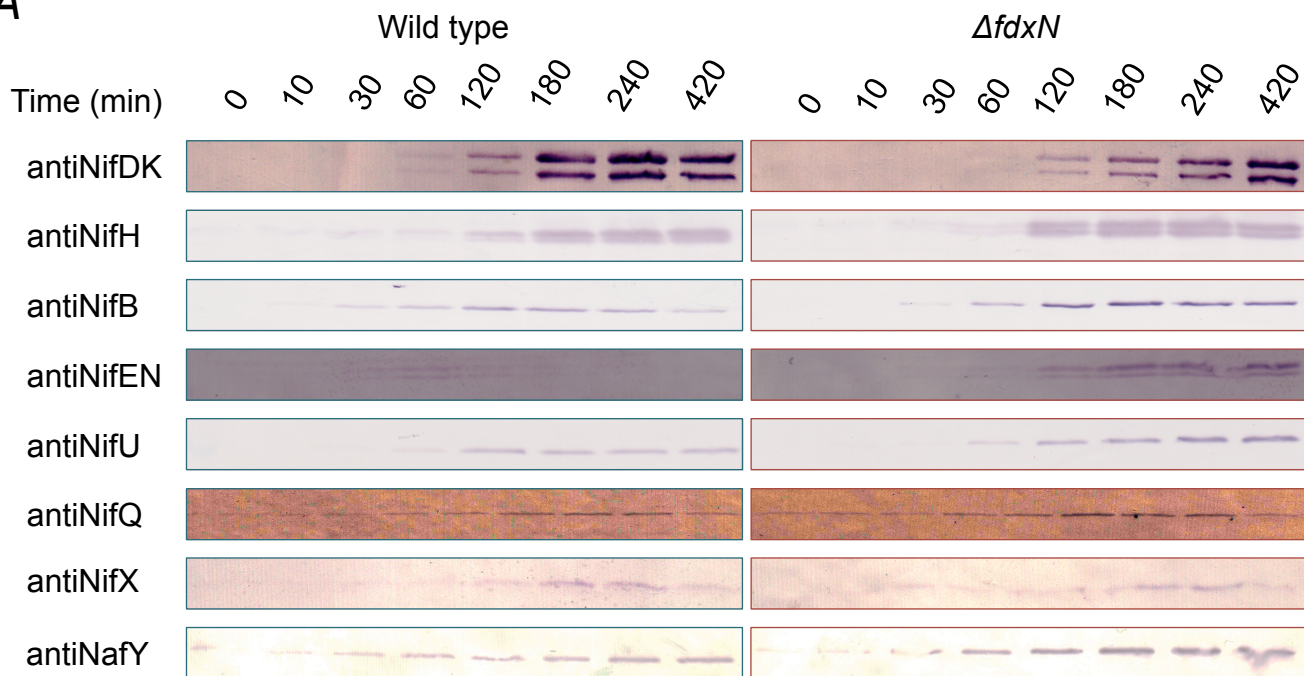
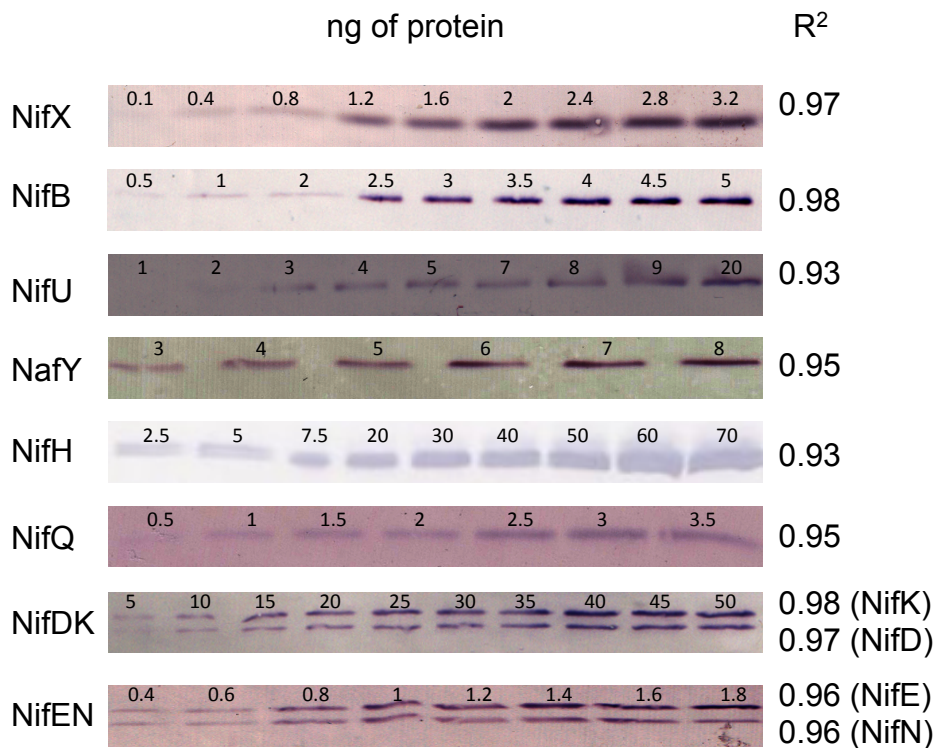


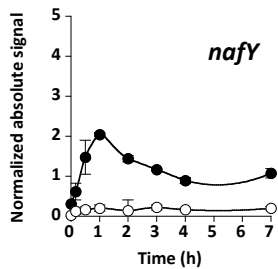
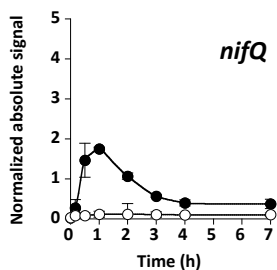
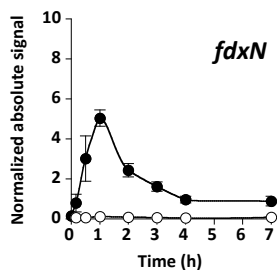
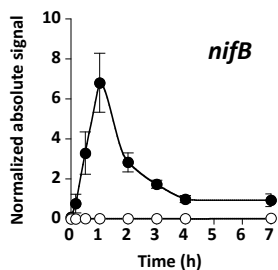
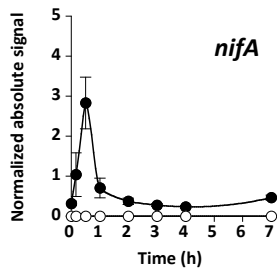
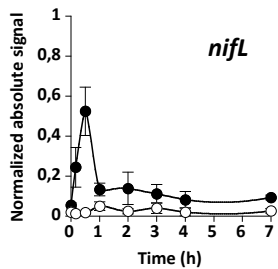
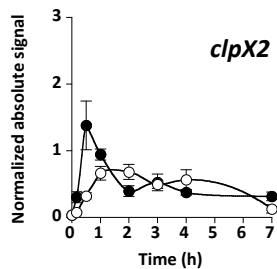
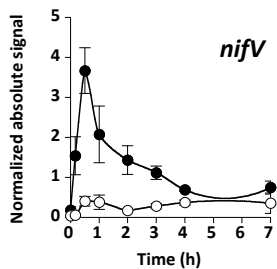
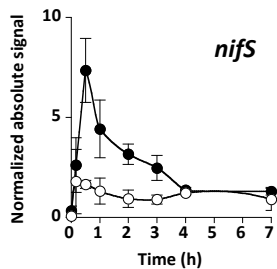
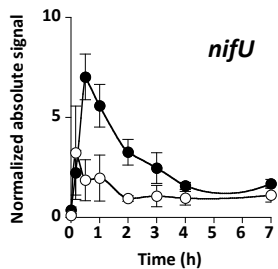
