Diversity of Nitrogen Cycling Genes at a Midwest Long Term Ecological Research Site with Different Management Practices

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KEYWORDS: nitrous oxide, *nirK*, *nirS*, *norB*, *nosZ*

Abstract

Nitrogen fertilizer results in the release of nitrous oxide (N_2O), a concern because N_2O is an ozone-depleting substance and a greenhouse gas. Although the reduction of N_2O to nitrogen gas can control emissions, the factors impacting the enzymes involved have not been fully explored. The current study investigated the abundance and diversity of genes involved in nitrogen cycling (primarily denitrification) under four agricultural management practices (no tillage [NT], conventional tillage [CT], reduced input, biologically based). The work involved examining soil shotgun sequencing data for nine genes (*napA, narG, nirK, nirS, norB, nosZ, nirA, nirB, nifH*). For each gene, relative abundance values, diversity and richness indices and taxonomic classification were determined. Additionally, the genes associated with nitrogen metabolism (defined by the KEGG hierarchy) were examined. The data generated were statistically compared between the four management practices. The relative abundance of four genes (*nifH*, *nirK nirS* and *norB*) were significantly lower in the NT treatment compared to one or more of the other soils. The abundance values of *napA*, *narG*, *nifH*, *nirA* and *nirB* were not significantly different between NT and CT. The relative abundance of *nirS* was significantly higher in the CT treatment compared to the others. Diversity and richness values were higher for four of the nine genes (*napA, narG, nirA, nirB*). Based on *nirS/nirK* ratios, CT represents the highest N_2O consumption potential in four soils. In conclusion, the microbial communities involved in nitrogen metabolism were sensitive to different agricultural practices, which in turn, likely has implications for N_2O emissions.

Key Points

Four genes were less abundant in NT compared to one or more of the others soils (*nifH*, *nirK nirS*, *norB*).

The most abundant sequences for many of the genes classified within the *Proteobacteria*.

Higher diversity and richness indices were observed for four genes (*napA, narG, nirA, nirB*).

Based on *nirS/nirK* ratios, CT represents the highest N_2O consumption potential.

Introduction

An understanding of the terrestrial nitrogen cycle is important both for optimizing agricultural productivity as well as for minimizing environmental impacts, such as water pollution or global warming. Nitrous oxide (N_2O) is a predominant ozone-depleting substance and an important and potent greenhouse gas with a global warming potential over 100 years of ~298 and 11.9 times that of CO_2 and CH_4, respectively (Domeignoz-Horta et al. 2018; Ravishankara et al. 2009). The majority (almost 70%) of the total global N_2O atmospheric loading can be accounted for by terrestrial ecosystems, and at least 45% of this has been attributed to microbial cycling of nitrogen in agricultural systems (Rudy et al. 2008; Syakila and Kroeze 2011). The increasing use of nitrogen fertilizer in agricultural practice has accordingly increased N_2O production (Davidson, 2009). The nitrogen cycle involves two key microbial processes for the emission of N_2O from soils. During nitrification, bacteria produce N_2O during the first step, when ammonia is oxidized to nitrite via hydroxylamine (Prosser and Nicol 2012). Denitrification is another key microbial process for the release of N_2O, involving the respiratory reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and their subsequent reduction to gaseous forms (NO, N_2O, N_2). Although the microbial reduction of N_2O to nitrogen gas is vital for controlling emissions from terrestrial ecosystems, the determinants for a soil to act as a source or a sink remain uncertain (Butterbach-Bahl et al. 2013). Although the importance of nitrification is recognized for nitrous oxide emissions, the current work focused primarily on the enzymes involved in denitrification to favor content depth over breadth.

A number of enzymes are associated with denitrification, including those encoded by nitrate reductases (*napA*/*narG*), nitrite reductases (*nirk*/*nirS*); nitric oxide reductase (*norB*) and nitrous oxide reductase (*nosZ*) (Philippot et al. 2007). Many researchers have suggested that the abundance and diversity of such genes can impact N_2O emission rates. For instance, researchers found corrections between the relative abundance of *nosZ* and the potential N_2O production (Domeignoz-Horta et al. 2016). In another study, low N_2O emission rates were explained by soils properties (up to 59%), whereas high rates were explained by the abundance and diversity of the microbial communities (up to 68%) (Domeignoz-Horta et al. 2018). The same study found that the diversity of *nosZ* was important to explain the variation in N_2O emissions (Domeignoz-Horta et al. 2018). Others found that *nirK* gene copy numbers correlated with potential

denitrification, but *nirS* gene copy numbers did not (Attard et al. 2011). Further, researchers have provided evidence of higher *nirS*/*nirK* ratios and higher N_2O consumption (Jones et al. 2014).

Agricultural practices are also known to influence denitrification trends. Although the impact of no tillage (NT) on N_2O emissions has been widely investigated, the results have been varied. Some studies reported minimal differences of N_2O emissions between NT and conventional tillage (CT) soil (Kaharabata et al. 2003; Lee et al. 2006; Melero et al. 2011). For example, the potential denitrification rates and the ratios of N_2O/N_2 were similar in NT and CT after harvesting in a rainfed crop rotation system in Spain (Melero et al. 2011). Others found that NT stimulates denitrification (Baudoin et al. 2009; Calderon et al. 2001; Wang and Zou 2020). The denitrification enzyme activity and denitrification gene abundances (*nirK* and *nosZ*) were enhanced in NT in a soybean/rice crop system in Madagascar (Baudoin et al. 2009). Similar results for the increase of denitrification gene abundance in NT was also observed under sub-zero temperatures (Tatti et al. 2015). The general trend that NT favored the denitrification rates, the abundance of denitrifying genes and N_2O emission was demonstrated at a global scale (Wang and Zou 2020).

Research has also addressed the differential consequences of tillage management on the microbial community structure and diversity. CT has a positive influence on the bacterial richness and diversity in clay soil in central Italy (Pastorelli et al. 2013). However, some studies found opposite results. Minimal tillage enriched the microbial population and diversity relative to CT in a recent global meta-analysis (Li et al. 2020). The bacteria diversity (represented by all the alpha-diversity indices) was higher in the NT soils compared to CT soils in a winter wheat cropping system in northern China (Dong et al. 2017). They also found that *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria* and *Betaproteobacteria* were more abundant at class level in NT whereas CT had more sequences belong to *Acidobacteria*. In an experiment conducted in the agricultural fields in Indiana (USA), more DNA sequences related to the nitrogen metabolism were observed in the NT soils compared to CT soils, indicating the higher potential of nitrogen cycling (Smith et al. 2016).

Although researchers have previously studied the impacts of various agricultural management practices on denitrification and N_2O emission, the information on the taxonomic distributions and functional sequences related to nitrogen metabolism under different managements in the

field-crop ecosystems is still limited, especially in the U.S Midwest. The objective of this research was to investigate the impact of four agricultural management on the abundance and diversity of microbial communities regulating nitrogen cycling (primarily denitrification). The work focused on the agricultural sites at the Long Term Ecological Research (LTER) Site at Kellogg Biological Station (KBS), in southwest Michigan, southwest of the campus of Michigan State University (MSU). This LTER has field-crop ecosystems typical of the U.S. Midwest. The work is unique because it examines the key functional genes for nitrogen cycling over four long-term systems and detects a wider range of sequences through high throughput shotgun sequencing.

Methods

Sample Collection, DNA Extraction and Shotgun Sequencing

The DNA examined in the current work was generated from a previous study by our group (Thelusmond et al. 2019), involving an examination of the genes associated with xenobiotic biodegradation. Our previous work did not investigate the genes involved in nitrogen cycling. Briefly, four soils were collected from 5 sampling stations in 6 replicate plots for Treatments 1, 2, 3 and 4 within the Michigan State University Main Cropping System Experiment at Kellogg Biological Station Long-Term Ecological Research (KBS LTER) (42°24'N, 85°23'W). The agricultural management practices for each Treatment are illustrated in Table 1 and for additional information see https://lter.kbs.msu.edu/research/site-description-and-maps/. The physical and chemical characteristics of the soils were previously determined (A & L Great Lakes Laboratories, Inc., Fort Wayne, IN) with all being classified as loam soils. DNA extraction was completed using the DNA extraction kit (DNeasy PowerLyzer PowerSoil Kit, Mo Bio, USA) according to the manual protocol. Shotgun sequencing was performed with the Illumina HiSeq 4000 (2 × 150 bp) platform at the Research Technology Support Facility (RTSF) at Michigan State University (MSU), as previously described (Thelusmond et al. 2019).

Processing, DIAMOND Alignment, Diversity Analysis and Enrichments in Each Soil

Low quality sequences and Illumina adapters were removed from the HiSeq fastq.gz files using Trimmomatic with the Paired End Mode settings (Bolger et al. 2014) (Version 0.36). Protein

sequences for each of the nine genes were collected from the FunGene website (http://fungene.cme.msu.edu/) using a filter minimum HMM coverage of 70% (Cole et al. 2011; Fish et al. 2013). Following this, the FunGene Pipeline Dereplicator tool was used to dereplicate these sequences (Cole et al. 2011; Fish et al. 2013). Table S1 provides a summary of the sequences obtained at each step.

The dereplicated sequences were then aligned against the trimmomatic files using DIAMOND (double index alignment of next-generation sequencing data) (Version 2.0.1) (Buchfink et al. 2015). Only reads that exhibited an identity of ≥ 60 % and an alignment length ≥ 49 amino acids to the reference sequences were retained. For each, relative abundance values were calculated using the number of aligned reads divided by the total number of sequences for each sample. The relative abundance values were then normalized by (divided by) the number of dereplicated reference sequences for each gene. Diversity indices (Chao 1, Chao2, Inverse Simpson and Shannon values) were determined (using the number of aligned reads for each gene) using EstimateS (Version 8.2.0) (Colwell 2006). The accession numbers of sequences statistically enriched in each soil (as described below) were determined. The R package Taxonomizr (Sherrill-Mix 2009) was used with R (Version 3.5.1) (R_Core_Team 2018) in RStudio (Version 0.9.24) (RStudio_Team 2020) to determine the taxonomic classification of each sequence. The data were illustrated with bar charts in Excel (Version 2010).

Phylogenetic Trees

The 50 most abundant sequences for each gene, averaged across all samples, were determined in Excel. The list of accession number for each were uploaded to COBALT: constraint-based alignment tool for multiple protein sequences (https://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi) (Papadopoulos and Agarwala 2007). The downloaded alignments (fasta plus gaps) from COBALT were then submitted for MAFFT (multiple alignment using fast Fourier transform) alignment using an online server (https://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019) (Version 7). Trees, also obtained from the same website, by the Neighbor-Joining method were exported in Newick format. The downloaded tree files were uploaded to the Interactive Tree of Life (https://itol.embl.de) (Letunic

and Bork 2019) (Version 5.5.1). Sequences were colored depending on their classification and relative abundance values were added using the Datasets function called simple bar chart.

MG-RAST Analysis

Shotgun sequences were also analyzed by MG-RAST (Meta Genome Rapid Annotation using Subsystem Technology, Version 4.0.2) (Meyer et al. 2008). The processing pipeline includes removing artificial replicate sequences by dereplication and removing low quality sequencing by using SolexaQA (Cox et al. 2010). The taxonomic analysis included RefSeq (Pruitt et al. 2005) database and the KEGG (Kanehisa 2002) database. The sequences are available publicly on the MG-RAST and the summary of the MG-RAST data is presented in Table S2.

Statistical Analysis

RStudio was used to perform a number of statistical tests, as follows (Version 0.9.24) (RStudio_Team 2020). One-way ANOVA or Kruskal-Wallis tests were performed using the "aov" or "kruskal.test" functions as implemented in R package "car" (Fox et al. 2020) to determine if there were statistically significant differences between 1) relative abundance of functional genes obtained by DIAMOND and 2) richness and diversity values (Chao 1, Chao2, Inverse Simpson and Shannon values). First, Levene's test was carried out to assess the homogeneity of variance of the data using the "leveneTest" function in the R package "car" (Fox et al. 2020). The Shapiro-Wilk test was conducted to evaluate the normality of the data using the "shapiro_test" function in the R package 'rstatix' (Kassambara 2020). When the *p* values from both of the Levene's and the Shapiro-Wilk tests were more than 0.05, the differences between the means were determined by one-way ANOVA. When the *p* values from the one-way ANOVA were less than 0.05, multiple pairwise comparison between the means were performed using Tukey's Honest Significant Difference test using the "TukeyHSD" function in the "stats" R package. When the *p* values from the Shapiro-Wilk test were less than 0.05, the non-parametric alternative to a one-way ANOVA, the test Kruskal-Wallis (function "kruskal.test" in the "stats" package), was used. When *p* values were less than 0.05 for the Kruskal-Wallis text, Dunn's test, using the "dunnTest" function in the R package "FSA", (Ogle et al. 2020)) was utilized to determine differences between means. Spearman's Rank Correlation test was carried out to

explore the strength of correlation between the relative abundance of different genes using the "cor.test" function (with method = "spearman") in the R package "stats".

Principle component analysis (PCA) was performed within Microsoft Excel using XLSTAT (Addinsoft 2020) (Version 2020.3.1) to visualize the effect of the addition of pharmaceuticals on the gene relative abundance in different managed soils. STAMP (Statistical Analysis of Taxonomic and Functional Profiles, Version 2.1.3) (Parks et al. 2014) was used to statistically analyze the MG-RAST data. Specifically, extended error bars were generated to illustrate significant differences (Welch's two-sided *t*-test, two group analysis option, $p < 0.05$) in the gene relative abundance for the genes associated with nitrogen metabolism (as defined by the KEGG hierarchy). The data (generated in MG-RAST, six metagenomes for each soil) were analyzed using STAMP with the two group analysis option (each soil compared to the other three soils) and Welch's two sided t-test ($p<0.05$).

Analysis of Assembled Sequences

Shotgun sequences processed by Trimmomatic were assembled with Megahit (Li et al. 2016) (Version 1.2.4) with the pair end plus single end option (minimum and maximum kmer size were 27 and 127 with a kmer size step of 10). TaxIds for the FunGene *nifH* database (as described above, except no dereplication occurred) were obtained with the R package taxonomizr (Sherrill-Mix 2009), RStudio (Team 2020) (Version 0.9.24) and R (Team 2018) (Version 3.5.1). The analysis targeted *nifH* because no significant differences were found between soils in the analysis described above (before assembly). Following the deletion of duplicate values, the taxids obtained were used to analyze the assembled reads using the NCBI nucleotide database (nt) with the taxids option in BLASTN (Altschul et al. 1990) (Version 2.10.0-Linux_x86_64). BLASTN command lines also included the following options: identity ≥ 60 %, evalue ≤ 1×10^{-5}. The txt files generated from BLASTN were imported into Megan (Huson et al. 2016) (community edition Version 6.19.7). In Megan, the option "Compare" was used to combine all twenty-four data sets and then the combined dataset (at species level) was exported (using the STAMP export option) for analysis in STAMP. Additionally, the assembled contigs were aligned against the entire nt database using BLASTN without the taxids option (identity ≥ 60 %, evalue ≤ 1×10^{-5}).

The output files were first imported and then in Megan, following this, the file was exported into STAMP to compare the communities between soils.

Results

Abundance and Diversity of Functional Genes

The relative abundance of genes associated with nitrogen fixation, denitrification or dissimilatory nitrate reduction in all four management systems are presented (Figure 1A), with the lowest abundance for *nifH* and highest for *nirK*. Two sets of genes were observed to have the approximately same level of abundance in the soils: the nitrate reductase genes *napA* and *narG*; and the nitrite reductase genes *nirA* and *nirB*. The distribution of the relative abundance of *nirK*, *norB* and *nosZ* were not as tightly grouped compared to the other genes, suggesting a greater spread in abundance of these genes across the metagenomes.

Principle component analysis of the functional genes (Figure 1B) indicated the nitrite reductase gene *nirK* was positively correlated with the nitrite reductase gene *nirS*, nitric oxide reductase gene *norB* and nitrous oxide reductase gene *nosZ*. Further, the nitrate reductase gene *napA* was positively correlated with the nitrite reductase gene *nirA*. In contrast, the nitrite reductase gene *nirB* did not appear to correlate with any other gene. The addition of pharmaceuticals impacted the functional genes in two treatments (conventional tillage and reduced input soils).

The average relative abundance of the twelve genes across the four management systems is displayed in Figure 2. Four genes (*nifH*, *nirK nirS* and *norB*) were significantly lower in the NT treatment compared to one or more of the other treatments. The average relative abundance of *nirS* was significantly higher in the CT treatment compared to the other treatments. It was also interesting to note that the average relative abundance of *nosZ* was approximately 50% lower in the NT soil compared to the other soils, although the difference was not statistically confirmed. The results of the statistical analysis tests (Levene's test, Shapiro-Wilk, One-way ANOVA, Tukey's Honest Significant Difference, Kruskal-Wallis, Dunn's test) on these data sets are summarized (Tables S3-S5).

Figure 3 illustrates correlations between gene relative abundance percentages across all samples with all statistically significant positive correlations (Spearman's rank test) being boxed in red. The abundance *napA* significantly correlated with six genes (*narG, nirA, nirB, nirK, norB, nosZ*), as did the abundance of *narG* (*napA, nifH, nirA, nirB, nirK, norB)*. In contrast, *nifH* correlated with two genes (*narG* and *nirK*). The abundance of both *nirA* and *nirB* correlated with *napA, narG* and to each other. Additionally, *nirK*, *nirS*, *norB*, *nosZ* all correlated positively to each other. The *p*-values and Spearman's correlation coefficients for the Spearman's rank tests are shown in Tables S6 & S7.

The values of richness estimators (Chao 1 and Chao 2) and diversity indexes (Shannon and Inverse Simpson) determined by EstimateS are summarized (Figure 4). The results of the statistical analysis (Levene's test, Shapiro-Wilk, One-way ANOVA, Tukey's Honest Significant Difference, Kruskal-Wallis, Dunn's test) on this data set are also summarized (Tables S8-S17).

Overall, higher Chao 1 and Chao2 values (~8000-9000) were found for four genes (*napA, narG, nirA, nirB*), whereas lower values (~500-1500) were estimated for the five other genes (*nifH, nirK, nirS, norB, nosZ*). For Chao 2 no significant differences were found between the four treatments for all genes. The only significant difference for Chao 1 between treatments was for *nirS, nirK* and *nifH*. Chao1 values were higher in both the CT treatment and the biological based treatment compared to the NT treatment for *nirS*. For *nirK*, Choa 1 was lower for the CT treatment compared to the reduced input treatment. For *nifH*, the Choa 1 value in the reduced input treatment was lower than the biological based treatment.

Overall, the average values for Shannon and Inverse Simpson were higher (~1000-2000 and ~7.2-8.1) for four genes (*napA, narG, nirA, nirB*) compared to the rest (~100-400 and ~5.4-6.4). For the Inverse Simpson values, at least one significant difference between treatments was noted for six genes (*napA, nirA, nirB, nirK, nirS, norB*), with the most notable number of differences between treatments being for *nirK, nirS* and *norB*. For *nirK* and *norB*, Inverse Simpson values were significantly higher in the NT treatment compared to the other treatments. For *nirA* and *nirB*, Inverse Simpson values were significantly higher in the reduced input treatment compared to the CT and NT treatments. For the Shannon Index values, at least one significant difference was found between at least two treatment for all genes except *nifH* and *nirB*. For *napA*, Shannon Index (and Inverse Simpson) values were significantly higher in the reduced input treatment

compared to the CT and NT treatments. For *nosZ*, Shannon values were significantly lower in CT treatment compared to the other treatments. It was also interesting to note that the Shannon values of *nirA* were higher in the reduced input treatment than in the conventional and no tillage treatments.

The abundance of the genes associated with the nitrogen metabolism (as defined in the KEGG hierarchy) were investigated to determine if there were significant differences between management systems. For this, each dataset was compared individually with the other three datasets (Figure 5). Only one gene (*norF*; nitric-oxide reductase NorF protein) was more abundant in the CT soil compared to the other three (Figure 5A). In contrast, six genes were more abundant in the NT soil compared to the other three soils (*nirA*; ferredoxin-nitrite reductase, *cynT*, *can*; carbonic anhydrase, nitronate monooxygenase, nitrate reductase (NADH), *nrfD*; protein NrfD and *hao*; hydroxylamine oxidase) (Figure 5B). Three genes (*nirB*; nitrite reductase (NAD(P)H) large subunit, *nosZ*; nitrous-oxide reductase, and *nirD*; nitrite reductase (NAD(P)H) small subunit (Figure 5C)) and one gene (nitronate monooxygenase) were dominant in the reduced input soil and biological based soil, respectively (Figure 5D).

