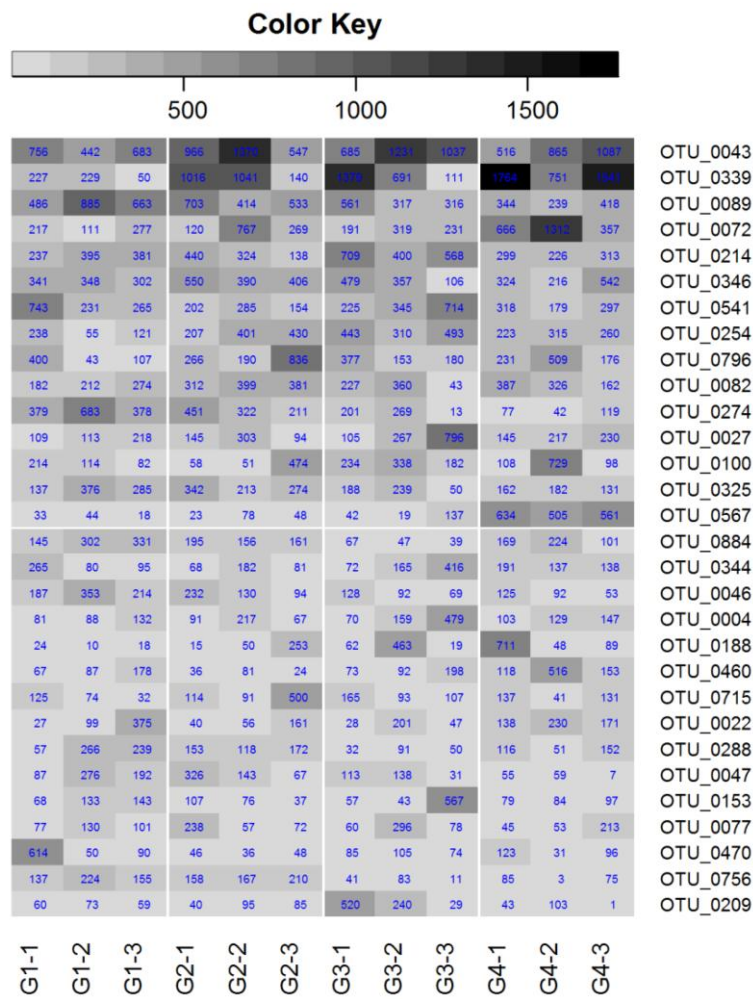
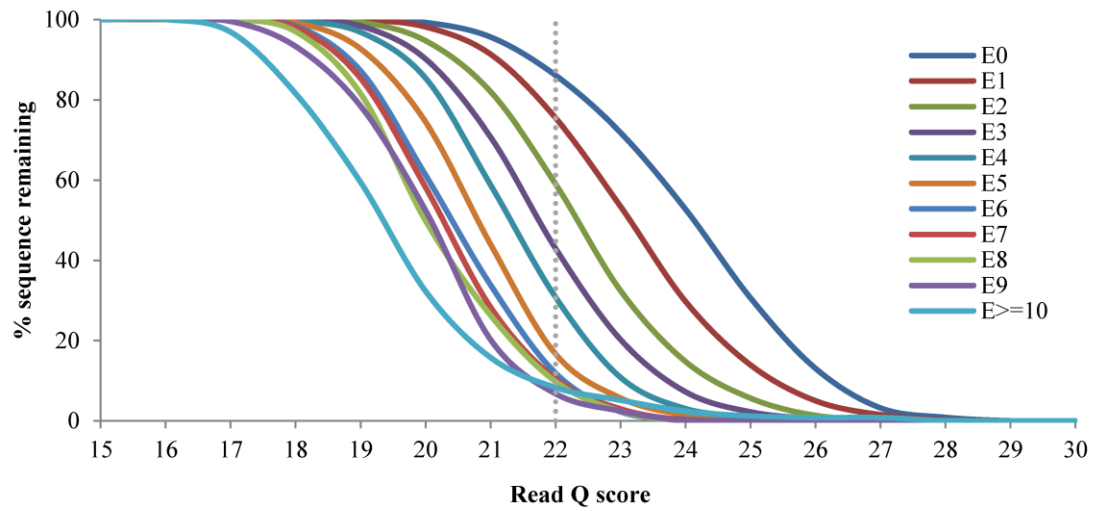


**FIG S1** Rarefaction curves of the 12 bulk soil samples using PGM 318 chip with Hi-Q Sequencing Kit. G1 and G2 showed more OTU<sub>s0.05</sub> than G3 and G4. Sequence number (x axis) plotted against OTU number (y axis). The downstream analyses were based on subsample of 22,616 sequences per sample (the vertical dashed line).