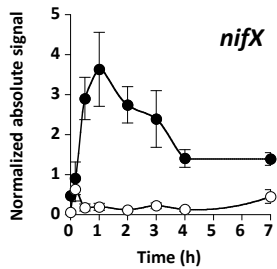
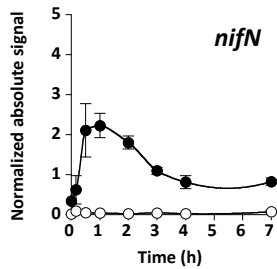
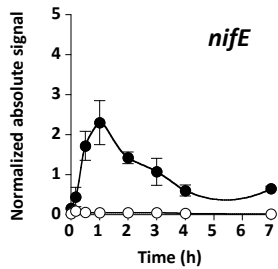
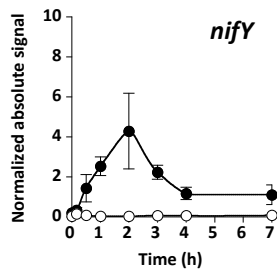
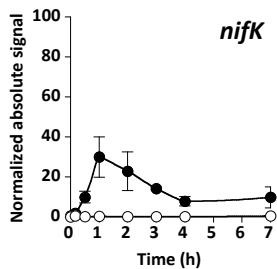
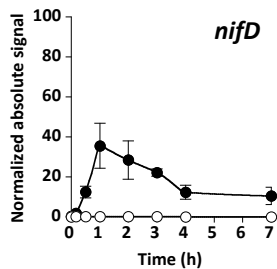
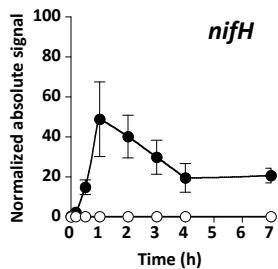
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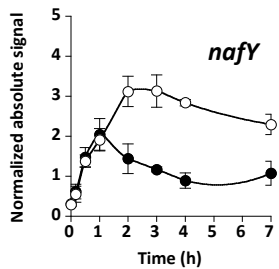
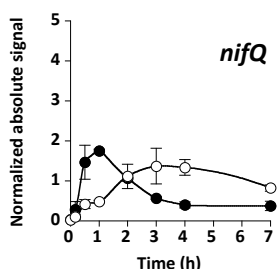
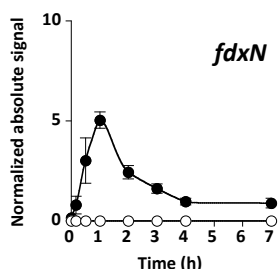
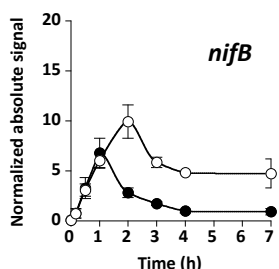