Phylotypes Associated with Nitrogen Metabolism

The phylotypes (at the class level) associated with the nitrogen metabolism genes have been summarized (Figures 6 and 7). The CT soil was dominated by *Betaproteobacteria* for *napA*, *narG*, *nirA*, *nirB*, *nirK*, *nirS* and *norB* and by *Cytophagia* for *nosZ*. Further, in many cases (*napA*, *narG*, *nirA*, *nirB*, *nirK, norB*) *Betaproteobacteria* were more abundant in the CT soil compared to the other three soils. For several genes (*napA*, *narG*, *nirK*, *nosZ*), *Alphaproteobacteria* were more abundant in the NT soil compared to the other soils. While for *nirA* and *nirB*, *Alphaproteobacteria* were more abundant in the biological based soil compared to the other three soils. For the genes *napA*, *narG*, *nirK* and *norB*, *Actinobacteria* were more abundant in the NT soil compared to the other three soils. For *nirA* and *nirB*, *Actinobacteria* was approximately at the same level in biological based soil compared to the NT soil while somewhat lower in the conventional tillage and reduced input soils. Additional trends included the dominance of the *Gammaproteobacteria* for two of the four soils for *norB* as well as the dominance of unclassified sequences and *Flavobacteriia* across various soils for *nosZ.*

As no significant differences were noted for *nifH* for the above analysis, differences were investigated for this gene within the assembled contigs. Significant differences at the genera level associated with *nifH* gene between the CT and the other three soils are shown (Figure S1). The genus *Frankia* was significantly more abundant in the NT, reduced input and biological based soils compared to the CT soils. Several genera were enriched for this gene in the CT soil compared to the NT soil (e.g. *Rubrivivax, Leptothrix, Cupriavidus*). Also, two (*Paraburkholderia* and *Burkholderia*) were more enriched in the CT compared to the reduced input soils (Figure S1B). Four genera were more highly enriched in the comparison between the CT and the biological based soil for this gene (Figure S1C).

Comparison of Microbial Communities

When the entire microbial community from the assembled contigs was compared between treatments significant differences were found and are illustrated at the genus level (Figure S2). No enriched genera were found in the NT soil compared to the CT soil. Four genera (*Nocardioides*, *Mycobacterium*, *Nakamurella* and *Microvirga*) were enriched in both the reduced input and biological based compared to the CT soil. The other more abundant genera identified in the reduced input compared to the CT soil were *Pseudonocardia* and *Archangium*. The other enriched genera identified in the biological based compared to the CT soil included *Candidatus Nitrosotalea*, *Nitrospira*, *Bradyrhizobium, Actinoplanes, Nonomuraea, Skermanella, Sulfuritortus, Pigmentiphaga and Variibacter.*

Phylogeny of Most Abundant Sequences

The phylogenetic relationships of the representative sequences (fifty most abundant sequences, before contigs were assembled) for the genes related to nitrogen metabolism in the four soils are shown (Figure 8). The three most abundant sequences for *napA* and *narG* were the same sequences and classified as *Betaproteobacteria* and *Alphaproteobacteria*. Similarly, the three most abundant sequences for *nirA* and *nirB* were the same and belonged to *Deltaproteobacteria*, *Opitutae* and the unclassified. The three most abundant sequences for *norB* belonged to *Alphaproteobacteria* and were phylogenetically close to each other. For *nirK* and *nirS*, both the majority of the fifty most abundant sequences and the three most abundant sequences belonged to *Betaproteobacteria*. Moreover, the predominant representative sequences belonged to

Betaproteobacteria for *nirB*, *nirK* and *nirS* and belonged to *Alphaproteobacteria*, and *Flavobacteriia* for *nifH* and *nosZ*, respectively.

Discussion

The influence of different agricultural management practices on nitrogen metabolism is important for understanding N_2O emissions from agricultural soils. Here, the taxonomic and functional profiles of the soil microbial communities associated with nitrogen metabolism, primarily denitrification, were characterized in Mid-West agricultural soils under four different management practices. From the nine nitrogen metabolism genes examined in the soil metagenomes, the most abundant was *nirK*. Denitrifying microorganisms contain either a Cu-nitrite reductase or a cytochrome cd_1 nitrite reductase, encoded by *nirK* and *nirS* respectively (Zumft 1997). In the current study, *nirK* was approximately 17.9 times more abundant than *nirS* when all of the soil metagenomes were considered together. Further, consistent with other researchers, *nirK* and *nirS* gene abundance were significantly correlated (Enwall et al. 2010). Others have also reported higher levels of *nirK* compared to *nirS* in soil metagenomes, for example, *nirK* was up to 3.8 times more abundant than *nirS* in 35 from 37 soils (Jones et al. 2014). Additionally, *nirK* was more abundant compared to *nirS* during agricultural waste composting (Zhang et al. 2015a). In another study, *nirK* copy numbers were approximately two orders of magnitude higher than *nirS*, regardless of tillage treatment (Kim et al. 2021). These two genes are considered to be mutually exclusive, representing two ecologically distinct denitrifying communities (Enwall et al. 2010; Jones and Hallin 2010). To date, no microorganism has been reported to contain both types of reductases. It has been suggested that higher *nirS/nirK* ratios may indicate higher N_2O consumption trends (Jones et al. 2014). Based on this, in the current study, CT represents the highest N_2O consumption potential in four soils examined. Specifically, the average *nirS/nirK* ratios were 0.074, 0.045, 0.045 and 0.056 for CT, NT, reduced input and biological based, respectively.

Concerning other genes impacting N_2O depletion and formation, here, *nosZ* was less abundant compared to *norB* and (*nirK*+*nirS*). Others have also reported that *nir* gene abundance can exceed that of *nosZ* by up to one order of magnitude (Garcia-Lledo et al. 2011; Hallin et al. 2009; Philippot et al. 2011). This may be explained by the absence of *nosZ* in nearly one-third of genomes which contained *nir* and *nor* genes (Jones et al. 2008) and because *nosZ* has been found on plasmids (Zumft 1997).

When considering the different management practices, the abundance of *napA*, *narG*, *nifH*, *nirA* and *nirB* was not significantly different between NT and CT. The same trend was reported for *nifH* and *narG* by others (Liu et al. 2016). In contrast, we found that *nirK, nirS* and *norB* were statistically significantly lower in the NT compared to the CT treatment. Others have reported an increase in the abundance of denitrifying genes in response to NT (Baudoin et al. 2009; Wang and Zou 2020) or minimal tillage (Kaurin et al. 2018). However, in an experiment across arable soils, the abundance of *nirS*- and *nirK*- denitrifiers were not significantly different between agricultural practices (Domeignoz-Horta, Philippot et al. 2018). Similarly, in another study, the abundance of *nirK* and *nirS* did not differ between NT and CT (Puerta et al. 2019). The authors speculated that NT could have promoted denitrification in the form of higher activity but not the abundance of denitrifying genes. NT was reported to greatly increase the RNA/DNA ratios for *nirS* and *nosZ* denitrifiers (Tatti et al. 2015). They hypothesized that anoxic conditions (e.g., water content) contributed more to the *nirS* and *nosZ* transcription under NT compared to CT (Tatti et al. 2015). NT tends to reduce the oxygen level below the surface (Pastorelli et al. 2013) and increase the water-filled pore space because of greater soil moisture and bulk density (Wang and Zou 2020). These two factors may contribute to the potential enhanced anaerobic denitrification in NT soil. No correlation was found between denitrification enzyme activity and the abundances of *nirK*- and *nirS*- denitrifiers (Yin et al. 2014). More information is needed to determine the real impact of lower *nirK, nirS* and *norB* gene abundances in NT in the current system.

The microbial community richness (Chao1 and Chao2) and diversity (Shannon index and Inverse of Simpson) indices were generally higher for the genes associated with nitrate reduction (*napA* and *narG)* and dissimilatory nitrite reduction (*nirA* and *nirB*) compared to the other genes. For *nirK*, *norB* and *nosZ* for at least one and up to three richness and diversity indexes were

significantly greater in NT soil compared to CT soil, indicating a potential higher species richness and diversity in the current NT soil for these genes. In other research, higher alpha diversity of soil bacterial community was found in NT treatment compared to tilled treatment (Dong et al. 2017; Liu et al. 2020). Similarly, the richness and diversity of bacteria (characterized by phospholipid fatty acids analysis) were greater in NT over CT soil (Zhang et al. 2015b). This may be due to crop residues under the soil surface in NT soils being utilized as food sources (Zhang et al. 2015b). Another possible reason is that NT soil contains larger soil aggregates which could provide more organic matter for the microorganisms, therefore enhancing the bacterial diversity (Peixoto et al. 2006). It was demonstrated that denitrification activity was greatly influenced by denitrifier diversity but not the abundance. Using a dilution approach to manipulate the soil microbial community, researchers found that a decrease in the potential denitrification activity could be a result of denitrifier diversity loss and not the lower denitrifier biomass (Philippot et al. 2013). These trends could suggest that the NT examined in the current study may have a higher denitrification potential due to higher *norB* and *nosZ* diversity, although more research is needed to confirm this hypothesis.

It is interesting to note that Chao 1, Shannon and Inverse of Simpson were significantly higher in CT soil compared to NT soils for *nirS*. Inversely, for *nirK*, Shannon and Inverse of Simpson were significantly higher in NT soil over CT soil. A previous study reported that diversity indices targeting *nirS* were more sensitive to environmental factors compared to *nirK* (e.g., ammonium content, total organic carbon and total N) (Li et al. 2017). It was also found that *nirS*-denitrifiers rely more on the full anaerobic conditions than *nirK*-denitrifiers (Yuan et al. 2012). The greater diversity of *nirK* in NT in the current study could indicate oxygen levels and other environmental conditions in NT soil may be more favorable for *nirK*-denitrifiers than *nirS*-denitrifiers.

Several trends were noted concerning the taxonomy of the microorganisms associated with the functional genes studied. For example, the most abundant sequences classified within the *Proteobacteria* (primarily *Betaproteobacteria*) for *nirK* and *nirS*. Further, NT illustrated equal or more abundant levels of *Betaproteobacteria* (phylum *Proteobacteria*) compared to CT soil for a number of the genes examined (*nirK*, *nirS, norB and nosZ*). In other systems, *Betaproteobacteria* often dominates microbial populations due to high growth rates under available carbon substrates

(Jenkins et al. 2010). For *nifH*, *Frankia* (phylum *Actinobacteria*) was enriched in NT reduced input and biologically based soils compared to the CT soil. *Frankia* is a typical nitrogen-fixed organism both in free-living and symbiotic conditions (Sellstedt and Richau 2013).

Two phyla, *Actinobacteria* and *Acidobacteria,* were notably less enriched or absent in CT for several genes (*napA*, *narG, nirA*, *nirB* and *nirK)* compared to the other three soils. Relating these results to previous research, others have examined soil microbial communities under different management systems. For example, one-time tillage increased the abundance of *Actinobacteria* and *Acidobacteria* in an acidic Solonetz with a 19-year NT management in Australia (Liu et al. 2016). In another study, the abundance of *Acidobacteria* was higher in CT over NT, with the pH of 7.4 and 7.5, respectively (Dong et al. 2017). *Acidobacteria* are acidophilic and could be favored by slightly to moderately acidic growth conditions (Sait et al. 2006). Moreover, *Acidobacteria* exhibit the functional ability of the degradation of plant-derived organic matter (Naumoff and Dedysh 2012) and thus play an important role in the decomposition of organic matter (Rampelotto et al. 2013).

For *nosZ*, the most abundant sequences belonged to the *Bacteroidetes* (with the dominant class of *Flavobacteria*). Others have reported *Bacteroidetes* display copiotrophic characteristics and are favored by increased nutrient availability (McHugh and Schwartz 2015). We found *Flavobacteria* was absent in NT soil but dominated in CT soil for *nosZ*. Consistent with these results, microbial community studies have reported more *Bacteroidetes* in CT compared to NT soil (Yin et al. 2017). *Bacteroidetes* were also more dominant in one soil compared to the same soil under non-disturbed grass systems (Acosta-Martinez et al. 2008). However, others have reported that *Bacteroidetes* were more abundant under NT compared to CT in winter wheat cropping system (Dong et al. 2017) and non-disturbed grass system in comparison with agricultural rotation system (Zhang et al. 2014). Besides, NT increased the abundance of *Flavobacteria* compared to the tilled treatment under semi-arid conditions (Liu et al. 2020). Notably, the above studies did not examine the taxonomy of the microorganisms linked with *nosZ* and so it is difficult to conclude if our results are typical of NT compared to CT soils.

Recommended future research to build on the current genomic analysis should include correlations between gene and transcript counts and nitrous oxide emissions. As with all molecular methods, the genomic analysis approach used in the current work has several notable

limitations. One shortcoming involves the analysis of genomic DNA rather than messenger RNA, which would indicate actual activity instead of potential activity. Future research involving transcriptomics to confirm the results of the current study would be beneficial. Additionally, the results obtained are dependent on the analysis thresholds for example, protein sequences for each of the nine genes were collected from the FunGene using a filter minimum HMM coverage of 70%. Increasing or decreasing this threshold would have impacted the downstream analysis. Also, changes in the read alignment identity (set at ≥ 60 %) and alignment length (≥ 49 amino acids) will also effect the results obtained. It is also important to note that the current analysis did not involve nitrification enzymes, which are also highly relevant for an understanding of nitrous oxide emissions.

In conclusion, the agricultural management practices investigated here impacted gene abundance as well as the taxonomy of microorganisms associated with the nitrogen metabolism. From the nine genes examined, *nirK* was the most abundant and *nifH* was the least abundant. The *nirS/nirK* ratios were highest for the CT system, which may indicate a greater potential for N_2O consumption. Three genes (*nirK, nirS* and *norB*) were statistically significantly lower in the NT compared to the CT treatment. The microbial community richness and diversity indices were generally higher for the genes associated with nitrate reduction (*napA* and *narG)* and dissimilatory nitrite reduction (*nirA* and *nirB*) compared to the other genes. For *nirK*, *norB* and *nosZ* a number of the richness and diversity indexes were significantly greater in NT soil compared to CT soil, indicating a potentially a higher denitrification potential. A number of trends were noted for the taxonomy of the functional genes across agricultural systems. The genus *Frankia* was significantly more abundant in the NT, reduced input and biological based soils compared to the CT soils. The CT soil was dominated by *Betaproteobacteria* for seven genes and by *Cytophagia* for *nosZ.* Also, for six of these genes, *Betaproteobacteria* were more abundant in the CT soil compared to the other three soils. *Alphaproteobacteria* were more abundant in the NT soil compared to the other soils for several genes. While for *nirA* and *nirB*, *Alphaproteobacteria* were more abundant in the biological based soil compared to the other three soils. For *napA*, *narG*, *nirK* and *norB*, *Actinobacteria* were more abundant in the NT soil compared to the other three soils. Overall, these results suggest microbial communities involved in nitrogen metabolism are sensitive to varying soil conditions, which in turn, likely has important implications for N_2O emissions.

Acknowledgements

This work was, in part, supported by USDA (Agriculture and Food Research Initiative, Number 2014-67024) and KBS LTER (Kellogg Biological Station Long-Term Ecological Research). Thanks to Stacey VanderWulp from MSU for providing the soil samples from KBS LTER.

Authors' contributions

AC and ZL conceived and designed research. AC and ZL analyzed the data. ZL wrote the manuscript and AC edited the manuscript. All authors read and approved the manuscript.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available on the MG-RAST website (details for the MG-RAST data can be found in Table S2).

Compliance with ethical standards

Conflict of interest

All authors declare no conflict of interest.

Ethical statement

This work was, in part, supported by USDA (Agriculture and Food Research Initiative, Number 2014-67024) and KBS LTER (Kellogg Biological Station Long-Term Ecological Research).

Human and animal rights and informed consent

This article does not contain any studies with human participants or animals performed by any of the authors

References

Acosta-Martinez V, Dowd S, Sun Y, Allen V (2008) Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Bio Biochem 40(11):2762-2770 doi:10.1016/j.soilbio.2008.07.022

Addinsoft (2020) XLSTAT statistical and data analysis solution., 2020.3.1 edn, New York, USA, p https://www.xlstat.com

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403-10 doi:10.1016/S0022-2836(05)80360-2

Attard E, Recous S, Chabbi A, De Berranger C, Guillaumaud N, Labreuche J, Philippot L, Schmid B, Le Roux X (2011) Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. Glob Change Biol 17(5):1975-1989 doi:10.1111/j.1365-2486.2010.02340.x

Baudoin E, Philippot L, Cheneby D, Chapuis-Lardy L, Fromin N, Bru D, Rabary B, Brauman A (2009) Direct seeding mulch-based cropping increases both the activity and the abundance of denitrifier communities in a tropical soil. Soil Bio Biochem 41(8):1703-1709 doi:10.1016/j.soilbio.2009.05.015

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. 30(15):2114-20 doi:10.1093/bioinformatics/btu170

Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods 12(1):59-60 doi:10.1038/nmeth.3176

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philosophical Transactions of the Royal Society B-Biological Sciences 368(1621) doi:10.1098/rstb.2013.0122

Calderon FJ, Jackson LE, Scow KM, Rolston DE (2001) Short-term dynamics of nitrogen, microbial activity, and phospholipid fatty acids after tillage. Soil Sci Soc Am J 65(1):118-126 doi:10.2136/sssaj2001.651118x

Cole JR, Wang Q, Chai B, Tiedje JM (2011) The Ribosomal Database Project: Sequences and software for high-throughput rRNA analysis. Nuc Acids Res 42:D633–D642 doi:10.1002/9781118010518.ch36

Colwell RK (2006) EstimateS : statistical estimation of species richness and shared species from simples, version 8.0. http://purloclcorg/estimates

Cox MP, Peterson DA, Biggs PJ (2010) SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data. Bmc Bioinformatics 11:6 doi:10.1186/1471-2105-11-485

Domeignoz-Horta LA, Philippot L, Peyrard C, Bru D, Breuil MC, Bizouard F, Justes E, Mary B, Leonard J, Spor A (2018) Peaks of in situ N_2O emissions are influenced by N_2O-producing and reducing microbial communities across arable soils. Glob Change Biol 24(1):360-370 doi:10.1111/gcb.13853

Domeignoz-Horta LA, Putz M, Spor A, Bru D, Breuil MC, Hallin S, Philippot L (2016) Non-denitrifying nitrous oxide-reducing bacteria - An effective N2O sink in soil. Soil Biology & Biochemistry 103:376-379 doi:10.1016/j.soilbio.2016.09.010

Dong W, Liu E, Yan C, Tian J, Zhang H, Zhang Y (2017) Impact of no tillage vs. conventional tillage on the soil bacterial community structure in a winter wheat cropping succession in northern China. Eur J Soil Bio 80:35-42 doi:10.1016/j.ejsobi.2017.03.001

Enwall K, Throback IN, Stenberg M, Soderstrom M, Hallin S (2010) Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management. Appl Env Micro 76(7):2243-2250 doi:10.1128/Aem.02197-09

Fish JA, Chai BL, Wang Q, Sun YN, Brown CT, Tiedje JM, Cole JR (2013) FunGene: the functional gene pipeline and repository. Front Microbiol 4:14 doi:10.3389/fmicb.2013.00291

Fox J, Weisberg S, Price B, Adler D, Bates D, Baud-Bovy G, Bolker B, Ellison S, Firth D, Friendly M, Gorjanc G, Graves S, Heiberger R, Krivitsky P, Laboissiere R, Maechler M, Monette G, Murdoch D, Nilsson H, Ogle D, Ripley B, Venables W, Walker S, Winsemius D, Zeileis A, R-Core (2020) Package'car': Companion Applied Regression. 3.0-8 edn

Garcia-Lledo A, Vilar-Sanz A, Trias R, Hallin S, Baneras L (2011) Genetic potential for N2O emissions from the sediment of a free water surface constructed wetland. Water Res 45(17):5621-5632 doi:10.1016/j.watres.2011.08.025