**FIG S2** Heatmap of the 30 most abundant OTU<sub>s0.05</sub> of the 12 bulk soil samples, accounting for 33% (90,033 sequences) of all sequences. Values in heatmap cells are the actual OTU<sub>0.05</sub> numbers, and separation by white lines was based on different cropping systems and the top 15<sup>th</sup> OTU<sub>0.05</sub>.



**FIG S3** Percentage of reads with specified number of errors (E0-E $\geq$ 10) by read Q score in M-R4-318, the representative of PGM chip 318 runs with Sequencing 400 Kit.

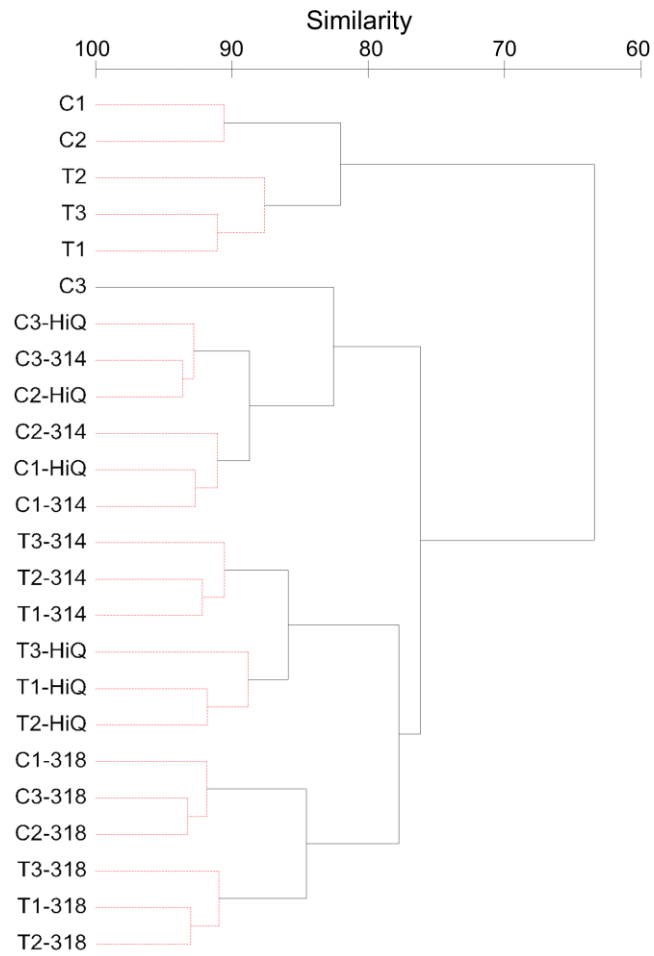


FIG S4 Complete linkage clustering of *nifH*-harboring bacterial community structure at OTU<sub>0.05</sub> level using SIMPROF (similarity profile analysis) at 95% confidence.

**TABLE S1** Cropping history of G1-G4 at Arlington, WI. G2, G3 and G4 were in the corn-soybean-canola rotation since 2008, and were changed to a continuous corn + cover crop system and a corn-soybean + cover crop system in 2012. Cover crop was planted after harvesting the corn or soybean.

Systems	2008	2009	2010	2011	2012	2013
G1	Corn	Corn	Corn	Corn	Corn	Corn
G2	Corn	Soybean	Canola	Corn	Corn+covercrop	Corn+covercrop
G3	Soybean	Canola	Corn	Soybean	Corn+covercrop	Soybean+covercrop
G4	Canola	Corn	Soybean	Canola	Soybean+covercrop	Corn+covercrop

**TABLE S2** Soil properties of these 12 bulk soils from four different cropping systems; mean values of three replicates. CEC stands for Cation Exchange Capacity with unit of milli equivalents/100 g. Units for P, K, Ca, and Mg are mg/kg.

Sample	pH	OM (%)	P	K	Ca	Mg	CEC
G1-1	6.4	2.9	44	168	1638	435	15
G1-2	6.4	3.7	224	211	1909	428	17.5
G1-3	6.6	3.6	196	240	1538	443	15
G2-1	6.3	2.9	31	127	1612	446	15
G2-2	6.7	2.8	69	126	1358	737	16
G2-3	6.9	3.1	153	179	1653	513	15
G3-1	6.7	3.5	33	132	1892	502	17
G3-2	6.8	3.2	70	125	1303	592	15
G3-3	7.1	2.8	177	142	1732	511	15
G4-1	6.9	3.3	104	148	1882	484	17
G4-2	6.9	2.9	115	155	413	368	7
G4-3	6.7	3.7	201	202	1652	471	16

**TABLE S3** Diazotrophic primer sets with Ion Torrent adapters (A or trP1) and IonXpress barcodes (red). The green *nifH* primer (Poly F and R) can be replaced with other gene-specific primers for other functional gene studies.