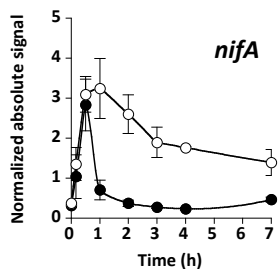
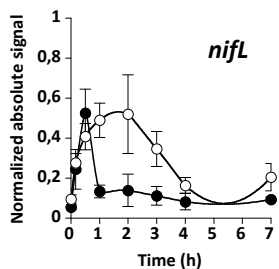
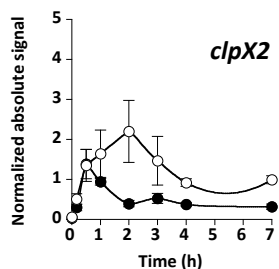
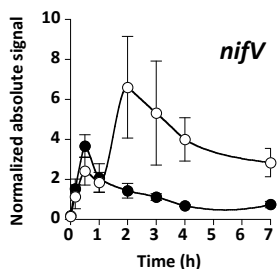
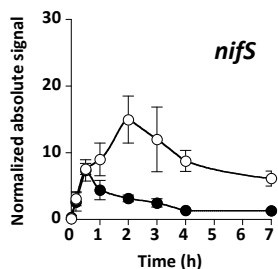
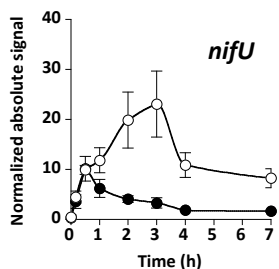
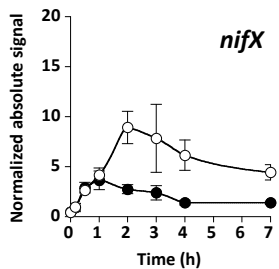
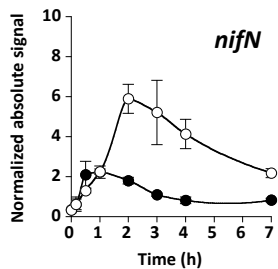
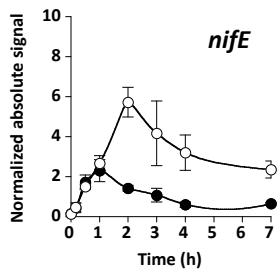
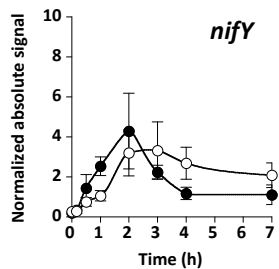
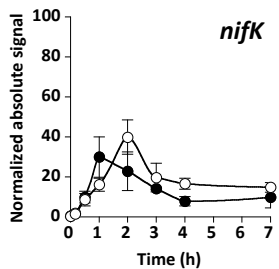
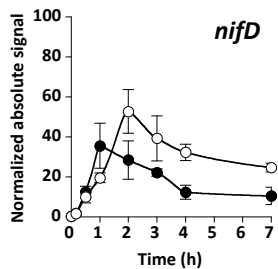
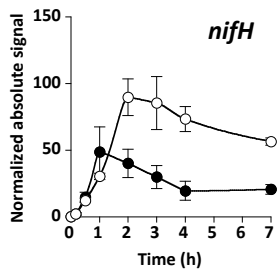