Hallin S, Jones CM, Schloter M, Philippot L (2009) Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J 3(5):597-605 doi:10.1038/ismej.2008.128
Huson DH, Beier S, Flade I, Gorska A, El-Hadidi M, Mitra S, Ruscheweyh HJ, Tappu R (2016) MEGAN Community Edition - Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. PLoS Comput Biol 12(6):e1004957 doi:10.1371/journal.pcbi.1004957
Jenkins SN, Rushton SP, Lanyon CV, Whiteley AS, Waite IS, Brookes PC, Kemmitt S, Evershed RP, O'Donnell AG (2010) Taxon-specific responses of soil bacteria to the addition of low level C inputs. 42(9):1624-1631 doi:10.1016/j.soilbio.2010.06.002
Jones CM, Hallin S (2010) Ecological and evolutionary factors underlying global and local assembly of denitrifier communities. Isme Journal 4(5):633-641 doi:10.1038/ismej.2009.152
Jones CM, Spor A, Brennan FP, Breuil MC, Bru D, Lemanceau P, Griffiths B, Hallin S, Philippot L (2014) Recently identified microbial guild mediates soil N2O sink capacity. Nat Climate Change 4(9):801-805 doi:10.1038/nclimate2301
Jones CM, Stres B, Rosenquist M, Hallin S (2008) Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. Molecular Biology and Evolution 25(9):1955-1966 doi:10.1093/molbev/msn146
Kaharabata SK, Drury CF, Priesack E, Desjardins RL, McKenney DJ, Tan CS, Reynolds D (2003) Comparing measured and Expert-N predicted N2O emissions from conventional till and no till corn treatments. Nut Cycl Agroeco 66(2):107-118 doi:10.1023/a:1023978830307
Kanehisa M (2002) The KEGG database. In Silico Sim Bio Pro 247:91-103
Kassambara A (2020) rstatix: Pipe-Friendly Framework for Basic Statistical Tests. Version 0.6.0, https://rpkgs.datanovia.com/rstatix/ edn
Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20(4):1160-1166 doi:10.1093/bib/bbx108
Kaurin A, Mihelic R, Kastelec D, Grcman H, Bru D, Philippot L, Suhadolc M (2018) Resilience of bacteria, archaea, fungi and N-cycling microbial guilds under plough and conservation tillage, to agricultural drought. Soil Biology & Biochemistry 120:233-245 doi:10.1016/j.soilbio.2018.02.007
Kim N, Riggins CW, Rodriguez-Zas S, Zabaloy MC, Villamil MB (2021) Long-term residue removal under tillage decreases *amoA*-nitrifiers and stimulates *nirS*-denitrifier groups in the soil. Appl Soil Ecol 157 doi:10.1016/j.apsoil.2020.103730
Lee J, Six J, King AP, Van Kessel C, Rolston DE (2006) Tillage and field scale controls on greenhouse gas emissions. J Env Qual 35(3):714-725 doi:10.2134/jeq2005.0337
Letunic I, Bork P (2019) Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nuc Acids Res 47(W1):W256-W259 doi:10.1093/nar/gkz239
Li D, Luo R, Liu CM, Leung CM, Ting HF, Sadakane K, Yamashita H, Lam TW (2016) MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102:3-11 doi:10.1016/j.ymeth.2016.02.020
Li F, Li M, Shi W, Li H, Sun Z, Gao Z (2017) Distinct distribution patterns of proteobacterial *nirK*- and *nirS*-type denitrifiers in the Yellow River estuary, China. Can J Micro 63(8):708-718 doi:10.1139/cjm-2017-0053
Li Y, Zhang Q, Cai Y, Yang Q, Chang SX (2020) Minimum tillage and residue retention increase soil microbial population size and diversity: Implications for conservation tillage. Sci Total Env 716 doi:10.1016/j.scitotenv.2020.137164
Liu C, Li LL, Xie JH, Coulter JA, Zhang RZ, Luo ZZ, Cai LQ, Wang LL, Gopalakrishnan S (2020) Soil bacterial diversity and potential functions are regulated by long-term conservation tillage and straw mulching. Microorganisms 8(6) doi:10.3390/microorganisms8060836

Liu H, Carvalhais LC, Crawford M, Dang YP, Dennis PG, Schenk PM (2016) Strategic tillage increased the relative abundance of *Acidobacteria* but did not impact on overall soil microbial properties of a 19-year no-till Solonetz. Bio Fert Soils 52(7):1021-1035 doi:10.1007/s00374-016-1138-0

McHugh TA, Schwartz E (2015) Changes in plant community composition and reduced precipitation have limited effects on the structure of soil bacterial and fungal communities present in a semiarid grassland. Plant Soil 388(1-2):175-186 doi:10.1007/s11104-014-2269-4

Melero S, Perez-de-Mora A, Manuel Murillo J, Buegger F, Kleinedam K, Kublik S, Vanderlinden K, Moreno F, Schloter M (2011) Denitrification in a vertisol under long-term tillage and no-tillage management in dryland agricultural systems: Key genes and potential rates. Appl Soil Ecol 47(3):221-225 doi:10.1016/j.apsoil.2010.12.003

Meyer F, Paarmann D, D'Souza M, Olson R, Glass EM, Kubal M, Paczian T, Rodriguez A, Stevens R, Wilke A, Wilkening J, Edwards RA (2008) The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. Bmc Bioinformatics 9:8 doi:10.1186/1471-2105-9-386

Naumoff DG, Dedysh SN (2012) Lateral gene transfer between the *Bacteroidetes* and *Acidobacteria*: The case of alpha-L-rhamnosidases. FEMS Lett 586(21):3843-3851 doi:10.1016/j.febslet.2012.09.005

Ogle D, Wheeler P, Dinno A (2020) FSA: Simple Fisheries Stock Assessment Methods. Version 0.8.30 edn

Papadopoulos JS, Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics 23(9):1073-9 doi:10.1093/bioinformatics/btm076

Parks DH, Tyson GW, Hugenholtz P, Beiko RG (2014) STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30(21):3123-3124 doi:10.1093/bioinformatics/btu494

Pastorelli R, Vignozzi N, Landi S, Piccolo R, Orsini R, Seddaiu G, Roggero PP, Pagliai M (2013) Consequences on macroporosity and bacterial diversity of adopting a no-tillage farming system in a clayish soil of Central Italy. Soil Bio Biochem 66:78-93 doi:10.1016/j.soilbio.2013.06.015

Peixoto RS, Coutinho HLC, Madari B, Machado PLOA, Rumjanek NG, Van Elsas JD, Seldin L, Rosado AS (2006) Soil aggregation and bacterial community structure as affected by tillage and cover cropping in the Brazilian Cerrados. Soil Till Res 90(1-2):16-28 doi:10.1016/j.still.2005.08.001

Philippot L, Andert J, Jones CM, Bru D, Hallin S (2011) Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N2O emissions from soil. Glob Change Bio 17(3):1497-1504 doi:10.1111/j.1365-2486.2010.02334.x

Philippot L, Hallin S, Schloter M (2007) Ecology of denitrifying prokaryotes in agricultural soil. In: Sparks DL (ed) Advances in Agronomy, Vol 96. Advances in Agronomy, vol 96. Elsevier Academic Press Inc, San Diego, pp 249-305

Philippot L, Spor A, Henault C, Bru D, Bizouard F, Jones CM, Sarr A, Maron P-A (2013) Loss in microbial diversity affects nitrogen cycling in soil. ISME J 7(8):1609-1619 doi:10.1038/ismej.2013.34

Prosser JI, Nicol GW (2012) Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends in Microbiology 20(11):523-531 doi:10.1016/j.tim.2012.08.001

Pruitt KD, Tatusova T, Maglott DR (2005) NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res 33:D501-D504 doi:10.1093/nar/gki025

Puerta VL, Six J, Wittwer R, van der Heijden M, Pereira EIP (2019) Comparable bacterial-mediated nitrogen supply and losses under organic reduced tillage and conventional intensive tillage. Eur J Soil Sci 95 doi:10.1016/j.ejsobi.2019.103121

R_Core_Team (2018) R: A language and environment for statistical computing. vol URL https://www.R-project.org/, Vienna, Austria.

Rampelotto PH, Ferreira AdS, Muller Barboza AD, Wurdig Roesch LF (2013) Changes in diversity, abundance, and structure of soil bacterial communities in Brazilian Savanna under different land use systems. Micro Ecol 66(3):593-607 doi:10.1007/s00248-013-0235-y

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous Oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st Century. Science 326(5949):123-125 doi:10.1126/science.1176985

RStudio_Team (2020) RStudio: Integrated Development for R. RStudio, PBC. Boston, MA pURL http://www.rstudio.com/

Rudy AP, Harris CK, Thomas BJ, Worosz MR, Kaplan SC, O'Donnell EC (2008) The political ecology of Southwest Michigan Agriculture, 1837-200. In: Redman CL, Foster DR (eds) Agrarian landscapes in transition. Oxford University Press, New York

Sait M, Davis KER, Janssen PH (2006) Effect of pH on isolation and distribution of members of *subdivision 1* of the phylum *Acidobacteria* occurring in soil. Appl Env Micro 72(3):1852-1857 doi:10.1128/aem.72.3.1852-1857.2006

Sellstedt A, Richau KH (2013) Aspects of nitrogen-fixing *Actinobacteria*, in particular free-living and symbiotic *Frankia*. FEMS Micro Lett 342(2):179-186 doi:10.1111/1574-6968.12116

Sherrill-Mix S (2009) taxonomizr: Functions to Work with NCBI Accessions and Taxonomy., R package Version 0.5.3 edn

Smith CR, Blair PL, Boyd C, Cody B, Hazel A, Hedrick A, Kathuria H, Khurana P, Kramer B, Muterspaw K, Peck C, Sells E, Skinner J, Tegeler C, Wolfe Z (2016) Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. Ecol Evol 6(22):8075-8084 doi:10.1002/ece3.2553

Syakila A, Kroeze C (2011) The global nitrous oxide budget revisited. Greenhouse Gas Meas Man 1:17-26

Tatti E, Goyer C, Burton DL, Wertz S, Zebarth BJ, Chantigny M, Filion M (2015) Tillage management and seasonal effects on denitrifier community abundance, gene expression and structure over winter. Micro Ecol 70(3):795-808 doi:10.1007/s00248-015-0591-x

Team R (2020) RStudio: Integrated Development for R. RStudio, PBC. Boston, MA

Team RC (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Thelusmond JR, Strathmann TJ, Cupples AM (2019) Carbamazepine, triclocarban and triclosan biodegradation and the phylotypes and functional genes associated with xenobiotic degradation in four agricultural soils. Sci Total Environ 657:1138-1149 doi:10.1016/j.scitotenv.2018.12.145

Wang J, Zou J (2020) No-till increases soil denitrification via its positive effects on the activity and abundance of the denitrifying community. Soil Bio Biochem 142 doi:10.1016/j.soilbio.2020.107706

Yin C, Fan F, Song A, Li Z, Yu W, Liang Y (2014) Different denitrification potential of aquic brown soil in Northeast China under inorganic and organic fertilization accompanied by distinct changes of *nirS*- and *nirK*-denitrifying bacterial community. Eur J Soil Bio 65:47-56 doi:10.1016/j.ejsobi.2014.09.003

Yin CT, Mueth N, Hulbert S, Schlatter D, Paulitz TC, Schroeder K, Prescott A, Dhingra A (2017) Bacterial communities on wheat grown under long-term conventional tillage and no-till in the Pacific Northwest of the United States. Phytobiomes J 1(2):83-90 doi:10.1094/pbiomes-09-16-0008-r

Yuan Q, Liu P, Lu Y (2012) Differential responses of *nirK*- and *nirS*-carrying bacteria to denitrifying conditions in the anoxic rice field soil. Env Micro Rep 4(1):113-122 doi:10.1111/j.1758-2229.2011.00311.x

Zhang L, Zeng G, Zhang J, Chen Y, Yu M, Lu L, Li H, Zhu Y, Yuan Y, Huang A, He L (2015a) Response of denitrifying genes coding for nitrite (*nirK* or *nirS*) and nitrous oxide (*nosZ*) reductases to different

physico-chemical parameters during agricultural waste composting. Appl Micro Biotec 99(9):4059-4070 doi:10.1007/s00253-014-6293-3
Zhang S, Li Q, Lü Y, Sun X, Jia S, Zhang X, Liang W (2015b) Conservation tillage positively influences the microflora and microfauna in the black soil of Northeast China. Soil Till Res 149:46 - 52 doi:https://doi.org/10.1016/j.still.2015.01.001
Zhang W, Wu XK, Liu GX, Dong ZB, Zhang GS, Chen T, Dyson PJ (2014) Tag-encoded pyrosequencing analysis of bacterial diversity within different alpine grassland ecosystems of the Qinghai-Tibet Plateau, China. Environ Earth Sci 72(3):779-786 doi:10.1007/s12665-013-3001-z
Zumft WG (1997) Cell biology and molecular basis of denitrification. Micro Mole Bio Rev 61(4):533-+ doi:10.1128/.61.4.533-616.1997

Table and Figure Legends

Table 1. Summary of the KBS agricultural management approaches for the four soils examined.

Figure 1. Box and whisker plot of relative abundance of genes (A) and Principle Component Analysis of the genes across the four management practices (with or without pharmaceuticals added) (B).

Figure 2. Average relative abundance values (%, as determined by DIAMOND) for each soil (n=6) with standard deviations illustrated with the bars. Values that are statistically significantly different (ANOVA or Kruskal-Wallis test, p<0.05) are shown with different letters. Letters are missing for *nosZ* because the statistical assumptions were not met for either test (unequal variance). Note, all y-axis have different scales.

Figure 3. Scatterplots comparing relative abundance values of all genes across all samples. Correlations that were statistically significant (Spearman's rank test, p<0.05) are boxed in red.

Figure 4. Average index diversity values and richness estimators for each soil (as determined by EstimateS, n=6) with standard deviations. Values that are statistically significantly different (ANOVA or Kruskal-Wallis test, p<0.05) are shown with different letters. In some cases, letters are missing because the statistical assumptions were not met for either test. Note, the scale on the y-axis differs between graphs.

Figure 5. Extended error bars illustrating the differences between each treatment compared to the other three treatments for the genes associated with nitrogen metabolism (as defined by the KEGG hierarchy). The data (generated in MG-RAST, six metagenomes for each soil) were analyzed using STAMP with the two group analysis option (each soil compared to the other three soils) and Welch's two sided t-test (p<0.05).

Figure 6. Phylotypes enriched in each soil associated with the genes *napA, narG, nirA* and *nirB* at the level of class. All y-axis have the same scale.

Figure 7. Phylotypes enriched in each soil associated with the genes *nirK, nirS, norB* and *nosZ* at the level of class. Note, the y-axis scales on each are different. There was minimal enrichment for any soil for *nifH*, therefore no graph was generated.

Figure 8. Neighbour-Joining phylogenetic trees of fifty most abundant sequences in each soil associated with the genes *napA*, *narG*, *nifH*, *nirA*, *nirB*, *nirK, nirS, norB* and *nosZ*. Note, the bar charts illustrate the relative abundance (%) of the sequences in each soil. The three most abundant sequences are highlighted in yellow.

The legends for the tables and figures are listed after the references in the manuscript document (as requested by the journal)

Table 1.

Conventional	This system is practiced by most farmers in this region. Tilled corn–soybean–winter wheat (c–s–w) rotation; standard chemical inputs, chisel-plowed, no cover crops, no manure or compost
No-till	No-till c–s–w rotation; standard chemical inputs, permanent no-till, no cover crops, no manure or compost
Reduced Input	Biologically based c–s–w rotation managed to reduce synthetic chemical inputs; chisel-plowed, winter cover crop of red clover or annual rye, no manure or compost
Biologically Based	Biologically based c–s–w rotation managed without synthetic chemical inputs; chisel-plowed, mechanical weed control, winter cover crop of red clover or annual rye, no manure or compost; USDA-certified organic

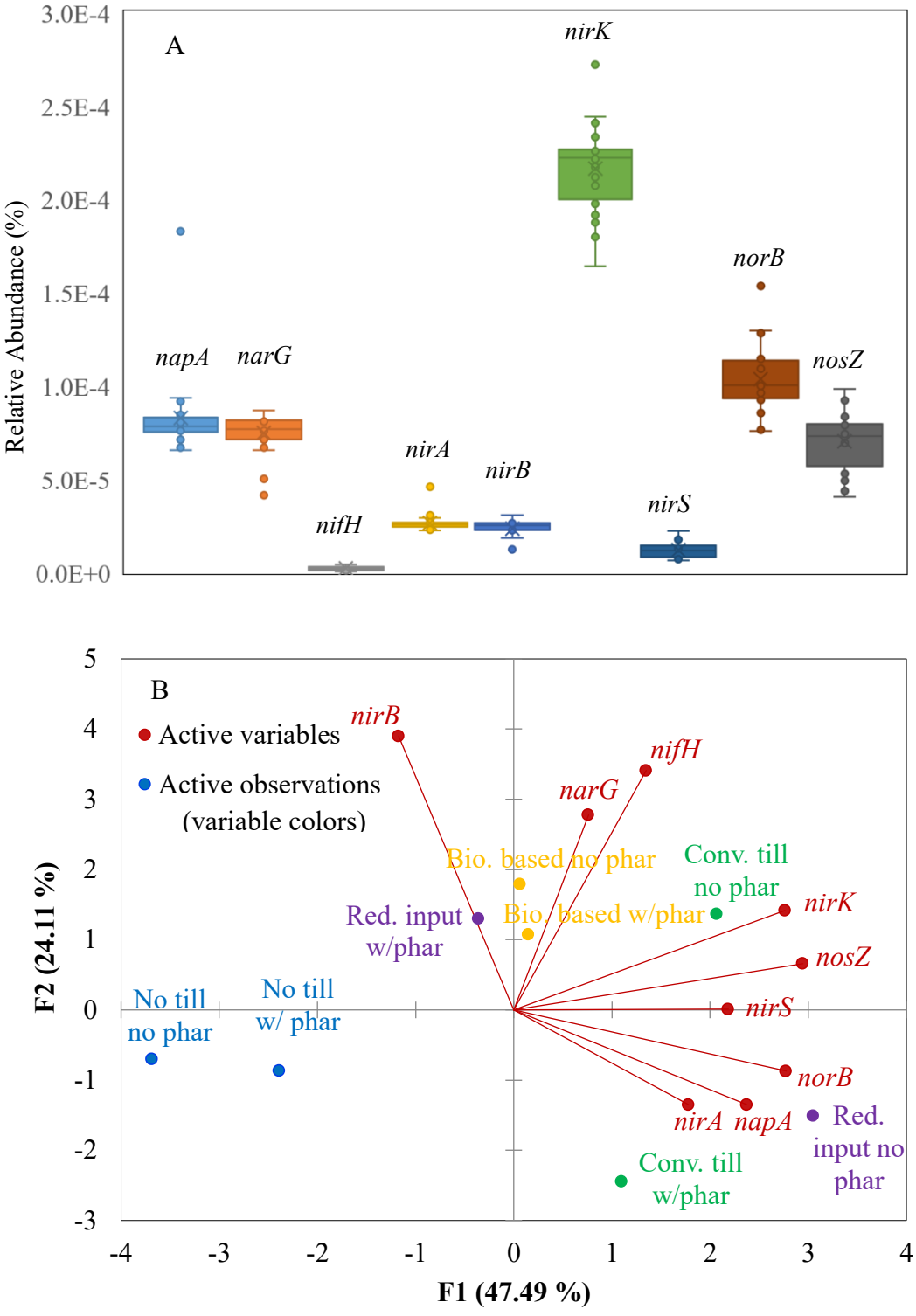

A
Relative Abundance (%)
3.0E-4
2.5E-4
2.0E-4
1.5E-4
1.0E-4
5.0E-5
0.0E+0
napA
narG
nifH
nirA
nirB
nirK
nirS
norB
nosZ
B
Active variables
Active observations
(variable colors)
nirB
nifH
narG
Bio. based no phar
Conv. till
no phar
Red. input
w/phar
Bio. based w/phar
nirK
nosZ
nirS
No till
no phar
No till
w/ phar
norB
nirA
napA
Red.
input no
phar
Conv. till
w/phar
F2 (24.11 %)
F1 (47.49 %)

Figure 1.