Forward	A adaptor-IonXpress- <i>nifH</i> forward Primer
nifH-F-1	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAAC <b>TGCGAYCCSAARGCBGACTC</b>
nifH-F-2	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGAGA <b>ACTGCGAYCCSAARGCBGACTC</b>
nifH-F-3	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGAGGAT <b>CTGCGAYCCSAARGCBGACTC</b>
nifH-F-4	CCATCTCATCCCTGCGTGTCTCCGACTCAGTACCAAGAT <b>CTGCGAYCCSAARGCBGACTC</b>
nifH-F-5	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGAAGGA <b>ACTGCGAYCCSAARGCBGACTC</b>
nifH-F-6	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGT <b>CTGCGAYCCSAARGCBGACTC</b>

nifH-F-7	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGTGATTCTGCGAYCCSAARGCBGACTC
nifH-F-8	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGGATAACTGCGAYCCSAARGCBGACTC
nifH-F-9	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACTGCGAYCCSAARGCBGACTC
nifH-F-10	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACCGAACTGCGAYCCSAARGCBGACTC
nifH-F-11	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTCTGAATCTGCGAYCCSAARGCBGACTC
nifH-F-12	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAGGTGGTTCGCGAYCCSAARGCBGACTC
nifH-F-13	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAACGGACTGCGAYCCSAARGCBGACTC
nifH-F-14	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGTGTCTGCGAYCCSAARGCBGACTC
nifH-F-15	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGAGGTCTGCGAYCCSAARGCBGACTC
nifH-F-16	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGATGACTGCGAYCCSAARGCBGACTC
nifH-F-17	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTATTCTGCTGCGAYCCSAARGCBGACTC
nifH-F-18	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGGCAATTGCTGCGAYCCSAARGCBGACTC
nifH-F-19	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAGTCTGGACTGCGAYCCSAARGCBGACTC
nifH-F-20	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGATCCATCTGCGAYCCSAARGCBGACTC
nifH-F-21	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCAATTACTGCGAYCCSAARGCBGACTC
nifH-F-22	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCGAGACGCTGCGAYCCSAARGCBGACTC
nifH-F-23	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCCACGAACTGCGAYCCSAARGCBGACTC
nifH-F-24	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCTCATTCTGCGAYCCSAARGCBGACTC
nifH-F-25	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTGAGATACTGCGAYCCSAARGCBGACTC
nifH-F-26	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTACAACCTCTGCGAYCCSAARGCBGACTC
nifH-F-27	CCATCTCATCCCTGCGTGTCTCCGACTCAGAACCATCCGCTGCGAYCCSAARGCBGACTC
nifH-F-28	CCATCTCATCCCTGCGTGTCTCCGACTCAGATCCGGAACTGCGAYCCSAARGCBGACTC
nifH-F-29	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGACCACTCTGCGAYCCSAARGCBGACTC
nifH-F-30	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAGGTTATCTGCGAYCCSAARGCBGACTC
nifH-F-31	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCAAGCTGCTGCGAYCCSAARGCBGACTC
nifH-F-32	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTACACACTGCGAYCCSAARGCBGACTC
nifH-F-33	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTCATTGAACTGCGAYCCSAARGCBGACTC
nifH-F-34	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGCATCGTCTGCGAYCCSAARGCBGACTC
nifH-F-35	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGCCATTGCTGCGAYCCSAARGCBGACTC
nifH-F-36	CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGGAATCGTCTGCGAYCCSAARGCBGACTC
nifH-F-37	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTGAGAATGTCTGCGAYCCSAARGCBGACTC
nifH-F-38	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGGACGGACTGCGAYCCSAARGCBGACTC
nifH-F-39	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAACAATCGGCTGCGAYCCSAARGCBGACTC
nifH-F-40	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGACATAATCTGCGAYCCSAARGCBGACTC
nifH-F-41	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCCACTTCGCTGCGAYCCSAARGCBGACTC
nifH-F-42	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCACGAATCTGCGAYCCSAARGCBGACTC
nifH-F-43	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTGACACCGCTGCGAYCCSAARGCBGACTC
nifH-F-44	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCAGCTGCGAYCCSAARGCBGACTC
nifH-F-45	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGGAGCTTCCTCTGCGAYCCSAARGCBGACTC
nifH-F-46	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCCGAACCTGCGAYCCSAARGCBGACTC
nifH-F-47	CCATCTCATCCCTGCGTGTCTCCGACTCAGTAAGGCAACCACTGCGAYCCSAARGCBGACTC
nifH-F-48	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTAAGAGACTGCGAYCCSAARGCBGACTC
nifH-F-49	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTAACATAACTGCGAYCCSAARGCBGACTC