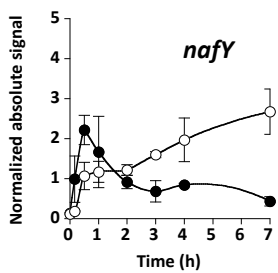
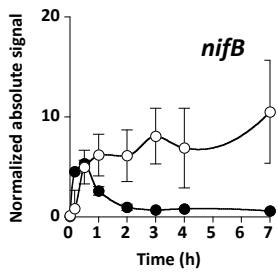
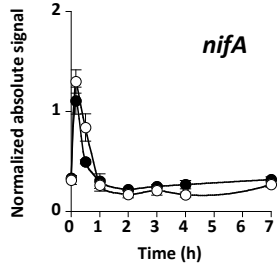
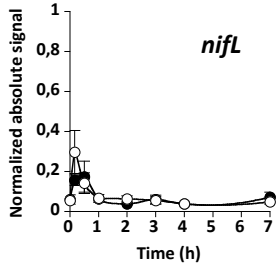
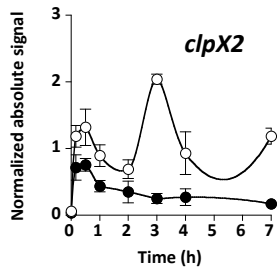
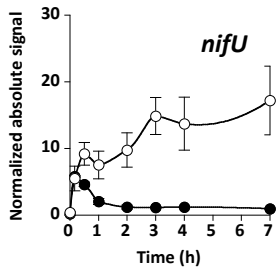
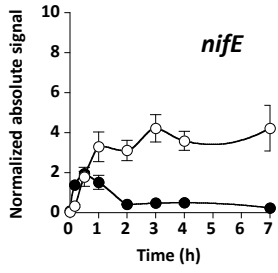
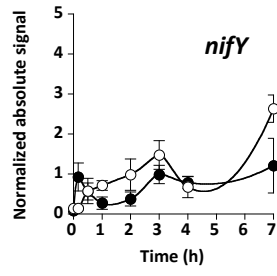
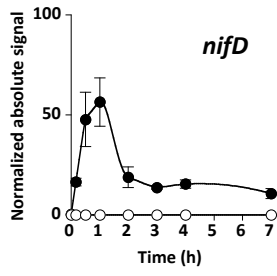
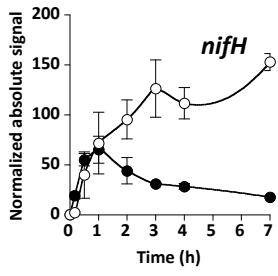
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A**B**