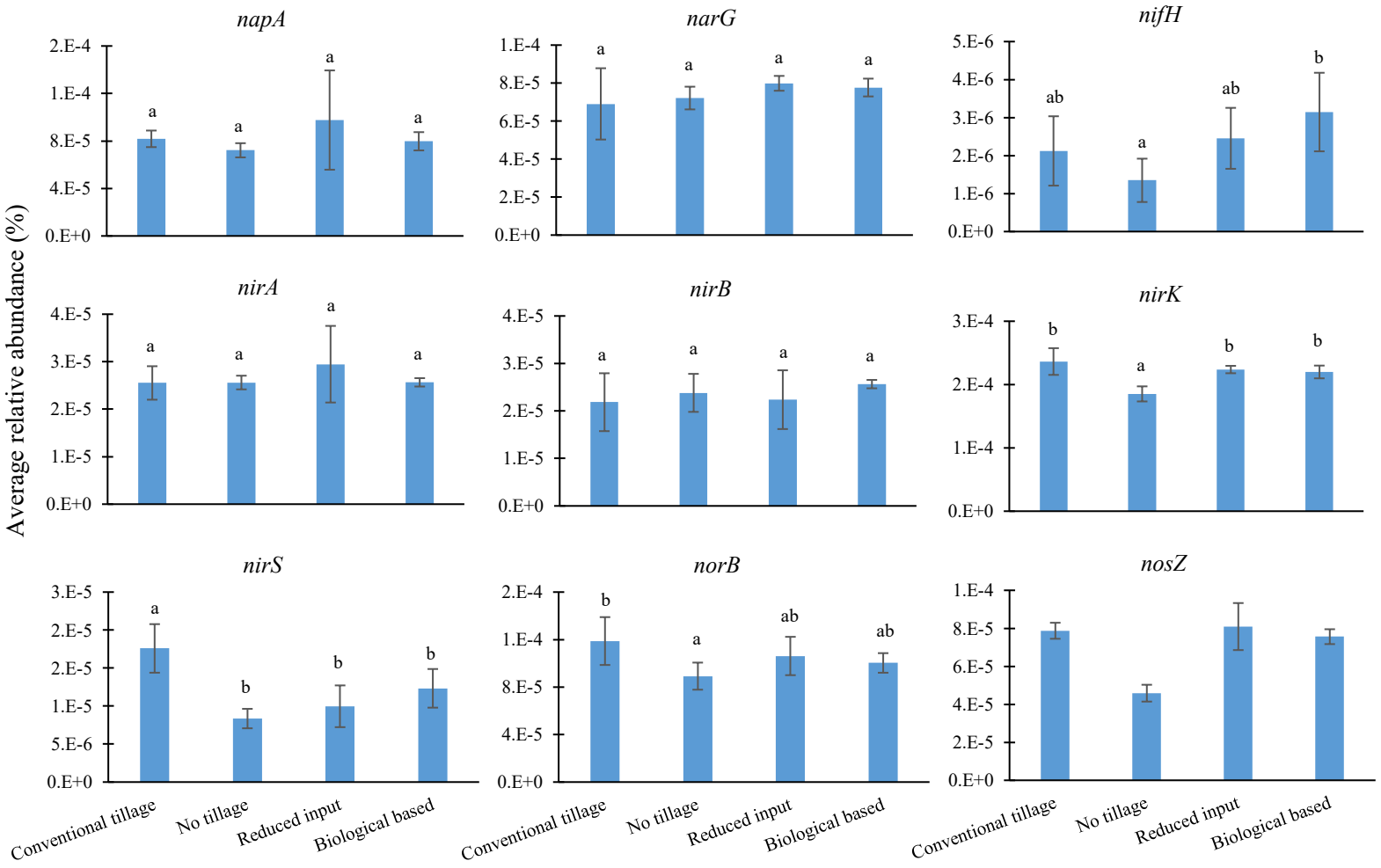

napA
narG
nifH
nirA
nirB
nirK
nirS
norB
nosZ
Average relative abundance (%)
Conventional tillage
No tillage
Reduced input
Biological based

Figure 2.

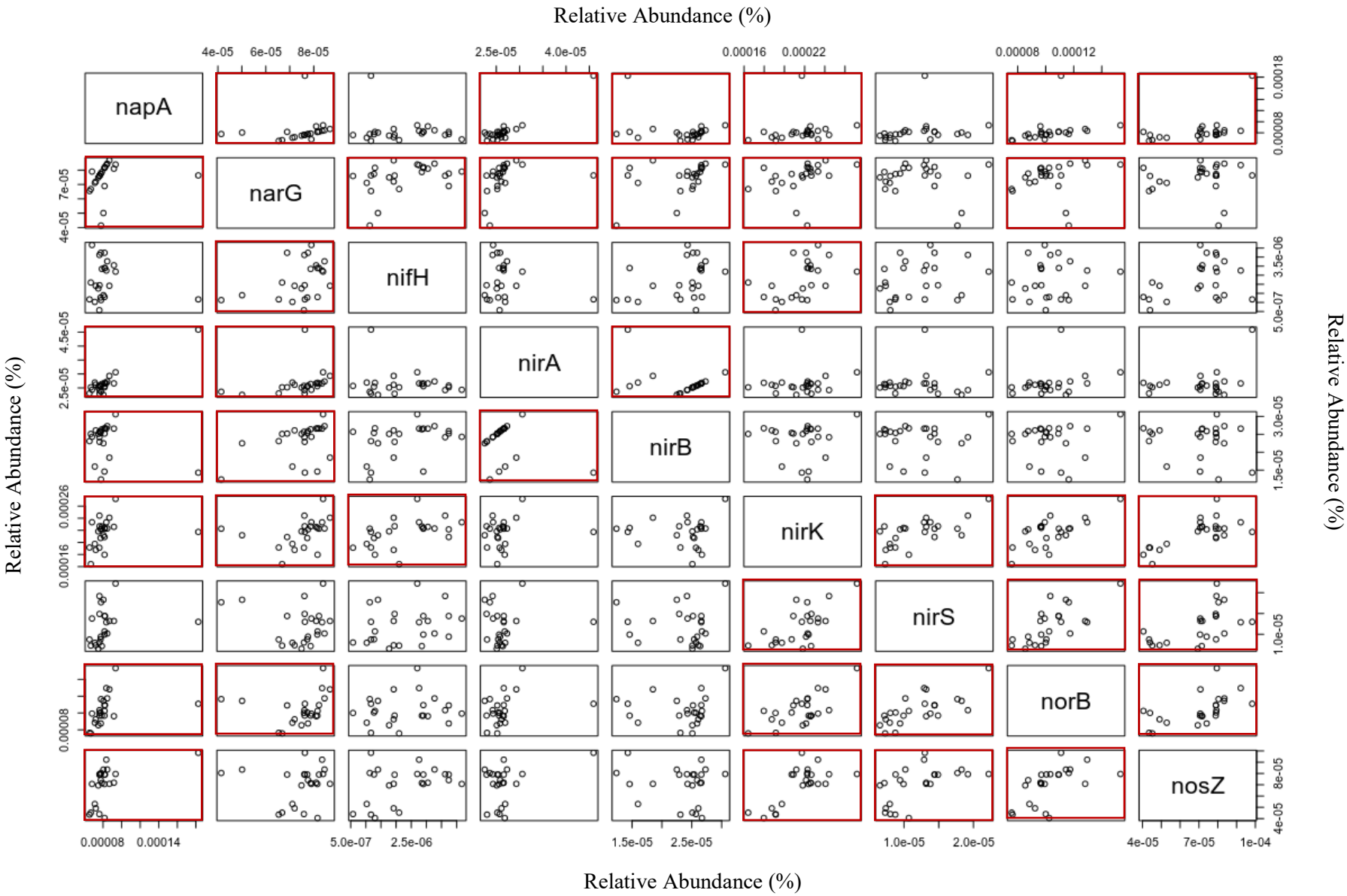

Relative Abundance (%)
napA
narG
nifH
nirA
nirB
nirK
nirS
norB
nosZ
Relative Abundance (%)
Relative Abundance (%)
Relative Abundance (%)

Figure 3.

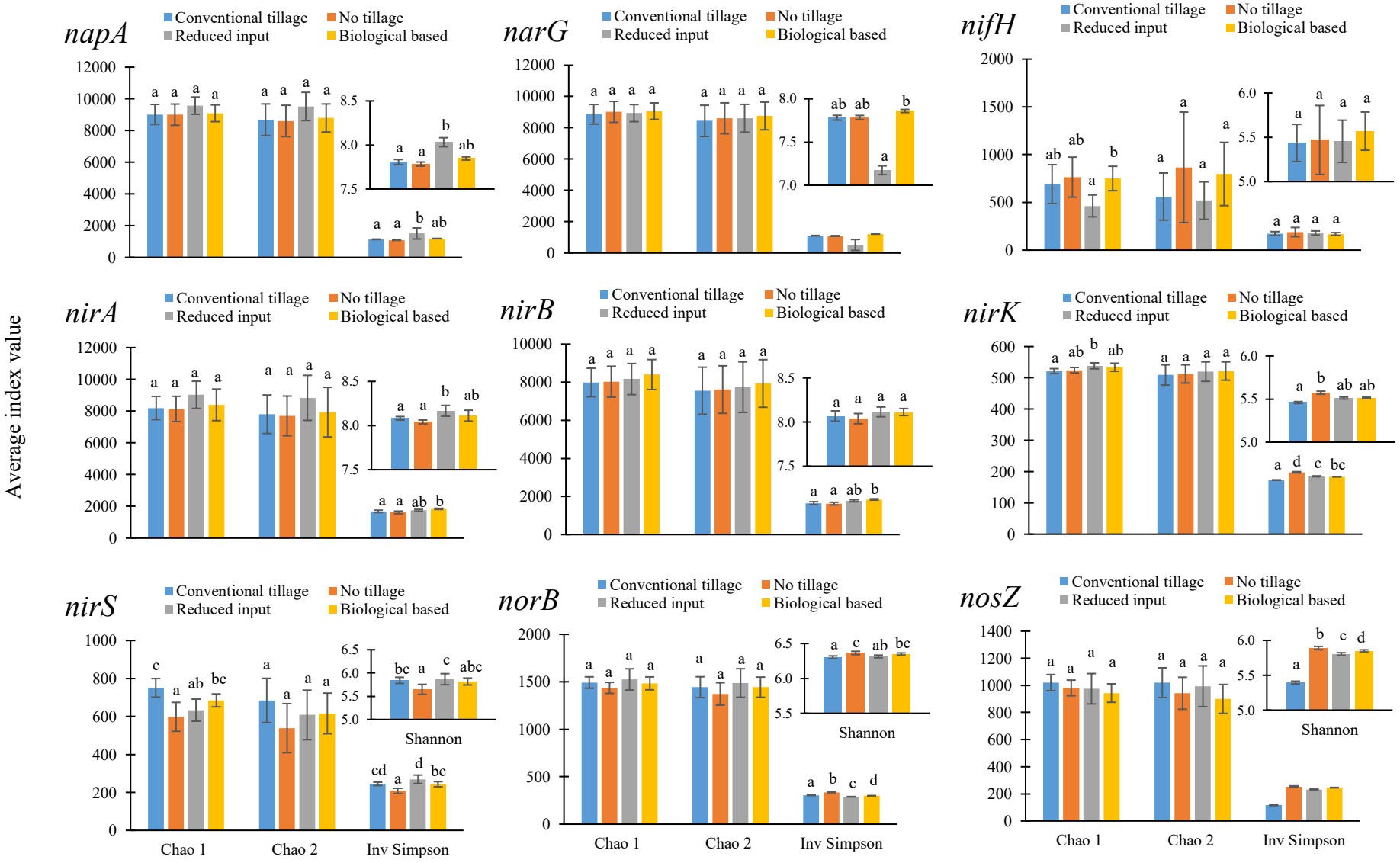
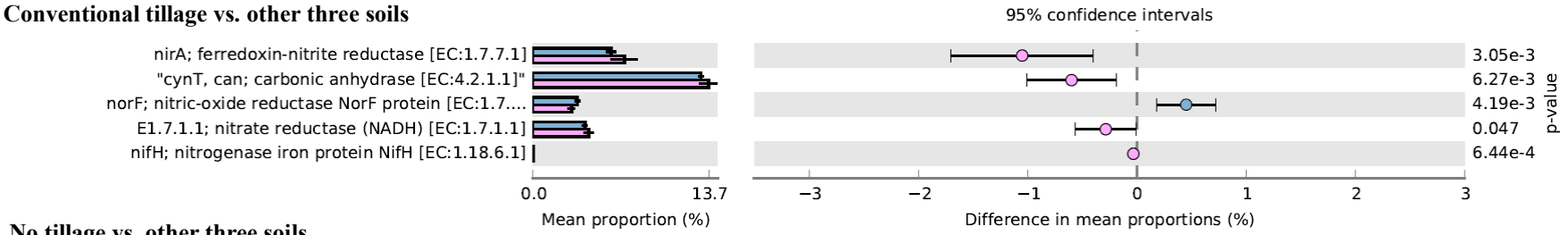
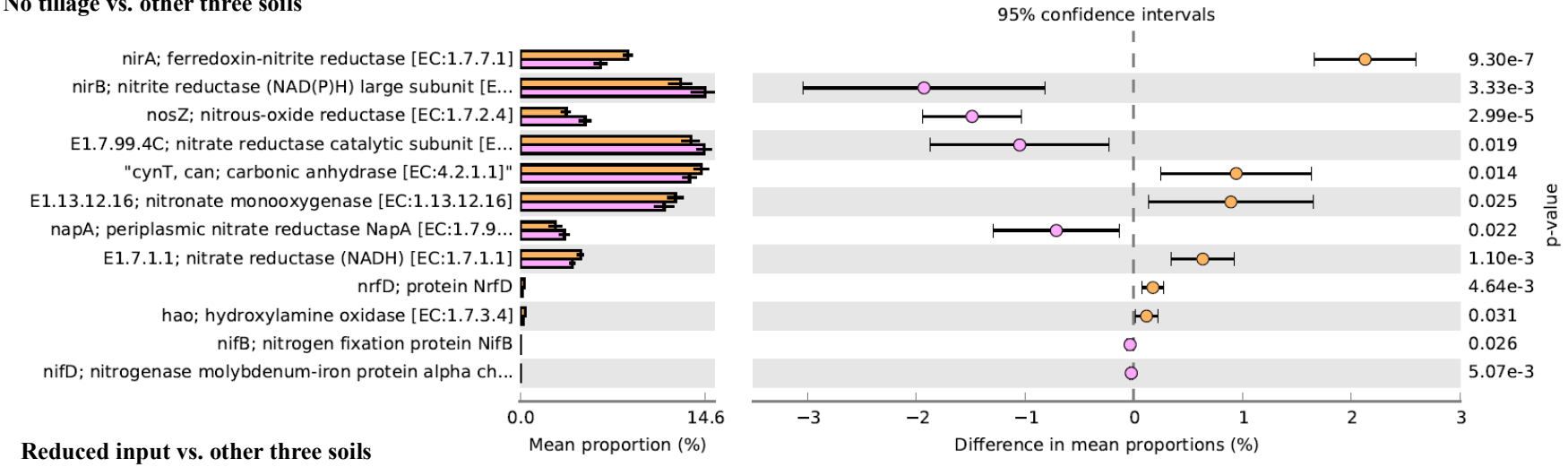

napA
narG
nifH
nirA
nirB
nirK
nirS
norB
nosZ
Conventional tillage
No tillage
Reduced input
Biological based
Average index value
Chao 1
Chao 2
Inv Simpson
Shannon

Figure 4.

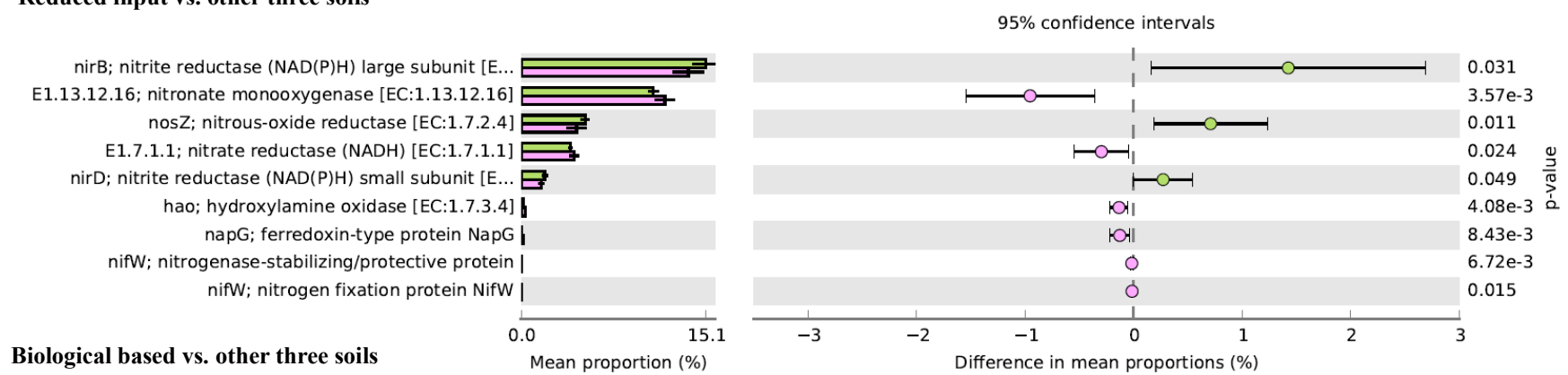
Conventional tillage vs. other three soils



No tillage vs. other three soils



Reduced input vs. other three soils



Biological based vs. other three soils

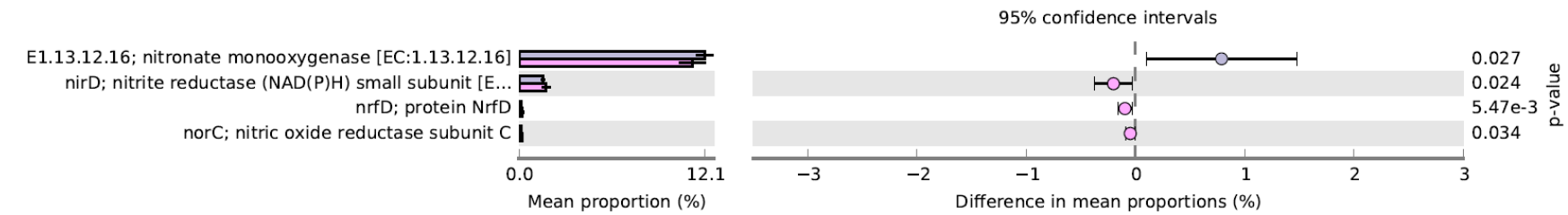


Figure 5.

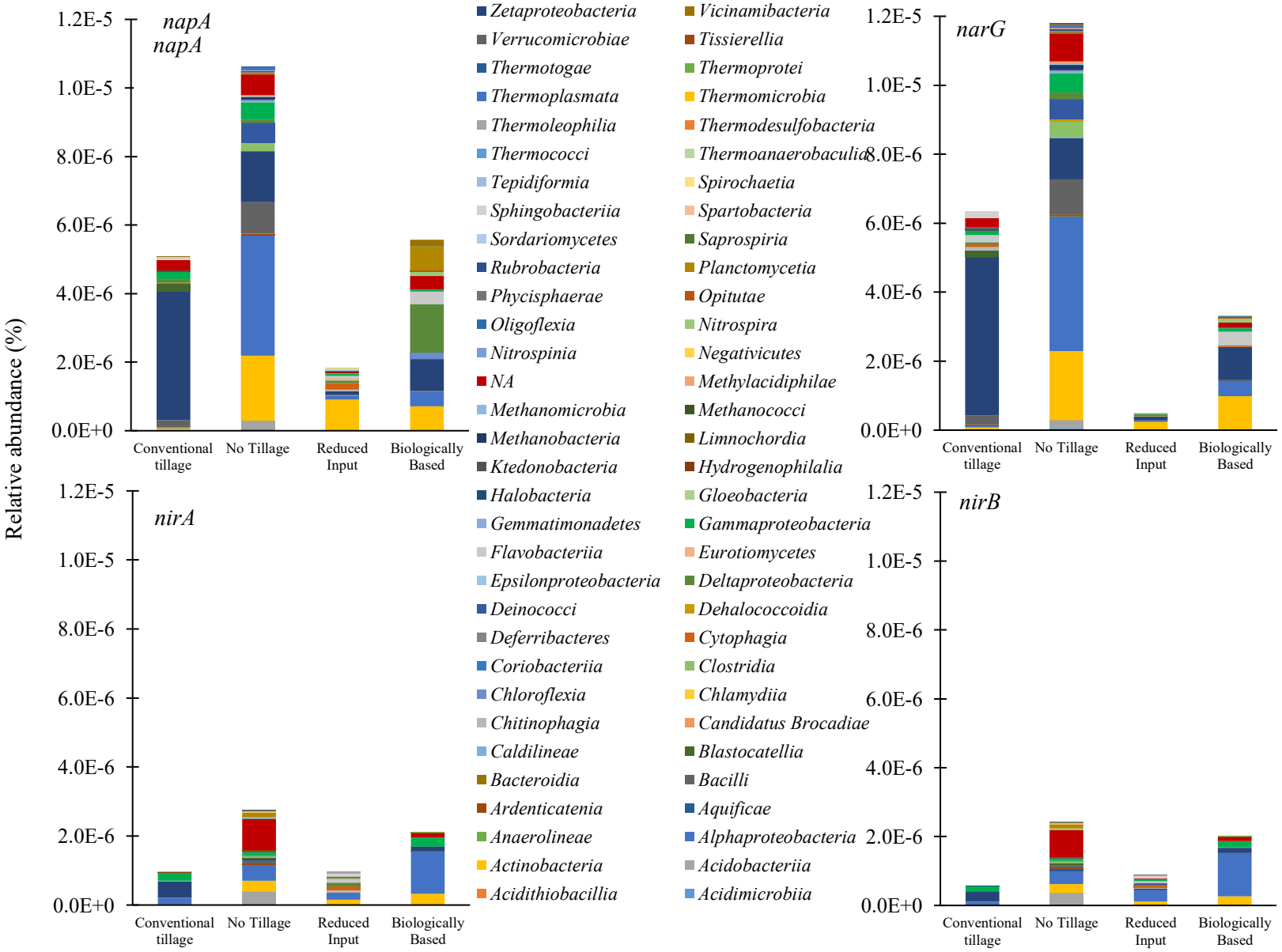

napA
napA
narG
nirA
nirB
Relative abundance (%)
1.2E-5
1.0E-5
8.0E-6
6.0E-6
4.0E-6
2.0E-6
0.0E+0
Conventional tillage
No Tillage
Reduced Input
Biologically Based
Zetaproteobacteria
Verrucomicrobiae
Thermotogae
Thermoplasmata
Thermoleophilia
Thermococci
Tepidiformia
Sphingobacteriia
Sordariomycetes
Rubrobacteria
Phycisphaerae
Oligoflexia
Nitrospinia
NA
Methanomicrobia
Methanobacteria
Ktedonobacteria
Halobacteria
Gemmatimonadetes
Flavobacteriia
Epsilonproteobacteria
Deinococci
Deferribacteres
Coriobacteriia
Chloroflexia
Chitinophagia
Caldilineae
Bacteroidia
Ardenticatenia
Anaerolineae
Actinobacteria
Acidithiobacillia
Vicinamibacteria
Tissierellia
Thermoprotei
Thermomicrobia
Thermodesulfobacteria
Thermoanaerobaculia
Spirochaetia
Spartobacteria
Saprospiria
Planctomycetia
Opitutae
Nitrospira
Negativicutes
Methylacidiphilae
Methanococci
Limnochordia
Hydrogenophilalia
Gloeobacteria
Gammaproteobacteria
Eurotiomycetes
Deltaproteobacteria
Dehalococcoidia
Cytophagia
Clostridia
Chlamydiia
Candidatus Brocadiae
Blastocatellia
Bacilli
Aquificae
Alphaproteobacteria
Acidobacteriia
Acidimicrobiia

Figure 6.