nifH-F-50	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGACAATGGCTGCGAYCCSAARGCBGACTC
nifH-F-51	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGAGCCTATTCTGCGAYCCSAARGCBGACTC
nifH-F-52	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCATGGAACGCGAYCCSAARGCBGACTC
nifH-F-53	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGGCAATCCTCTGCGAYCCSAARGCBGACTC
nifH-F-54	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGGAGAATCGCTGCGAYCCSAARGCBGACTC
nifH-F-55	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCACCTCCTCTGCGAYCCSAARGCBGACTC
nifH-F-56	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGCATTAATTCTGCGAYCCSAARGCBGACTC
nifH-F-57	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGGCAACGGCTGCGAYCCSAARGCBGACTC
nifH-F-58	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGAACACTGCGAYCCSAARGCBGACTC
nifH-F-59	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTGATGTTCTGCGAYCCSAARGCBGACTC
nifH-F-60	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTAGCTCTTCTGCGAYCCSAARGCBGACTC
nifH-F-61	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCACTCGGATCTGCGAYCCSAARGCBGACTC
nifH-F-62	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTTGCTTCACTGCGAYCCSAARGCBGACTC
nifH-F-63	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTTAGAGTTCGCGAYCCSAARGCBGACTC
nifH-F-64	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTGAGTTCCGACTGCGAYCCSAARGCBGACTC
nifH-F-65	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTTGCCACATCTGCGAYCCSAARGCBGACTC
nifH-F-66	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGCAATCATCTGCGAYCCSAARGCBGACTC
nifH-F-67	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCTACCAGTCTGCGAYCCSAARGCBGACTC
nifH-F-68	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCAAGAAGTTCGCGAYCCSAARGCBGACTC
nifH-F-69	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTCAATTGGCTGCGAYCCSAARGCBGACTC
nifH-F-70	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTACTGGTCTGCGAYCCSAARGCBGACTC
nifH-F-71	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGGCTCCGACTGCGAYCCSAARGCBGACTC
nifH-F-72	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAAGGCCACACTGCGAYCCSAARGCBGACTC
nifH-F-73	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGCTGTCTGCGAYCCSAARGCBGACTC
nifH-F-74	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGATCGGTTCTGCGAYCCSAARGCBGACTC
nifH-F-75	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCAGGAATACTGCGAYCCSAARGCBGACTC
nifH-F-76	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGAAGAACCCTCTGCGAYCCSAARGCBGACTC
nifH-F-77	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGAAGCGATTCTGCGAYCCSAARGCBGACTC
nifH-F-78	CCATCTCATCCCTGCGTGTCTCCGACTCAGCAGCCAATTCTCTGCGAYCCSAARGCBGACTC
nifH-F-79	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTGGTTGTCGCGAYCCSAARGCBGACTC
nifH-F-80	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCGAAGGCAGGCTGCGAYCCSAARGCBGACTC
nifH-F-81	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCTGCCATTCTGCGAYCCSAARGCBGACTC
nifH-F-82	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGCATCTCTGCGAYCCSAARGCBGACTC
nifH-F-83	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAGGACATTCTGCGAYCCSAARGCBGACTC
nifH-F-84	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTCCATAACTGCGAYCCSAARGCBGACTC
nifH-F-85	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCAGCCTCAACTGCGAYCCSAARGCBGACTC
nifH-F-86	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTGGTTATTCTGCGAYCCSAARGCBGACTC
nifH-F-87	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTGGCTGGACTGCGAYCCSAARGCBGACTC
nifH-F-88	CCATCTCATCCCTGCGTGTCTCCGACTCAGCCGAACACTTCTGCGAYCCSAARGCBGACTC
nifH-F-89	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCTGAATCTCTGCGAYCCSAARGCBGACTC
nifH-F-90	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAACCCACGGCTGCGAYCCSAARGCBGACTC
nifH-F-91	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGAAGGATGCTGCGAYCCSAARGCBGACTC
nifH-F-92	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTAGGAACCGCTGCGAYCCSAARGCBGACTC

nifH-F-93	CCATCTCATCCCTGCGTGTCTCCGACTCAGCTTGTC <del>CAATC</del> TGCGAYCCSAARGCBGACTC
nifH-F-94	CCATCTCATCCCTGCGTGTCTCCGACTCAGTCCGACAAGCTGCGAYCCSAARGCBGACTC
nifH-F-95	CCATCTCATCCCTGCGTGTCTCCGACTCAGCGGACAGATCTGCGAYCCSAARGCBGACTC
nifH-F-96	CCATCTCATCCCTGCGTGTCTCCGACTCAGTTAAGCGGCTGCGAYCCSAARGCBGACTC
Reverse	trP1- <i>nifH</i> reverse primer
nifH-R	CCTCTCTATGGGCAGTCGGTGATATSGCCATCATYTCRCCGGA