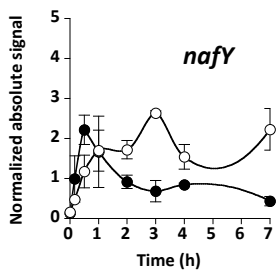
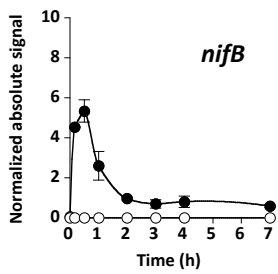
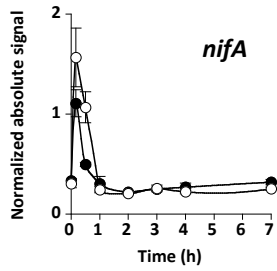
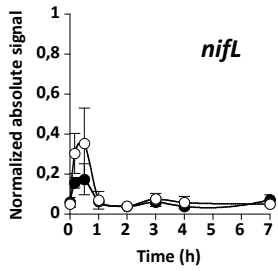
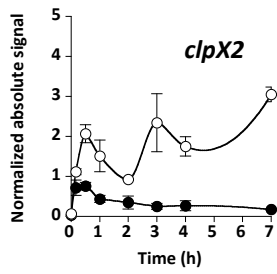
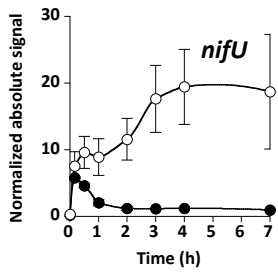
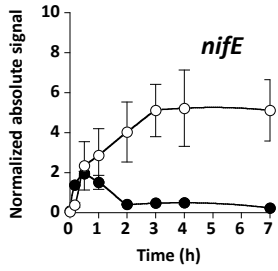
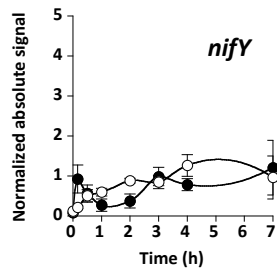
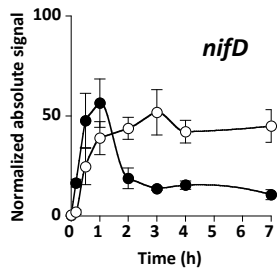
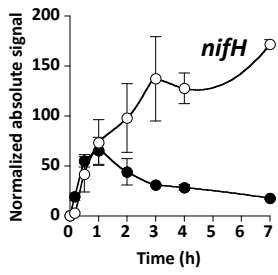


Table S1. List of primers used for Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) experiments.