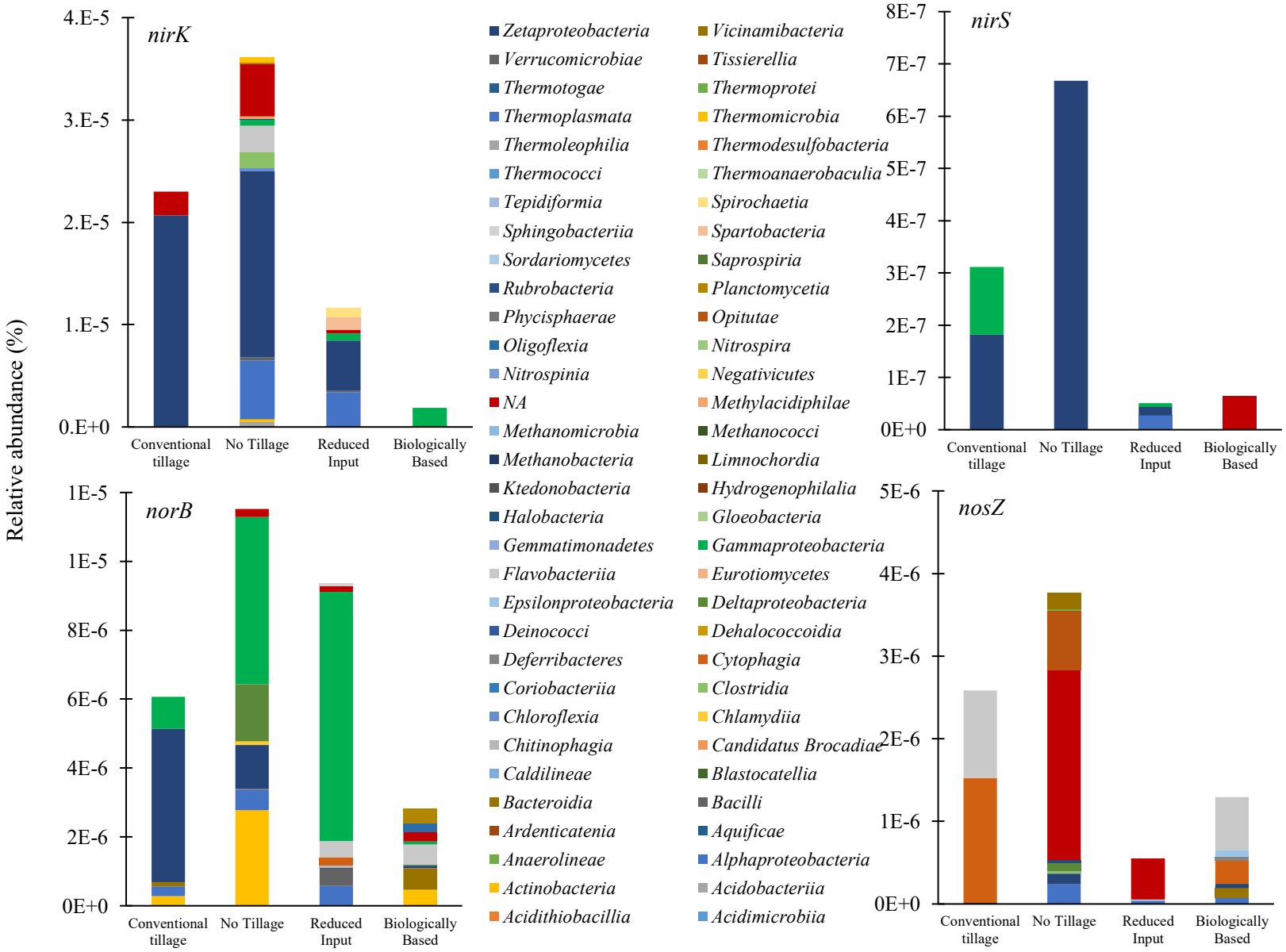
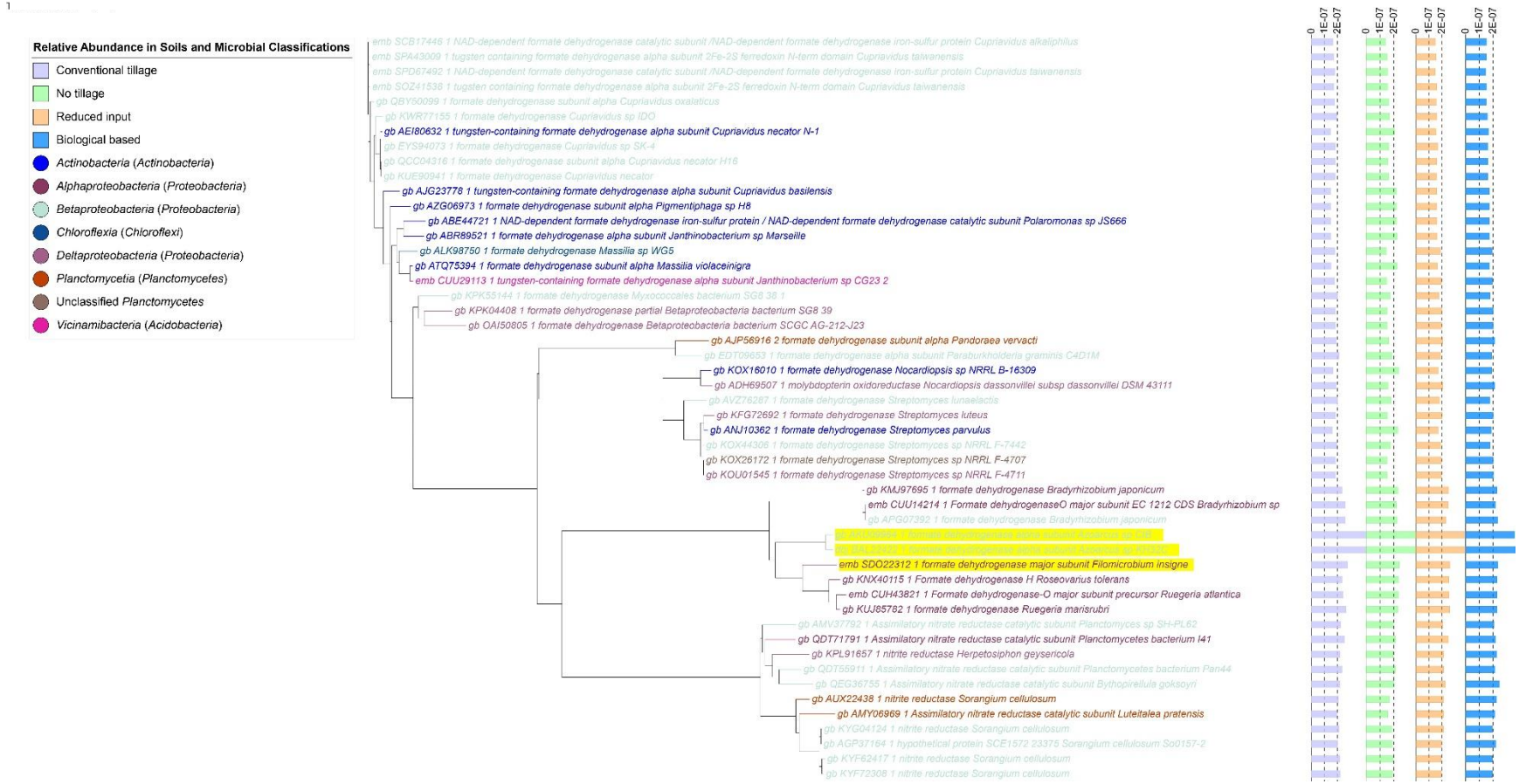

Relative abundance (%)
nirK
4.E-5
3.E-5
2.E-5
1.E-5
0.E+0
Conventional tillage
No Tillage
Reduced Input
Biologically Based
nirS
8E-7
7E-7
6E-7
5E-7
4E-7
3E-7
2E-7
1E-7
0E+0
norB
1E-5
8E-6
6E-6
4E-6
2E-6
0E+0
nosZ
5E-6
4E-6
3E-6
2E-6
1E-6
0E+0
Zetaproteobacteria
Verrucomicrobiae
Thermotogae
Thermoplasmata
Thermoleophilia
Thermococci
Tepidiformia
Sphingobacteriia
Sordariomycetes
Rubrobacteria
Phycisphaerae
Oligoflexia
Nitrospinia
NA
Methanomicrobia
Methanobacteria
Ktedonobacteria
Halobacteria
Gemmatimonadetes
Flavobacteriia
Epsilonproteobacteria
Deinococci
Deferribacteres
Coriobacteriia
Chloroflexia
Chitinophagia
Caldilineae
Bacteroidia
Ardenticatenia
Anaerolineae
Actinobacteria
Acidithiobacillia
Vicinamibacteria
Tissierellia
Thermoprotei
Thermomicrobia
Thermodesulfobacteria
Thermoanaerobaculia
Spirochaetia
Spartobacteria
Saprospiria
Planctomycetia
Opitutae
Nitrospira
Negativicutes
Methylacidiphilae
Methanococci
Limnochordia
Hydrogenophilalia
Gloeobacteria
Gammaproteobacteria
Eurotiomycetes
Deltaproteobacteria
Dehalococcoidia
Cytophagia
Clostridia
Chlamydiia
Candidatus Brocadiae
Blastocatellia
Bacilli
Aquificae
Alphaproteobacteria
Acidobacteriia
Acidimicrobiia

Figure 7.

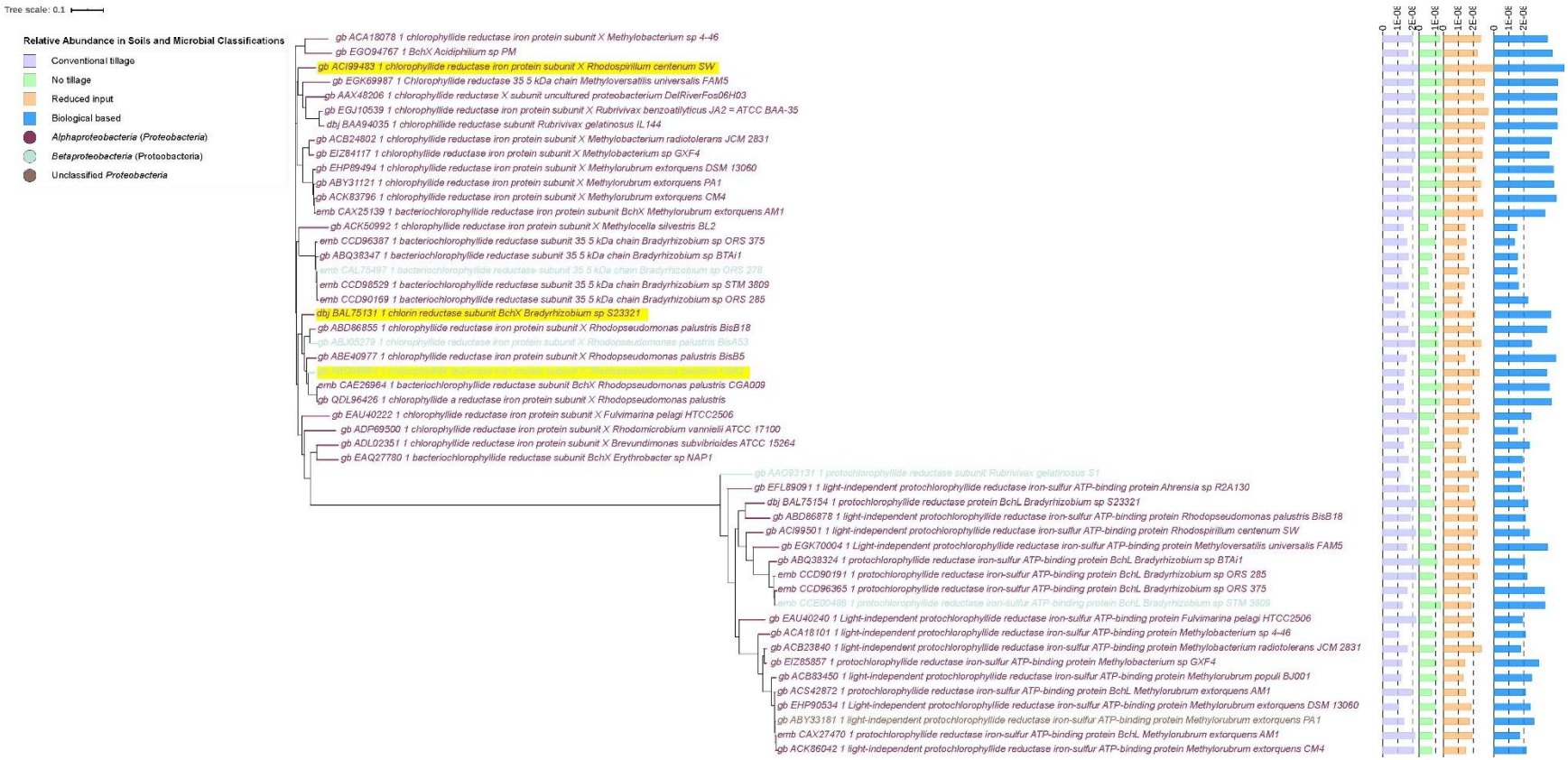
(1) *napA*


Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological based
Actinobacteria (Actinobacteria)
Alphaproteobacteria (Proteobacteria)
Betaproteobacteria (Proteobacteria)
Chloroflexia (Chloroflexi)
Deltaproteobacteria (Proteobacteria)
Planctomycetia (Planctomycetes)
Unclassified Planctomycetes
Vicinamibacteria (Acidobacteria)
emb SCB17446 1 NAD-dependent formate dehydrogenase catalytic subunit /NAD-dependent formate dehydrogenase iron-sulfur protein Cupriavidus alkaliphilus
emb SPA43009 1 tungsten containing formate dehydrogenase alpha subunit 2Fe-2S ferredoxin N-term domain Cupriavidus taiwanensis
emb SPD67492 1 NAD-dependent formate dehydrogenase catalytic subunit /NAD-dependent formate dehydrogenase iron-sulfur protein Cupriavidus taiwanensis
emb SOZ41538 1 tungsten containing formate dehydrogenase alpha subunit 2Fe-2S ferredoxin N-term domain Cupriavidus taiwanensis
gb QBY50099 1 formate dehydrogenase subunit alpha Cupriavidus oxalaticus
gb KWR77155 1 formate dehydrogenase Cupriavidus sp IDO
gb AEI80632 1 tungsten-containing formate dehydrogenase alpha subunit Cupriavidus necator N-1
gb EYS94073 1 formate dehydrogenase Cupriavidus sp SK-4
gb QCC04316 1 formate dehydrogenase subunit alpha Cupriavidus necator H16
gb KUE90941 1 formate dehydrogenase Cupriavidus necator
gb AJG23778 1 tungsten-containing formate dehydrogenase alpha subunit Cupriavidus basilensis
gb AZG06973 1 formate dehydrogenase subunit alpha Pigmentiphaga sp H8
gb ABE44721 1 NAD-dependent formate dehydrogenase iron-sulfur protein / NAD-dependent formate dehydrogenase catalytic subunit Polaromonas sp JS666
gb ABR89521 1 formate dehydrogenase alpha subunit Janthinobacterium sp Marseille
gb ALK98750 1 formate dehydrogenase Massilia sp WG5
gb ATQ75394 1 formate dehydrogenase subunit alpha Massilia violaceinigra
emb CUU29113 1 tungsten-containing formate dehydrogenase alpha subunit Janthinobacterium sp CG23 2
gb KPK55144 1 formate dehydrogenase Myxococcales bacterium SG8 38 1
gb KPK04408 1 formate dehydrogenase partial Betaproteobacteria bacterium SG8 39
gb OAI50805 1 formate dehydrogenase Betaproteobacteria bacterium SCGC AG-212-J23
gb AJP56916 2 formate dehydrogenase subunit alpha Pandoraea vervacti
gb EDT09653 1 formate dehydrogenase alpha subunit Paraburkholderia graminis C4D1M
gb KOX16010 1 formate dehydrogenase Nocardiopsis sp NRRL B-16309
gb ADH69507 1 molybdopterin oxidoreductase Nocardiopsis dassonvillei subsp dassonvillei DSM 43111
gb AVZ76287 1 formate dehydrogenase Streptomyces lunaelactis
gb KFG72692 1 formate dehydrogenase Streptomyces luteus
gb ANJ10362 1 formate dehydrogenase Streptomyces parvulus
gb KOX44306 1 formate dehydrogenase Streptomyces sp NRRL F-7442
gb KOX26172 1 formate dehydrogenase Streptomyces sp NRRL F-4707
gb KOU01545 1 formate dehydrogenase Streptomyces sp NRRL F-4711
gb KMJ97695 1 formate dehydrogenase Bradyrhizobium japonicum
emb CUU14214 1 Formate dehydrogenaseO major subunit EC 1212 CDS Bradyrhizobium sp
gb APG07392 1 formate dehydrogenase Bradyrhizobium japonicum
emb SDO22312 1 formate dehydrogenase major subunit Filomicrobium insigne
gb KNX40115 1 Formate dehydrogenase H Roseovarius tolerans
emb CUH43821 1 Formate dehydrogenase-O major subunit precursor Ruegeria atlantica
gb KUJ85782 1 formate dehydrogenase Ruegeria marisrubri
gb AMV37792 1 Assimilatory nitrate reductase catalytic subunit Planctomyces sp SH-PL62
gb QDT71791 1 Assimilatory nitrate reductase catalytic subunit Planctomycetes bacterium I41
gb KPL91657 1 nitrite reductase Herpetosiphon geysericola
gb QDT55911 1 Assimilatory nitrate reductase catalytic subunit Planctomycetes bacterium Pan44
gb QEG36755 1 Assimilatory nitrate reductase catalytic subunit Bythopirellula goksoyri
gb AUX22438 1 nitrite reductase Sorangium cellulosum
gb AMY06969 1 Assimilatory nitrate reductase catalytic subunit Luteitalea pratensis
gb KYG04124 1 nitrite reductase Sorangium cellulosum
gb AGP37164 1 hypothetical protein SCE1572 23375 Sorangium cellulosum So0157-2
gb KYF62417 1 nitrite reductase Sorangium cellulosum
gb KYF72308 1 nitrite reductase Sorangium cellulosum
0
1E-07
2E-07

(2) *narG*


Tree scale: 0.1
Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological based
Actinobacteria (Actinobacteria)
Alphaproteobacteria (Proteobacteria)
Betaproteobacteria (Proteobacteria)
Chloroflexia (Chloroflexia)
Deltaproteobacteria (Proteobacteria)
Planctomycetia (Planctomycetes)
Unclassified Planctomycetes
Vicinamibacteria (Acidobacteria)
gb EON20614 1 formate dehydrogenase subunit alpha Cupriavidus sp GA3-3
gb AEI80632 1 tungsten-containing formate dehydrogenase alpha subunit Cupriavidus necator N-1
gb EYS94073 1 formate dehydrogenase Cupriavidus sp SK-4
gb QCC04316 1 formate dehydrogenase subunit alpha Cupriavidus necator H16
gb AGW94307 1 formate dehydrogenase subunit alpha Ralstonia pickettii DTP0602
gb KWR77155 1 formate dehydrogenase Cupriavidus sp IDO
gb QBY50099 1 formate dehydrogenase subunit alpha Cupriavidus oxalaticus
emb SPK74491 1 Tugsten containing formate dehydrogenase alpha subunit 2Fe-2S ferredoxin N-term domain Cupriavidus taiwanensis
emb SPD57701 1 gi 1442878137 NAD-dependent formate dehydrogenase catalytic subunit /NAD-dependent formate dehydrogenase iron-sulfur protein plasmid Cupriavidus taiwanen
emb SPA55920 1 tugsten containing formate dehydrogenase alpha subunit 2Fe-2S ferredoxin N-term domain Cupriavidus taiwanensis
gb AMR81091 1 formate dehydrogenase Cupriavidus nantongensis
emb SPD67492 1 NAD-dependent formate dehydrogenase catalytic subunit /NAD-dependent formate dehydrogenase iron-sulfur protein Cupriavidus taiwanensis
gb KDP87972 1 formate dehydrogenase Cupriavidus sp SK-3
gb AZG06973 1 formate dehydrogenase subunit alpha Pigmentiphaga sp H8
gb ABR89521 1 formate dehydrogenase alpha subunit Janthinobacterium sp Marseille
gb KQQ87784 1 formate dehydrogenase Massilia sp Leaf139
gb ALK98750 1 formate dehydrogenase Massilia sp WG5
gb KPK55144 1 formate dehydrogenase Myxococcales bacterium SG8 38 1
gb KPK04408 1 formate dehydrogenase partial Betaproteobacteria bacterium SG8 39
gb OAI50805 1 formate dehydrogenase Betaproteobacteria bacterium SCGC AG-212-J23
gb AJP56916 2 formate dehydrogenase subunit alpha Pandoraea vervacti
gb EDT09653 1 formate dehydrogenase alpha subunit Paraburkholderia graminis C4D1M
gb KOX16010 1 formate dehydrogenase Nocardiopsis sp NRRL B-16309
gb ADH69507 1 molybdopterin oxidoreductase Nocardiopsis dassonvillei subsp dassonvillei DSM 43111
gb AVZ76287 1 formate dehydrogenase Streptomyces lunaelactis
gb KFG72692 1 formate dehydrogenase Streptomyces luteus
gb ANJ10362 1 formate dehydrogenase Streptomyces parvulus
gb KOX44306 1 formate dehydrogenase Streptomyces sp NRRL F-7442
gb KOX26172 1 formate dehydrogenase Streptomyces sp NRRL F-4707
gb KOU01545 1 formate dehydrogenase Streptomyces sp NRRL F-4711
gb KMJ97695 1 formate dehydrogenase Bradyrhizobium japonicum
emb CUU14214 1 Formate dehydrogenaseO major subunit EC 1212 CDS Bradyrhizobium sp
gb APG07392 1 formate dehydrogenase Bradyrhizobium japonicum
emb SDO22312 1 formate dehydrogenase major subunit Filomicrobium insigne
gb KNX40115 1 Formate dehydrogenase H Roseovarius tolerans
emb CUH43821 1 Formate dehydrogenase-O major subunit precursor Ruegeria atlantica
gb KUJ85782 1 formate dehydrogenase Ruegeria marisrubri
gb AMV37792 1 Assimilatory nitrate reductase catalytic subunit Planctomyces sp SH-PL62
gb QDT71791 1 Assimilatory nitrate reductase catalytic subunit Planctomycetes bacterium I41
gb KPL91657 1 nitrite reductase Herpetosiphon geysericola
gb QDT55911 1 Assimilatory nitrate reductase catalytic subunit Planctomycetes bacterium Pan44
gb QEG36755 1 Assimilatory nitrate reductase catalytic subunit Bythopirellula goksoyri
gb AUX22438 1 nitrite reductase Sorangium cellulosum
gb AMY06969 1 Assimilatory nitrate reductase catalytic subunit Luteitalea pratensis
gb KYG04124 1 nitrite reductase Sorangium cellulosum
gb AGP37164 1 hypothetical protein SCE1572 23375 Sorangium cellulosum So0157-2
gb KYF72308 1 nitrite reductase Sorangium cellulosum
gb KYF62417 1 nitrite reductase Sorangium cellulosum
0
1E-07
2E-07

(3) *nifH*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Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological based
Alphaproteobacteria (Proteobacteria)
Betaproteobacteria (Proteobacteria)
Unclassified Proteobacteria
gb ACA18078 1 chlorophyllide reductase iron protein subunit X Methylobacterium sp 4-46
gb EGQ94767 1 BchX Acidiphilium sp PM
gb ACI99483 1 chlorophyllide reductase iron protein subunit X Rhodospirillum centenum SW
gb EGK69987 1 Chlorophyllide reductase 35 5 kDa chain Methyloversatilis universalis FAM5
gb AAX48206 1 chlorophyllide reductase X subunit uncultured proteobacterium DelRiverFos06H03
gb EGJ10539 1 chlorophyllide reductase iron protein subunit X Rubrivivax benzoatilyticus JA2 = ATCC BAA-35
dbj BAA94035 1 chlorophillide reductase subunit Rubrivivax gelatinosus IL144
gb ACB24802 1 chlorophyllide reductase iron protein subunit X Methylobacterium radiotolerans JCM 2831
gb EIZ84117 1 chlorophyllide reductase iron protein subunit X Methylobacterium sp GXF4
gb EHP89494 1 chlorophyllide reductase iron protein subunit X Methylorubrum extorquens DSM 13060
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gb ACK50992 1 chlorophyllide reductase iron protein subunit X Methylocella silvestris BL2
emb CCD96387 1 bacteriochlorophyllide reductase subunit 35 5 kDa chain Bradyrhizobium sp ORS 375
gb ABQ38347 1 bacteriochlorophyllide reductase subunit 35 5 kDa chain Bradyrhizobium sp BTAi1
emb CAL75497 1 bacteriochlorophyllide reductase subunit 35 5 kDa chain Bradyrhizobium sp ORS 278
emb CCD98529 1 bacteriochlorophyllide reductase subunit 35 5 kDa chain Bradyrhizobium sp STM 3809
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gb ABJ05279 1 chlorophyllide reductase iron protein subunit X Rhodopseudomonas palustris BisA53
gb ABE40977 1 chlorophyllide reductase iron protein subunit X Rhodopseudomonas palustris BisB5
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gb EAU40222 1 chlorophyllide reductase iron protein subunit X Fulvimarina pelagi HTCC2506
gb ADP69500 1 chlorophyllide reductase iron protein subunit X Rhodomicrobium vannielii ATCC 17100
gb ADL02351 1 chlorophyllide reductase iron protein subunit X Brevundimonas subvibrioides ATCC 15264
gb EAQ27780 1 bacteriochlorophyllide reductase subunit BchX Erythrobacter sp NAP1
gb AAQ93131 1 protochlorophyllide reductase subunit Rubrivivax gelatinosus S1
gb EFL89091 1 light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Ahrensia sp R2A130
dbj BAL75154 1 protochlorophyllide reductase protein BchL Bradyrhizobium sp S23321
gb ABD86878 1 light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Rhodopseudomonas palustris BisB18
gb ACI99501 1 light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Rhodospirillum centenum SW
gb EGK70004 1 Light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Methyloversatilis universalis FAM5
gb ABQ38324 1 protochlorophyllide reductase iron-sulfur ATP-binding protein BchL Bradyrhizobium sp BTAi1
emb CCD90191 1 protochlorophyllide reductase iron-sulfur ATP-binding protein BchL Bradyrhizobium sp ORS 285
emb CCD96365 1 protochlorophyllide reductase iron-sulfur ATP-binding protein BchL Bradyrhizobium sp ORS 375
emb CCE00486 1 protochlorophyllide reductase iron-sulfur ATP-binding protein BchL Bradyrhizobium sp STM 3809
gb EAU40240 1 Light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Fulvimarina pelagi HTCC2506
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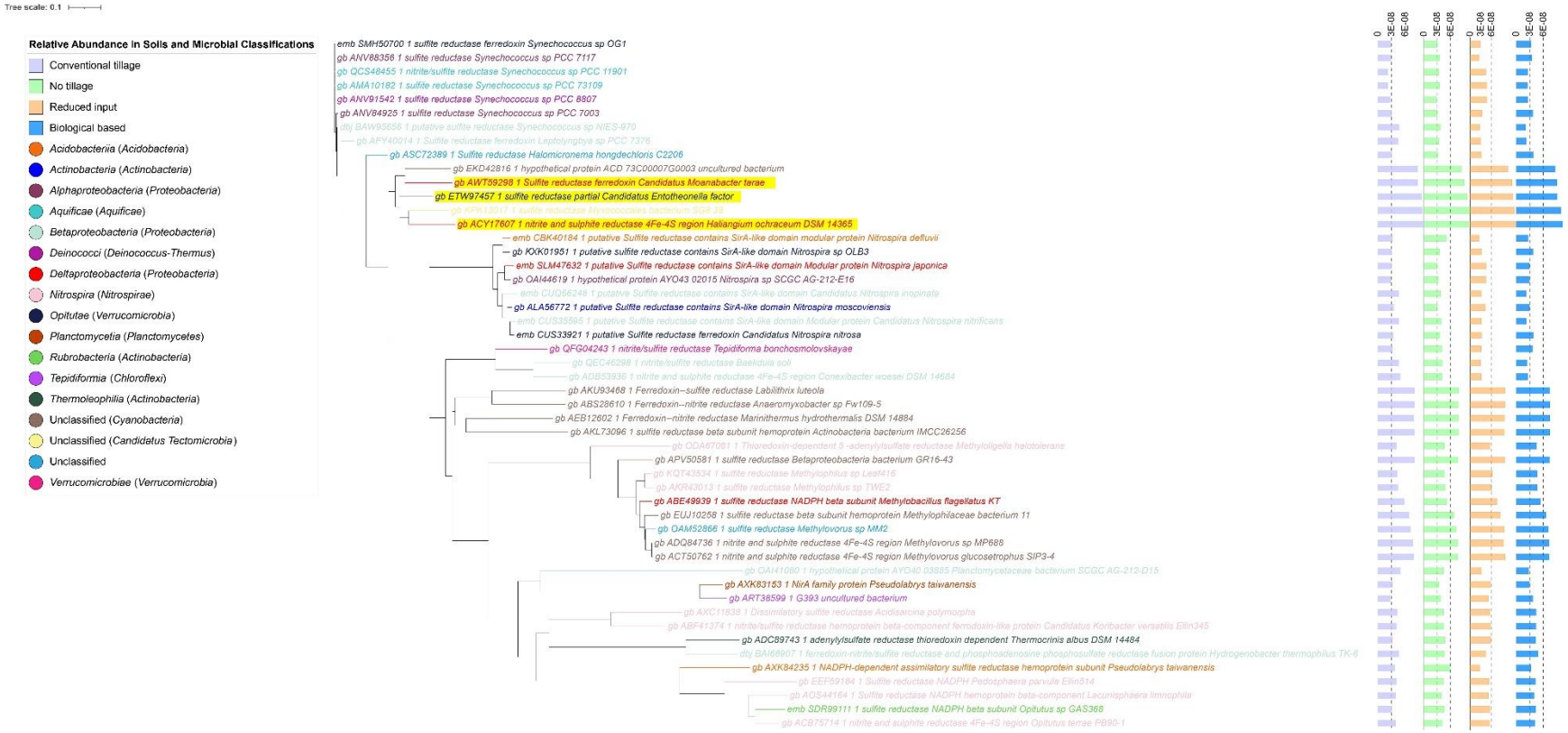
gb ACB23840 1 light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Methylobacterium radiotolerans JCM 2831
gb EIZ85857 1 protochlorophyllide reductase iron-sulfur ATP-binding protein Methylobacterium sp GXF4
gb ACB83450 1 light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Methylorubrum populi BJ001
gb ACS42872 1 protochlorophyllide reductase iron-sulfur ATP-binding protein BchL Methylorubrum extorquens AM1
gb EHP90534 1 Light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Methylorubrum extorquens DSM 13060
gb ABY33181 1 light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Methylorubrum extorquens PA1
emb CAX27470 1 protochlorophyllide reductase iron-sulfur ATP-binding protein BchL Methylorubrum extorquens AM1
gb ACK86042 1 light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein Methylorubrum extorquens CM4
0
1E-08
2E-08
0
1E-08
2E-08
0
1E-08
2E-08
0
1E-08
2E-08

(4) *nirA*


Tree scale: 0.1
Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological based
Acidobacteriia (Acidobacteria)
Actinobacteria (Actinobacteria)
Alphaproteobacteria (Proteobacteria)
Aquificae (Aquificae)
Betaproteobacteria (Proteobacteria)
Deinococci (Deinococcus-Thermus)
Deltaproteobacteria (Proteobacteria)
Nitrospira (Nitrospirae)
Opitutae (Verrucomicrobia)
Planctomycetia (Planctomycetes)
Rubrobacteria (Actinobacteria)
Tepidiformia (Chloroflexi)
Thermoleophilia (Actinobacteria)
Unclassified (Cyanobacteria)
Unclassified (Candidatus Tectomicrobia)
Unclassified
Verrucomicrobiae (Verrucomicrobia)
emb SMH50700 1 sulfite reductase ferredoxin Synechococcus sp OG1
gb ANV88356 1 sulfite reductase Synechococcus sp PCC 7117
gb QCS48455 1 nitrite/sulfite reductase Synechococcus sp PCC 11901
gb AMA10182 1 sulfite reductase Synechococcus sp PCC 73109
gb ANV91542 1 sulfite reductase Synechococcus sp PCC 8807
gb ANV84925 1 sulfite reductase Synechococcus sp PCC 7003
dbj BAW93656 1 putative sulfite reductase Synechococcus sp NIES-970
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gb ASC72389 1 Sulfite reductase Halomicronema hongdechloris C2206
gb EKD42816 1 hypothetical protein ACD 73C00007G0003 uncultured bacterium
gb AWT59298 1 Sulfite reductase ferredoxin Candidatus Moanabacter tarae
gb ETW97457 1 sulfite reductase partial Candidatus Entotheonella factor
gb KPR13017 1 sulfite reductase Myxococcales bacterium SI3 33
gb ACY17607 1 nitrite and sulphite reductase 4Fe-4S region Haliangium ochraceum DSM 14365
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gb KXK01951 1 putative sulfite reductase contains SirA-like domain Nitrospira sp OLB3
emb SLM47632 1 putative Sulfite reductase contains SirA-like domain Modular protein Nitrospira japonica
gb OAI44619 1 hypothetical protein AYO43 02015 Nitrospira sp SCGC AG-212-E16
emb CUQ66248 1 putative Sulfite reductase contains SirA-like domain Candidatus Nitrospira inopinata
gb ALA56772 1 putative Sulfite reductase contains SirA-like domain Nitrospira moscoviensis
emb CUS35595 1 putative Sulfite reductase contains SirA-like domain Modular protein Candidatus Nitrospira nitricaris
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gb AKU93468 1 Ferredoxin--sulfite reductase Labilithrix luteola
gb ABS28610 1 Ferredoxin--nitrite reductase Anaeromyxobacter sp Fw109-5
gb AEB12602 1 Ferredoxin--nitrite reductase Marinithermus hydrothermalis DSM 14884
gb AKL73096 1 sulfite reductase beta subunit hemoprotein Actinobacteria bacterium IMCC26256
gb ODA67081 1 Thioredoxin-dependent 5 -adenylylsulfate reductase Methyloligella halotolerans
gb APV50581 1 sulfite reductase Betaproteobacteria bacterium GR16-43
gb KQT43534 1 sulfite reductase Methylophilus sp Leaf416
gb AKR43013 1 sulfite reductase Methylophilus sp TWE2
gb ABE49939 1 sulfite reductase NADPH beta subunit Methylobacillus flagellatus KT
gb EUJ10258 1 sulfite reductase beta subunit hemoprotein Methylophilaceae bacterium 11
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gb AXK83153 1 NirA family protein Pseudolabrys taiwanensis
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gb AXC11838 1 Dissimilatory sulfite reductase Acidiaerobia polymorpha
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gb ADC89743 1 adenylylsulfate reductase thioredoxin dependent Thermocrinis albus DSM 14484
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gb AXK84235 1 NADPH-dependent assimilatory sulfite reductase hemoprotein subunit Pseudolabrys taiwanensis
gb EEF59184 1 Sulfite reductase NADPH Pedosphaera parvula Ellin514
gb AOS44164 1 Sulfite reductase NADPH hemoprotein beta-component Lacunisphaera limnophila
emb SDR99111 1 sulfite reductase NADPH beta subunit Opitutus sp GAS368
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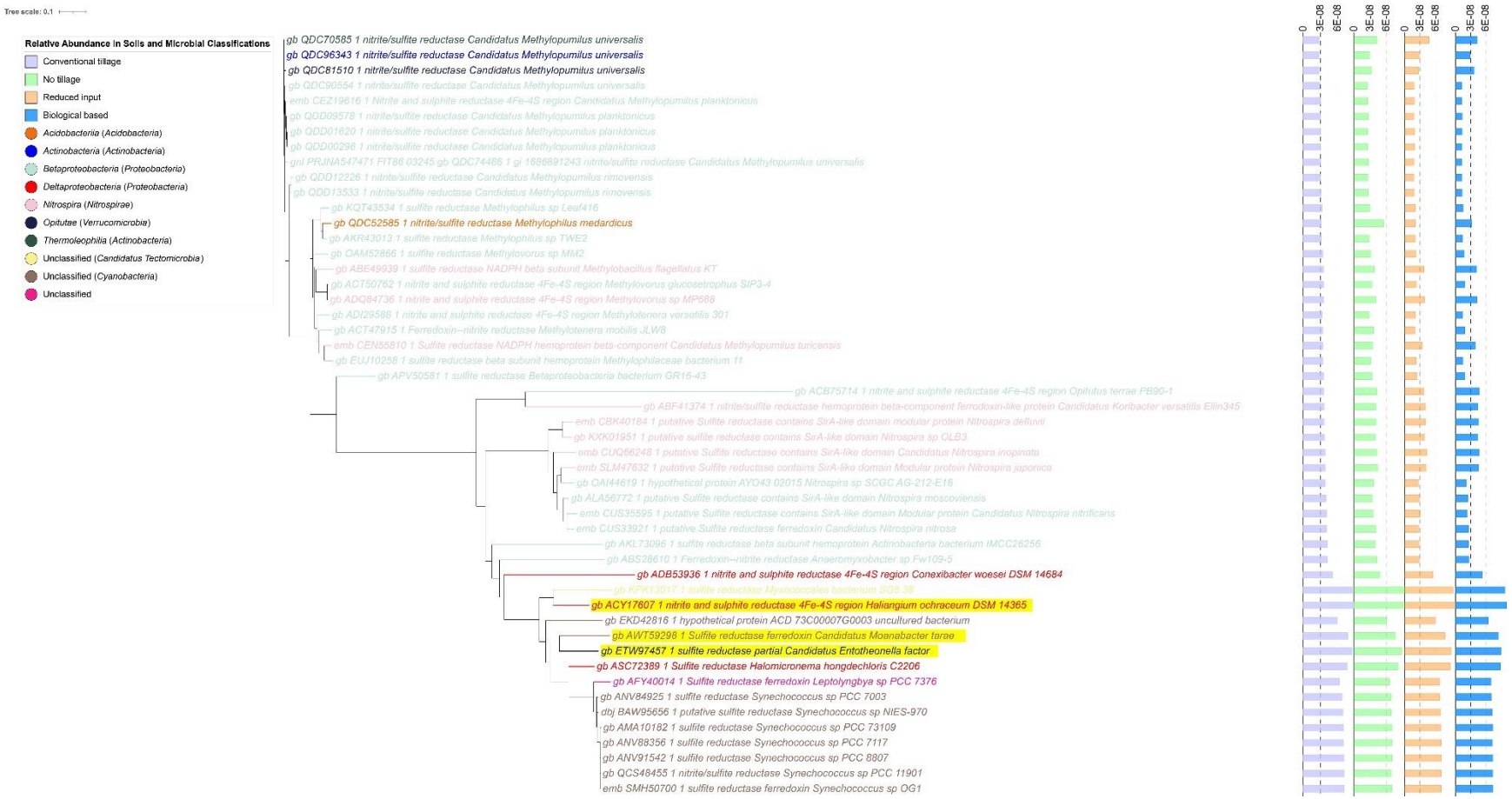
6E-08