Primer	Gene	Sequence (5'-3')
936	<i>nifH</i>	CCGCCAACAACATCTCCAA
937	<i>nifH</i>	TCTTCGCGGTTCGGTGTTAC
932	<i>nifD</i>	GCCGGCCGTCGTAACACTA
933	<i>nifD</i>	CACCGAACACGATGTCCTTCT
934	<i>nifK</i>	TGCGTTCGCTGGTCTTCAC
935	<i>nifK</i>	TCGAACTCCTTGCCCTTGTG
1156	<i>nifY</i>	CGCCTGGACGGCATCTT
1157	<i>nifY</i>	GGGTTCGCGTAGATCGATCA
942	<i>nifE</i>	CGTGCCCTTCTCGACAT
943	<i>nifE</i>	GGGCATTCCAGGGTGATG
944	<i>nifN</i>	TACCGCGGCAGCAGTCA
945	<i>nifN</i>	CCGGTTTTTGGCGATAGATC
1125	<i>nifX</i>	ACCCGACAATTGCAGGTATTG
1126	<i>nifX</i>	TCGGGATTGACCCCGTAGA
1121	<i>nifU</i>	AAGAGCACTTCTACAACCCCAAGA
1122	<i>nifU</i>	AGGCGCAGCGCATCAC
1123	<i>nifS</i>	CCTCAGCCTGTGCGATTACC
1124	<i>nifS</i>	GCTGGCGTAATGCTCCAGAT
1119	<i>nifV</i>	GCGTGATCATCGACGACACT
1120	<i>nifV</i>	CGGGCGATAGCGATCTTCT
1265	<i>clpX2</i>	GTTGGAAGCACTGGTCTCG
1266	<i>clpX2</i>	TGGGCATGGAAACCGATA
1263	<i>nifL</i>	CGAGACGCTGGAAAACCA
1264	<i>nifL</i>	GCCCAGGTCTGAAGGAGAT
1115	<i>nifA</i>	CGCTCGGCCAAACAGAAC
1116	<i>nifA</i>	CCAGGGCCTGCAATTGC
940	<i>nifB</i>	CTCATCGAGCAGCAGCAGAA
941	<i>nifB</i>	TTCAGGTGCTCGTCGTTGAC
1129	<i>fdxN</i>	AATTGTTGGGCATGTGTCGAT
1130	<i>fdxN</i>	TCACACTCGGTGCACTTGCT
1117	<i>nifQ</i>	TCCTGCTGATGCACAATTTCC
1118	<i>nifQ</i>	ATCCTGTTCGCACAGTTGCTT
1127	<i>nafY</i>	GTGAACATGAGTCGCGAAACC
1128	<i>nafY</i>	TGATGCAGGATTCCAGCAA
903	<i>16SrDNA</i>	GTAACGAGCGCAACCCTTGT
904	<i>16SrDNA</i>	CTTGACGTCATCCCCACCTT

Table S2. *nif* mRNA levels in cultures of *A. vinelandii* growing with NH_4^+ as nitrogen source.

Gene	NAS ^a	
	t=0 h	t=4 h ^b
<i>nifH</i>	0.35±0.17	0.16±0.11
<i>nifD</i>	0.33±0.12	0.22±0.09
<i>nifK</i>	0.53±0.13	0.28±0.11
<i>nifY</i>	0.20±0.08	0.07±0.01
<i>nifE</i>	0.15±0.07	0.09±0.02
<i>nifN</i>	0.34±0.14	0.25±0.05
<i>nifX</i>	0.47±0.15	0.42±0.06
<i>nifU</i>	0.36±0.22	0.13±0.05
<i>nifS</i>	0.32±0.23	0.09±0.04
<i>nifV</i>	0.18±0.08	0.08±0.03
<i>clpX2</i>	0.03±0.01	0.04±0.01
<i>nifL</i>	0.054±0.01	0.09±0.03
<i>nifA</i>	0.32±0.05	0.35±0.09
<i>nifB</i>	0.09±0.06	0.02±0.01
<i>fdxN</i>	0.15±0.09	0.05±0.01
<i>nifQ</i>	0.03±0.01	0.01±0.001
<i>nafY</i>	0.31±0.07	0.20±0.03

^a Normalized absolute signals

^b Cultures growing in the presence of NH_4^+ during 4h
Data is the average of at le