(5) *nirB*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Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological based
Acidobacteriia (Acidobacteria)
Actinobacteria (Actinobacteria)
Betaproteobacteria (Proteobacteria)
Deltaproteobacteria (Proteobacteria)
Nitrospira (Nitrospirae)
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emb CEZ19616 1 Nitrite and sulphite reductase 4Fe-4S region Candidatus Methylopumilus planktonicus
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gb QDD00296 1 nitrite/sulfite reductase Candidatus Methylopumilus planktonicus
gnl PRJNA547471 FIT86 03245 gb QDC74486 1 gi 1886891243 nitrite/sulfite reductase Candidatus Methylopumilus universalis
gb QDD12226 1 nitrite/sulfite reductase Candidatus Methylopumilus rimovensis
gb QDD13533 1 nitrite/sulfite reductase Candidatus Methylopumilus rimovensis
gb KQT43534 1 sulfite reductase Methylophilus sp Leaf416
gb QDC52585 1 nitrite/sulfite reductase Methylophilus medardicus
gb AKR43013 1 sulfite reductase Methylophilus sp TWE2
gb OAM52866 1 sulfite reductase Methylovorus sp MM2
gb ABE49939 1 sulfite reductase NADPH beta subunit Methylobacillus flagellatus KT
gb ACT50762 1 nitrite and sulphite reductase 4Fe-4S region Methylovorus glucosetrophus SIP3-4
gb ADQ84736 1 nitrite and sulphite reductase 4Fe-4S region Methylovorus sp MP688
gb ADI29588 1 nitrite and sulphite reductase 4Fe-4S region Methylotenera versatilis 301
gb ACT47915 1 Ferredoxin--nitrite reductase Methylotenera mobilis JLW8
emb CEN55810 1 Sulfite reductase NADPH hemoprotein beta-component Candidatus Methylopumilus turicensis
gb EUJ10258 1 sulfite reductase beta subunit hemoprotein Methylophilaceae bacterium 11
gb APV50581 1 sulfite reductase Betaproteobacteria bacterium GR16-43
gb ACB75714 1 nitrite and sulphite reductase 4Fe-4S region Opitutus terrae PB90-1
gb ABF41374 1 nitrite/sulfite reductase hemoprotein beta-component ferrodoxin-like protein Candidatus Koribacter versatilis Ellin345
emb CBK40184 1 putative Sulfite reductase contains SirA-like domain modular protein Nitrospira defluvii
gb KXK01951 1 putative sulfite reductase contains SirA-like domain Nitrospira sp OLB3
emb CUQ66248 1 putative Sulfite reductase contains SirA-like domain Candidatus Nitrospira inopinata
emb SLM47632 1 putative Sulfite reductase contains SirA-like domain Modular protein Nitrospira japonica
gb OAI44619 1 hypothetical protein AYO43 02015 Nitrospira sp SCGC AG-212-E16
gb ALA56772 1 putative Sulfite reductase contains SirA-like domain Nitrospira moscoviensis
emb CUS35595 1 putative Sulfite reductase contains SirA-like domain Modular protein Candidatus Nitrospira nitrificans
emb CUS33921 1 putative Sulfite reductase ferredoxin Candidatus Nitrospira nitrosa
gb AKL73096 1 sulfite reductase beta subunit hemoprotein Actinobacteria bacterium IMCC26256
gb ABS28610 1 Ferredoxin--nitrite reductase Anaeromyxobacter sp Fw109-5
gb ADB53936 1 nitrite and sulphite reductase 4Fe-4S region Conexibacter woesei DSM 14684
gb KPK13017 1 sulfite reductase Myxococcales bacterium SG8 38
gb ACY17607 1 nitrite and sulphite reductase 4Fe-4S region Haliangium ochraceum DSM 14365
gb EKD42816 1 hypothetical protein ACD 73C00007G0003 uncultured bacterium
gb AWT59298 1 Sulfite reductase ferredoxin Candidatus Moanabacter terrae
gb ETW97457 1 sulfite reductase partial Candidatus Entotheonella factor
gb ASC72389 1 Sulfite reductase Halomicronema hongdechloris C2206
gb AFY40014 1 Sulfite reductase ferredoxin Leptolyngbya sp PCC 7376
gb ANV84925 1 sulfite reductase Synechococcus sp PCC 7003
dbj BAW95656 1 putative sulfite reductase Synechococcus sp NIES-970
gb AMA10182 1 sulfite reductase Synechococcus sp PCC 73109
gb ANV88356 1 sulfite reductase Synechococcus sp PCC 7117
gb ANV91542 1 sulfite reductase Synechococcus sp PCC 8807
gb QCS48455 1 nitrite/sulfite reductase Synechococcus sp PCC 11901
emb SMH50700 1 sulfite reductase ferredoxin Synechococcus sp OG1
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6E-08

(6) *nirK*


Tree scale: 0.1
Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological based
Alphaproteobacteria (Proteobacteria)
Betaproteobacteria (Proteobacteria)
Candidatus Brocadiae (Planctomycetes)
Deltaproteobacteria (Proteobacteria)
Gammaproteobacteria (Proteobacteria)
Spartobacteria (Verrucomicrobia)
Sprochaeta (Spirochaetes)
Unclassified
gb KGD31297 1 nitrite reductase copper-containing Burkholderia pseudomallei
gb OMX29111 1 gnl WGS LWZA AQ923 05875 gi 1138426085 nitrite reductase copper-containing Burkholderia pseudomallei
gnl PRJNA339871 BGI47 20705 gb APZ21135 1 gi 1137400343 nitrite reductase copper-containing Burkholderia pseudomallei
gb OMV61124 1 gnl WGS LWXX AQ794 26675 gi 1138253294 nitrite reductase copper-containing Burkholderia pseudomallei
gb KOT19001 1 gnl WGS JPGF DM47 1763 gi 925276423 nitrite reductase copper-containing Burkholderia mallei
emb CPG99961 1 gi 899223453 outer membrane nitrite reductase Burkholderia pseudomallei
gb EIF53962 1 anaerobically induced outer membrane protein Burkholderia pseudomallei 1258a
gb OMT61893 1 gnl WGS LWWQ AQ761 27920 gi 1138045671 nitrite reductase copper-containing Burkholderia pseudomallei
gb EIF76035 1 anaerobically induced outer membrane protein Burkholderia pseudomallei 354a
gb AFI69817 1 anaerobically induced outer membrane protein Burkholderia pseudomallei 1026b
gb AII23703 1 nitrite reductase copper-containing Burkholderia thailandensis E254
emb CBJ52550 2 Copper-containing nitrite reductase NO-forming anaerobically induced outer membrane transmembrane protein Ralstonia solanacearum PSI07
gb EIL91553 1 major anaerobically induced outer membrane transmembrane protein Rhodanobacter fulvus Jip2
gb ADE28551 1 copper-containing nitrite reductase Rhodanobacter denitrificans
gb EIL98512 1 major anaerobically induced outer membrane transmembrane protein partial Rhodanobacter thiooxydans LCS2
emb CAD18654 1 probable major anaerobically induced outer membrane transmembrane protein Ralstonia solanacearum GMI1000
emb CBJ41149 1 Copper-containing nitrite reductase NO-forming anaerobically induced outer membrane transmembrane protein Ralstonia solanacearum CMR15
gb OCQ57187 1 gnl WGS LVKS AR463 09220 gi 1049020601 nitrite reductase copper-containing Ralstonia solanacearum
emb CAQ18424 1 gi 205587842 major anaerobically induced outer membrane protein Ralstonia solanacearum MolK2
gb OAI58357 1 membrane protein Ralstonia solanacearum
gb AMP76989 1 hypothetical protein RALBFv3 23065 Ralstonia solanacearum
gb EDY19063 1 nitrite reductase copper-containing Chthoniobacter flavus Ellin428
gb AEY02287 1 nitrite reductase copper-containing Oceanimonas sp GK1
gb ACV27434 1 nitrite reductase copper-containing Kangiella koreensis DSM 16069
gb AFM12745 1 dissimilatory nitrite reductase NO-forming copper type apoprotein Tumeriella parva DSM 21527
gb ABI66574 1 multicopper oxidase Maricaulis maris MCS10
gb EAU66216 1 multicopper oxidase type 3 Stigmatella aurantiaca DW4/3-1
gb ABF89931 1 multicopper oxidase domain protein Myxococcus xanthus DK 1622
gb AFE08400 1 multicopper oxidase domain-containing protein Corallococcus coralloides DSM 2259
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dbj GAB62123 1 multicopper oxidase Candidatus Jettenia caeni
gb ADG30159 1 multicopper oxidase type 3 Thiomonas intermedia K12
gb ACB65820 1 multicopper oxidase type 3 Burkholderia ambifaria MC40-6
gb AIO71817 1 multicopper oxidase family protein Burkholderia multivorans
gb AJY01560 1 multicopper oxidase family protein Burkholderia thailandensis 2002721643
gb ABN86571 1 multicopper oxidase domain protein Burkholderia pseudomallei 668
gb AIP07653 1 multicopper oxidase family protein Burkholderia pseudomallei
gb KGD22644 1 multicopper oxidase family protein Burkholderia pseudomallei
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emb CFT98547 1 multicopper oxidase domain-containing protein Burkholderia pseudomallei
gb EIF72816 1 multicopper oxidase Burkholderia pseudomallei 354e
gb AHK68411 1 multicopper oxidase family protein Burkholderia pseudomallei MSHR520
gb KGD25938 1 multicopper oxidase family protein Burkholderia pseudomallei
gb AJX10468 1 multicopper oxidase family protein Burkholderia pseudomallei 1026b
emb CDU31492 1 putative copper nitrite reductase protein Burkholderia pseudomallei
0
1E-06
2E-06

(7) *nirS*


Tree scale: 0.1
Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological based
Acidithiobacillia (Proteobacteria)
Betaproteobacteria (Proteobacteria)
Gammaproteobacteria (Proteobacteria)
Hydrogenophilalia (Proteobacteria)
Unclassified
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emb CUB07681 1 dissimilatory nitrite reductase NO-forming cytochrome cd1 type apoprotein Tepidiphilus thermophilus
emb CAI06598 1 Cytochrome cd1 nitrite reductase precursor Aromatoleum aromaticum EbN1
gb KON80814 1 nitrite reductase Azoarcus sp PA01
dbj BAL25833 1 cytochrome cd1 nitrite reductase precursor Azoarcus sp KH32C
gb AYH45152 1 nitrite reductase Azoarcus sp DN11
gb AKU13501 1 nitrite reductase Azoarcus sp CIB
gb ALT77177 1 nitrite reductase Paucibacter sp KCTC 42545
gb AWI52926 1 nitrite reductase Aquabacterium olei
gb ACF98225 1 putative cytochrome cd1 nitrite reductase NirS uncultured bacterium 2304
gb QBM27494 1 Nitrite reductase precursor Hydrogenophaga pseudoflava
gb KRH99151 1 nitrite reductase Curvibacter sp PAE-UM
gb AYM96229 1 nitrite reductase Acidovorax sp 1608163
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gb ACB33710 1 Hydroxylamine reductase Leptothrix cholodnii SP-6
gb KRB41143 1 nitrite reductase Acidovorax sp Root70
gb AVO49495 1 nitrite reductase Melaminivora sp SC2-9
gb AEB84718 1 Hydroxylamine reductase Alicycliphilus denitrificans K601
gb AVP57716 1 nitrite reductase Pulveribacter suum
gb ABM42092 1 dissimilatory nitrite reductase NO-forming cytochrome cd1 type apoprotein Acidovorax sp JS42
gb KLR56966 1 nitrite reductase Diaphorobacter sp J5-51
gb KFB70389 1 Nitrite reductase precursor Candidatus Accumulibacter sp BA-91
gb KFB75449 1 Nitrite reductase precursor Candidatus Accumulibacter sp SK-02
gb KFB69436 1 Nitrite reductase precursor Candidatus Accumulibacter sp SK-01
dbj BAV32586 1 nitrite reductase Sulfuricaulis limicola
gb KPK19352 1 nitrite reductase Betaproteobacteria bacterium SG8 41
gb AZP14686 1 c-type cytochrome Undibacterium parvum
gb QDZ30612 1 c-type cytochrome Noviherbaspirillum sp UKPF54
gb AZG13299 1 nitrite reductase Cupriavidus pauculus
gb AAZ64358 1 dissimilatory nitrite reductase NO-forming cytochrome cd1 type apoprotein Cupriavidus pinatubonensis JMP134
gb ACF98047 1 putative cytochrome cd1 nitrite reductase NirS uncultured bacterium 888
gb QDX81396 1 nitrite reductase Denitratisoma sp DHT3
gb OAJ71930 1 nitrite reductase Methylobacillus sp MM3
gb ADE11367 1 Nitrite reductase NO-forming Sideroxydans lithotrophicus ES-1
emb CUA82351 1 Cytochrome c mono-and diheme variants Gulbenkiania indica
emb CDI04496 1 Nitrite reductase Candidatus Competibacter denitrificans Run A D11
dbj BAP57172 1 nitrite reductase Thioploca ingrica
gb AUM12666 1 nitrite reductase Ketobacter alkanivorans
gb ACF98171 1 putative cytochrome cd1 nitrite reductase NirS uncultured bacterium 2303
gb KVW95095 1 cytochrome Cbb3 Thiobacillus denitrificans
gb OAJ71923 1 cytochrome C oxidase Cbb3 Methylobacillus sp MM3
gb KPK11656 1 cytochrome C oxidase Cbb3 Acidithiobacillales bacterium SG8 45
gb QDZ28973 1 cytochrome C oxidase Cbb3 Noviherbaspirillum sp UKPF54
dbj GAB72183 1 gi 393791211 hypothetical protein SCD 02137 Sulfuricella denitrificans skB26
dbj BAU46942 1 cytochrome Cbb3 Sulfurifustis variabilis
dbj BAV32595 1 cytochrome Cbb3 Sulfuricaulis limicola
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Tree scale: 0.1
Relative Abundance in Soils and Microbial Classifications
Conventional tillage
No tillage
Reduced input
Biological input
Acidobacteriia (Acidobacteria)
Alphaproteobacteria (Proteobacteria)
Betaproteobacteria (Proteobacteria)
Deltaproteobacteria (Proteobacteria)
Gammaproteobacteria (Proteobacteria)
Planctomycetia (Planctomycetes)
Unclassified (Planctomycetes)
Unclassified
gb QDT92642 1 Nitric oxide reductase subunit B Planctomycetes bacterium Pan161
gb QDV52281 1 Nitric oxide reductase subunit B Planctomycetes bacterium Enr17
gb APZ91073 1 Nitric oxide reductase subunit B Fuerstia marisgermanicae
gb QDU82529 1 Nitric oxide reductase subunit B Planctomycetes bacterium Pla110
gb QDT49102 1 Nitric oxide reductase subunit B Planctomycetes bacterium Pan258
gb QDU44771 1 Nitric oxide reductase subunit B Planctomycetes bacterium Mal52
gb QDV47064 1 Nitric oxide reductase subunit B Planctomycetes bacterium Enr13
gb QDV62542 1 Nitric oxide reductase subunit B Crateriforma conspicua
gb QEF97787 1 Nitric oxide reductase subunit B Planctomycetes bacterium
gb EMI24316 1 nitric oxide reductase subunit B transmembrane protein Rhodopirellula europaea SH398
gb EMB15770 1 nitric oxide reductase subunit B transmembrane protein Rhodopirellula europaea 6C
gb QDT32488 1 Nitric oxide reductase subunit B Planctomycetes bacterium Mal48
gb QDV30306 1 Nitric oxide reductase subunit B Planctopirus ephydatiae
gb QDV17254 1 Nitric oxide reductase subunit B Planctomycetes bacterium Pan153
gb QDT27684 1 Nitric oxide reductase subunit B Planctomycetes bacterium Enr10
gb QDT71782 1 Nitric oxide reductase subunit B Planctomycetes bacterium I41
gb QEG35187 1 Nitric oxide reductase subunit B Bythopirellula goksoyri
gb QDU25836 1 Nitric oxide reductase subunit B Planctomycetes bacterium ETA A8
gb QDU29046 1 Nitric oxide reductase subunit B Planctomycetes bacterium ETA A8
gb AMV27643 1 Nitric oxide reductase subunit B Gemmata sp SH-PL17
gb ABJ83921 1 putative nitric-oxide reductase Candidatus Solibacter usitatus Ellin6076
gb KUO62120 1 nitric oxide reductase large subunit bacterium BRH c32
gb QDL36755 1 nitric-oxide reductase large subunit Rhodoferax sediminis
gb ATQ70900 1 nitric-oxide reductase large subunit Methylosinus trichosporium OB3b
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gb EGP57068 1 cytochrome-c oxidase FixN chain Agrobacterium tumefaciens F2
gb ADY64481 1 cytochrome-c oxidase fixN chain Agrobacterium sp H13-3
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gb ANO52552 1 nitric oxide reductase large subunit Woeseia oceani
gb ADV26224 1 putative nitric-oxide reductase Pseudoxanthomonas suwonensis 11-1
gb AKC86351 1 nitric oxide reductase large subunit Pseudoxanthomonas suwonensis
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gb AUZ56621 1 nitric oxide reductase large subunit Stenotrophomonas acidaminiphila
gb ATB43141 1 nitric oxide reductase large subunit Cystobacter fuscus
gb QDV37632 1 Nitric oxide reductase subunit B Planctomycetes bacterium EIP
gb AMV40548 1 Nitric oxide reductase subunit B Planctomyces sp SH-PL62
gb QEH34254 1 Nitric oxide reductase subunit B Aquisphaera giovannonii
gb APW59926 1 Nitric oxide reductase subunit B Paludisphaera borealis
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gb KYF50198 1 nitric oxide reductase large subunit Sorangium cellulosum
gb AGP34548 1 hypothetical protein SCE1572 08535 Sorangium cellulosum So0157-2
gb KYF73765 1 nitric oxide reductase large subunit Sorangium cellulosum
gb KYF77729 1 nitric oxide reductase large subunit Sorangium cellulosum
gb ABS28027 1 putative nitric-oxide reductase Anaeromyxobacter sp Fw109-5
dbj GAO04078 1 nitric oxide reductase subunit B Anaeromyxobacter sp PSR-1
gb ABC83486 1 nitric oxide reductase NorZ apoprotein Anaeromyxobacter dehalogenans 2CP-C
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gb ACL67183 1 putative nitric-oxide reductase Anaeromyxobacter dehalogenans 2CP-1
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1E-06

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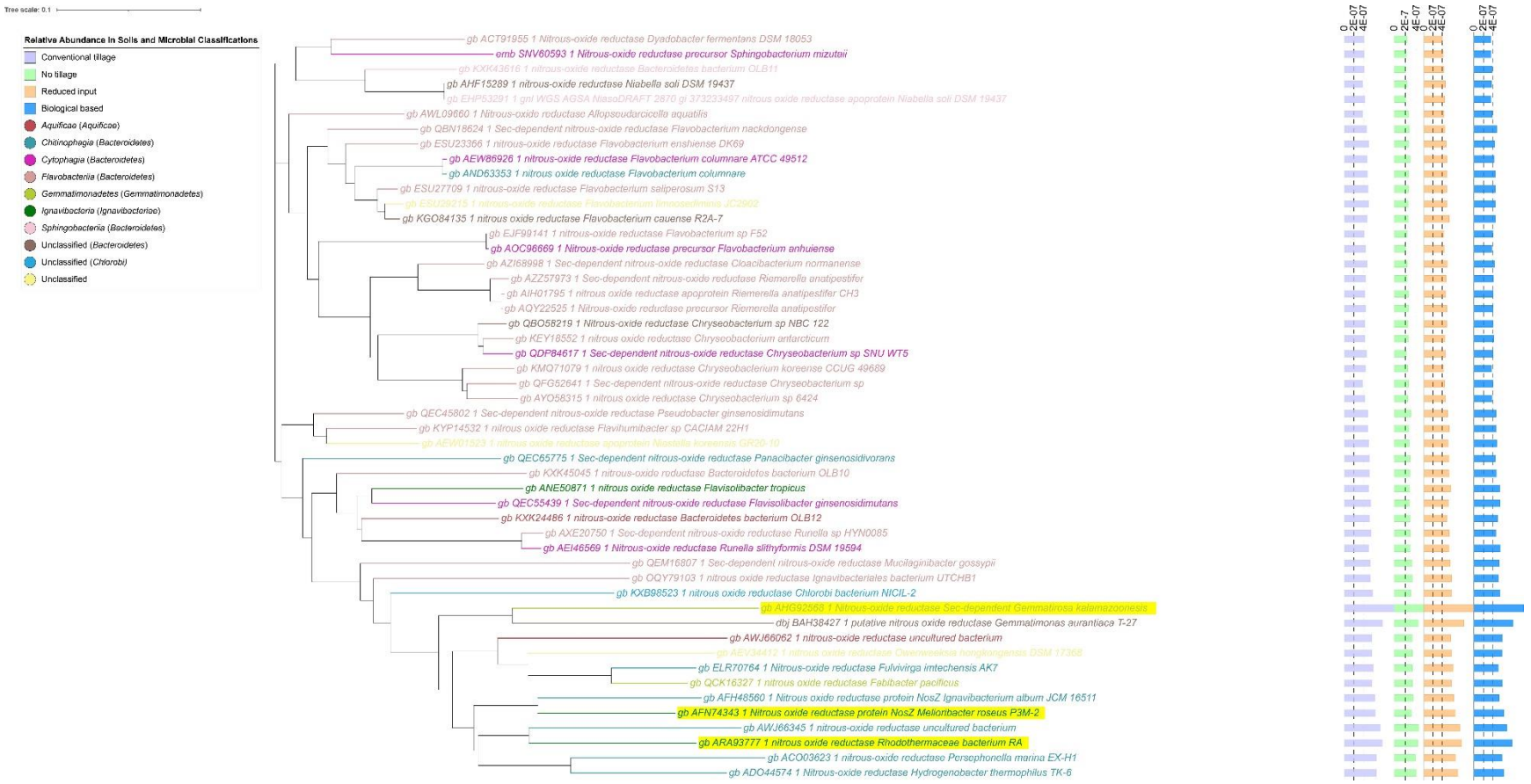

Figure 8.

Applied Microbiology and Biotechnology

Supplementary Section

Diversity of Nitrogen Cycling Genes at a Midwest Long Term Ecological Research Site with Different Management Practices

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cupplesa@egr.msu.edu

Table S1. FunGene and DIAMOND sequence data summary.

	On FunGene	Minimum HMM Coverage 70%	Dereplicated
napA	74937	40226	11395
narG	50753	49174	11395
nosZ	5304	3787	1266
norB	13238	7054	1778
nifH	19514	3474	1562
nirK	7988	3367	556
nirS	25330	3020	993
nirA	54085	51514	12955
nirB	90760	45767	12955

Table S2 MG-RAST sequence data summary.

Soil type	MG-RAST ID	Post QC: bp Count	Post QC: Sequences Count	Post QC: Mean Sequence Length bp
Conventional tillage	mgm4887245.3	1,049,991,462 bp	4,562,115	230 ± 37 bp
Conventional tillage	mgm4889385.3	865,087,512 bp	3,773,767	229 ± 38 bp
Conventional tillage	mgm4887247.3	873,760,585 bp	3,805,693	230 ± 38 bp
Conventional tillage	mgm4887261.3	1,042,733,021 bp	4,497,130	232 ± 37 bp
Conventional tillage	mgm4887259.3	1,186,811,683 bp	5,171,096	230 ± 37 bp
Conventional tillage	mgm4887263.3	1,049,806,246 bp	4,606,628	228 ± 38 bp
No tillage	mgm4887248.3	978,574,572 bp	4,289,260	228 ± 38 bp
No tillage	mgm4887249.3	1,021,883,457 bp	4,491,203	228 ± 38 bp
No tillage	mgm4887251.3	893,615,124 bp	3,901,326	229 ± 38 bp
No tillage	mgm4887262.3	1,052,482,005 bp	4,556,161	231 ± 37 bp
No tillage	mgm4887265.3	1,171,824,030 bp	5,106,093	229 ± 37 bp
No tillage	mgm4887264.3	1,151,447,486 bp	5,131,392	224 ± 39 bp
Reduced input	mgm4887252.3	1,020,227,225 bp	4,473,295	228 ± 38 bp
Reduced input	mgm4887253.3	1,156,421,815 bp	5,084,544	227 ± 38 bp
Reduced input	mgm4887254.3	845,604,740 bp	3,689,278	229 ± 38 bp
Reduced input	mgm4887267.3	904,740,521 bp	3,896,151	232 ± 37 bp
Reduced input	mgm4887266.3	1,216,560,266 bp	5,320,030	229 ± 38 bp
Reduced input	mgm4887268.3	923,078,351 bp	4,016,875	230 ± 37 bp
Biological based	mgm4887255.3	1,070,768,940 bp	4,666,479	229 ± 38 bp
Biological based	mgm4887256.3	1,048,398,089 bp	4,589,220	228 ± 38 bp
Biological based	mgm4887258.3	1,095,942,092 bp	4,834,482	227 ± 38 bp
Biological based	mgm4887270.3	1,410,382,064 bp	6,169,872	229 ± 38 bp
Biological based	mgm4887289.3	1,149,249,456 bp	5,008,186	229 ± 37 bp
Biological based	mgm4887290.3	1,303,754,397 bp	5,670,793	230 ± 37 bp

Table S3. P-values for statistical tests with the relative abundance of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk test**				**Levene's test**	**One-way ANOVA**	**Kruskal-Wallis test**
Null Hypothesis	**The sample distribution is normal**				**$\sigma_1=\sigma_2$**	**$\mu_1=\mu_2$**	**$Median_1=Median_2$**
Soil groups	**Conventional tillage**	**No tillage**	**Reduced input**	**Biological based**			
napA	1.57E-01	7.77E-01	**2.50E-04**	7.69E-01	5.35E-01	N/A	6.02E-02
narG	1.59E-01	7.66E-01	6.31E-01	1.71E-01	9.14E-02	2.74E-01	N/A
nifH	6.03E-01	7.31E-01	2.20E-01	2.44E-01	8.27E-01	**1.26E-02**	N/A
nirA	7.19E-02	2.20E-01	**3.40E-04**	5.73E-01	5.45E-01	N/A	4.81E-01
nirB	8.65E-01	**2.11E-02**	1.34E-02	5.69E-01	3.49E-01	N/A	4.09E-01
nirK	7.12E-01	3.27E-01	6.55E-01	5.24E-01	1.33E-01	**1.61E-05**	N/A
nirS	9.31E-01	1.16E-01	3.44E-01	4.63E-02	4.37E-01	**2.22E-05**	N/A
norB	5.97E-01	3.68E-01	8.43E-01	5.68E-01	3.81E-01	**1.85E-02**	N/A
nosZ	N/A	N/A	N/A	N/A	5.81E-03	N/A	N/A

Table S4. P-values for Tukey HSD test with the relative abundance of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Tukey's HSD test**								
Null Hypothesis	**$\mu_1=\mu_2$**								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	N/A	N/A	4.14E-01	N/A	N/A	**1.14E-05**	**2.07E-05**	**1.16E-02**	N/A
Conventional tillage - Reduced input	N/A	N/A	9.03E-01	N/A	N/A	3.84E-01	**2.33E-04**	4.65E-01	N/A
Conventional tillage - Biological based	N/A	N/A	1.89E-01	N/A	N/A	1.79E-01	**9.27E-03**	1.65E-01	N/A
No tillage - Reduced input	N/A	N/A	1.42E-01	N/A	N/A	**4.10E-04**	6.94E-01	2.22E-01	N/A
No tillage- Biological based	N/A	N/A	**7.59E-03**	N/A	N/A	**1.26E-03**	5.82E-02	5.68E-01	N/A
Reduced input - Biological based	N/A	N/A	5.06E-01	N/A	N/A	9.60E-01	3.88E-01	9.00E-01	N/A

Table S5. P-values for Dunn's test with the relative abundance of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Dunn's test**								
Null Hypothesis	**$\mu_1 = \mu_2$**								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Conventional tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Conventional tillage - Biological based	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
No tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
No tillage - Biological based	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Reduced input - Biological based	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table S6. Summary of the p values from Spearman's rank correlation tests with gene relative abundance data. Values in bold indicate a significant difference ($p \leq 0.05$).

Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
napA		**1.66E-07**	4.74E-01	**< 2.2e-16**	**1.43E-08**	**2.30E-06**	8.67E-02	**1.28E-08**	**4.26E-06**
narG	**1.66E-07**		**4.11E-02**	**2.74E-04**	**8.32E-03**	**3.30E-03**	4.70E-01	**3.12E-02**	3.63E-01
nifH	4.74E-01	**4.11E-02**		9.83E-01	7.64E-02	**7.37E-03**	2.02E-01	8.42E-01	2.86E-01
nirA	**< 2.2e-16**	**2.74E-04**	9.83E-01		**4.60E-02**	5.80E-01	6.15E-01	1.08E-01	5.87E-01
nirB	**1.43E-08**	**8.32E-03**	7.64E-02	**4.60E-02**		8.05E-01	6.89E-01	5.47E-01	9.87E-01
nirK	**2.30E-06**	**3.30E-03**	**7.37E-03**	5.80E-01	8.05E-01		**4.84E-03**	**1.98E-02**	**1.26E-02**
nirS	8.67E-02	4.70E-01	2.02E-01	6.15E-01	6.89E-01	**4.84E-03**		**2.12E-04**	**1.76E-03**
norB	**1.28E-08**	**3.12E-02**	8.42E-01	1.08E-01	5.47E-01	**1.98E-02**	**2.12E-04**		**7.65E-05**
nosZ	**4.26E-06**	3.63E-01	2.86E-01	5.87E-01	9.87E-01	**1.26E-02**	**1.76E-03**	**7.65E-05**	

Table S7. Summary of Spearman's correlation coefficient (rho) for Spearman's rank correlation test with gene relative abundance data. Rho values in bold indicate a statistically significant correlation ($p \leq 0.05$), as shown above.

Genes	*napA*	*narG*	*nifH*	*nirA*	*nirB*	*nirK*	*nirS*	*norB*	*nosZ*
napA		**8.48E-01**	1.54E-01	**9.81E-01**	**8.80E-01**	**8.03E-01**	3.57E-01	**8.81E-01**	**7.91E-01**
narG	**8.48E-01**		**4.20E-01**	**6.78E-01**	5.26E-01	**5.75E-01**	1.55E-01	**4.41E-01**	1.94E-01
nifH	1.54E-01	**4.20E-01**		-4.57E-03	3.69E-01	**5.33E-01**	2.70E-01	4.29E-02	2.27E-01
nirA	**9.81E-01**	**6.78E-01**	-4.57E-03		**4.11E-01**	1.19E-01	-1.08E-01	3.37E-01	1.17E-01
nirB	**8.80E-01**	5.26E-01	3.69E-01	**4.11E-01**		5.31E-02	-8.62E-02	1.29E-01	3.48E-03
nirK	**8.03E-01**	**5.75E-01**	**5.33E-01**	1.19E-01	5.31E-02		**5.55E-01**	**4.72E-01**	**5.01E-01**
nirS	3.57E-01	1.55E-01	2.70E-01	-1.08E-01	-8.62E-02	**5.55E-01**		**6.87E-01**	**6.04E-01**
norB	**8.81E-01**	**4.41E-01**	4.29E-02	3.37E-01	1.29E-01	**4.72E-01**	**6.87E-01**		**7.19E-01**
nosZ	**7.91E-01**	1.94E-01	2.27E-01	1.17E-01	3.48E-03	**5.01E-01**	**6.04E-01**	**7.19E-01**	

Table S8. P-values for statistical tests with the richness index Chao 1 of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk test**				**Levene's test**	**One-way ANOVA**	**Kruskal-Wallis test**
Null Hypothesis	**The sample distribution is normal**				**$\sigma_1=\sigma_2$**	**$\mu_1=\mu_2$**	**$Median_1=Median_2$**
Soil groups	**Conventional tillage**	**No tillage**	**Reduced input**	**Biological based**			
napA	4.89E-01	6.42E-01	2.90E-01	2.63E-01	9.37E-01	3.56E-01	N/A
narG	4.66E-01	5.43E-01	2.78E-01	2.82E-01	9.42E-01	9.37E-01	N/A
nifH	9.76E-01	**3.87E-03**	6.53E-01	9.76E-01	7.80E-01	N/A	**1.80E-02**
nirA	5.72E-01	4.34E-01	4.55E-01	5.53E-01	9.60E-01	2.65E-01	N/A
nirB	4.85E-01	4.86E-01	3.71E-01	5.47E-01	9.99E-01	7.99E-01	N/A
nirK	2.50E-01	**3.67E-02**	7.66E-02	**1.05E-03**	9.88E-01	N/A	**1.68E-02**
nirS	8.12E-01	1.16E-01	3.55E-01	8.13E-01	6.72E-01	**8.52E-04**	N/A
norB	9.10E-01	**2.92E-03**	9.44E-01	5.08E-01	2.25E-01	N/A	2.35E-01
nosZ	7.47E-01	4.99E-01	8.99E-01	4.85E-01	5.12E-01	5.86E-01	N/A

Table S9. P-values for Tukey's HSD test with the richness index Chao 1 of counts of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Tukey's HSD test**								
Null Hypothesis	**$\mu_1 = \mu_2$**								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	N/A	N/A	N/A	N/A	N/A	N/A	**7.37E-04**	N/A	N/A
Conventional tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	N/A	**8.59E-03**	N/A	N/A
Conventional tillage - Biological based	N/A	N/A	N/A	N/A	N/A	N/A	2.11E-01	N/A	N/A
No tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	N/A	7.05E-01	N/A	N/A
No tillage- Biological based	N/A	N/A	N/A	N/A	N/A	N/A	**6.50E-02**	N/A	N/A
Reduced input - Biological based	N/A	N/A	N/A	N/A	N/A	N/A	4.07E-01	N/A	N/A

Table S10. P-values for Dunn's test with the richness index Chao 1 of counts of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Dunn's test**								
Null Hypothesis	**$\mu_1 = \mu_2$**								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	N/A	N/A	1.00E+00	N/A	N/A	1.00E+00	N/A	N/A	N/A
Conventional tillage - Reduced input	N/A	N/A	1.33E-01	N/A	N/A	**2.91E-02**	N/A	N/A	N/A
Conventional tillage - Biological based	N/A	N/A	1.00E+00	N/A	N/A	2.03E-01	N/A	N/A	N/A
No tillage - Reduced input	N/A	N/A	5.39E-02	N/A	N/A	1.65E-01	N/A	N/A	N/A
No tillage - Biological based	N/A	N/A	1.00E+00	N/A	N/A	7.85E-01	N/A	N/A	N/A
Reduced input - Biological based	N/A	N/A	**3.30E-02**	N/A	N/A	1.00E+00	N/A	N/A	N/A

Table S11. P-values for statistical tests with the richness index Chao 2 of counts of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk test**				**Levene's test**	**One-way ANOVA**	**Kruskal-Wallis test**
Null Hypothesis	**The sample distribution is normal**				**$\sigma_1=\sigma_2$**	**$\mu_1=\mu_2$**	**$Median_1=Median_2$**
Soil groups	**Conventional tillage**	**No tillage**	**Reduced input**	**Biological based**			
napA	3.27E-01	4.60E-01	4.26E-01	2.38E-01	9.19E-01	4.17E-01	N/A
narG	2.88E-01	4.18E-01	2.19E-01	2.26E-01	9.91E-01	9.51E-01	N/A
nifH	7.54E-01	2.38E-01	1.62E-01	2.98E-01	5.93E-01	3.00E-01	**N/A**
nirA	3.24E-01	2.55E-01	4.23E-01	3.80E-01	9.32E-01	4.55E-01	N/A
nirB	2.55E-01	2.75E-01	2.57E-01	3.74E-01	9.98E-01	9.55E-01	N/A
nirK	5.32E-02	**7.63E-03**	**8.64E-03**	**6.93E-03**	9.95E-01	N/A	**2.18E-01**
nirS	3.70E-01	2.47E-01	2.80E-01	4.17E-01	9.87E-01	2.57E-01	N/A
norB	3.29E-01	6.27E-02	7.21E-01	1.90E-01	8.45E-01	4.62E-01	N/A
nosZ	2.30E-01	6.70E-01	7.22E-01	5.40E-01	6.34E-01	6.51E-01	N/A

Table S12. P-values for statistical tests with the Inverse Simpson values of the counts of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk test**				**Levene's test**	**One-way ANOVA**	**Kruskal-Wallis test**
Null Hypothesis	**The sample distribution is normal**				**$\sigma_1=\sigma_2$**	**$\mu_1=\mu_2$**	**$Median_1=Median_2$**
Soil groups	**Conventional tillage**	**No tillage**	**Reduced input**	**Biological based**			
napA	4.73E-01	**6.37E-03**	**5.07E-03**	**6.37E-03**	3.63E-01	N/A	**6.96E-05**
narG	N/A	N/A	N/A	N/A	**4.44E-05**	N/A	N/A
nifH	1.27E-01	2.66E-01	2.08E-01	1.60E-01	2.00E-01	6.52E-01	N/A
nirA	9.00E-02	1.25E-01	1.70E-01	**2.87E-02**	6.90E-01	N/A	**8.84E-04**
nirB	1.49E-01	2.33E-01	9.44E-02	**2.87E-02**	6.16E-01	N/A	**5.77E-04**
nirK	6.00E-02	9.95E-02	1.81E-01	2.05E-01	4.85E-01	**<2e-16**	N/A
nirS	2.26E-01	7.08E-02	1.79E-01	1.15E-01	6.44E-01	**1.57E-05**	N/A
norB	2.15E-01	7.04E-02	1.06E-01	8.94E-02	3.11E-01	**8.76E-15**	N/A
nosZ	N/A	N/A	N/A	N/A	**1.33E-04**	N/A	N/A

Table S13. P-values for Tukey's HSD test with the Inverse Simpson values of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Tukey's HSD test**								
Null Hypothesis	**$\mu_1 = \mu_2$**								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	**2.73E-03**	**0.00E+00**	N/A
Conventional tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	5.67E-02	**5.00E-07**	N/A
Conventional tillage - Biological based	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	9.98E-01	**2.62E-02**	N/A
No tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	**6.40E-06**	**0.00E+00**	N/A
No tillage- Biological based	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	**4.18E-03**	**0.00E+00**	N/A
Reduced input - Biological based	N/A	N/A	N/A	N/A	N/A	3.45E-01	**3.86E-02**	**3.18E-04**	N/A

Table S14. P-values for Dunn's test with the Inverse Simpson values of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Dunn's test**								
Null Hypothesis	**$\mu_1 = \mu_2$**								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	**8.35E-01**	N/A	N/A	1.00E+00	1.00E+00	N/A	N/A	N/A	N/A
Conventional tillage - Reduced input	**1.86E-02**	N/A	N/A	1.00E+00	4.31E-01	N/A	N/A	N/A	N/A
Conventional tillage - Biological based	**5.49E-05**	N/A	N/A	1.81E-01	1.12E-01	N/A	N/A	N/A	N/A
No tillage - Reduced input	**8.35E-01**	N/A	N/A	**2.87E-02**	**9.14E-03**	N/A	N/A	N/A	N/A
No tillage - Biological based	**1.86E-02**	N/A	N/A	**6.14E-04**	**1.18E-03**	N/A	N/A	N/A	N/A
Reduced input - Biological based	**8.35E-01**	N/A	N/A	5.15E-01	1.00E+00	N/A	N/A	N/A	N/A

Table S15. P-values for statistical tests with the Shannon diversity of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Shapiro-Wilk test**				**Levene's test**	**One-way ANOVA**	**Kruskal-Wallis test**
Null Hypothesis	**The sample distribution is normal**				**$\sigma_1=\sigma_2$**	**$\mu_1=\mu_2$**	**$Median_1=Median_2$**
Soil groups	**Conventional tillage**	**No tillage**	**Reduced input**	**Biological based**			
napA	**3.28E-02**	**3.17E-02**	2.50E-01	**3.28E-02**	2.09E-01	N/A	**2.03E-04**
narG	**2.47E-02**	**1.55E-02**	9.26E-01	6.07E-01	1.38E-01	N/A	**1.90E-04**
nifH	2.08E-01	2.63E-01	3.25E-01	3.08E-01	6.31E-01	8.35E-01	N/A
nirA	9.44E-02	6.95E-02	2.21E-01	1.31E-01	9.65E-01	**3.55E-03**	N/A
nirB	5.13E-02	5.13E-02	6.92E-02	2.07E-01	9.59E-01	5.85E-02	N/A
nirK	7.78E-02	5.51E-02	**3.29E-02**	9.11E-02	9.24E-01	N/A	**1.77E-04**
nirS	1.88E-01	1.35E-01	1.73E-01	1.52E-01	8.03E-01	**2.76E-03**	N/A
norB	1.61E-01	**1.55E-02**	7.96E-02	**1.01E-02**	9.52E-01	N/A	**1.44E-03**
nosZ	7.63E-01	5.08E-02	1.61E-01	7.03E-02	8.11E-01	**<2E-16**	N/A

Table S16. P-values for Tukey's HSD test with the Shannon diversity of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Tukey's HSD test**								
Null Hypothesis	**$\mu_1=\mu_2$**								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	**2.73E-03**	**0.00E+00**	N/A
Conventional tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	5.67E-02	**5.00E-07**	N/A
Conventional tillage - Biological based	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	9.98E-01	**2.62E-02**	N/A
No tillage - Reduced input	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	**6.40E-06**	**0.00E+00**	N/A
No tillage- Biological based	N/A	N/A	N/A	N/A	N/A	**0.00E+00**	**4.18E-03**	**0.00E+00**	N/A
Reduced input - Biological based	N/A	N/A	N/A	N/A	N/A	3.45E-01	**3.86E-02**	**3.18E-04**	N/A

Table S17. P-values for Dunn's test with the Shannon diversity of genes associated with nitrogen metabolism copies. "N/A" indicates the test was not appropriate and p-values in bold indicate a significant difference ($p \leq 0.05$).

Test	**Dunn's test**								
Null Hypothesis	$\mu_1 = \mu_2$								
Genes	***napA***	***narG***	***nifH***	***nirA***	***nirB***	***nirK***	***nirS***	***norB***	***nosZ***
Conventional tillage - No tillage	1.00E+00	1.00E+00	N/A	N/A	N/A	**4.88E-05**	N/A	**0.00E+00**	N/A
Conventional tillage - Reduced input	**1.37E-02**	1.75E-01	N/A	N/A	N/A	1.63E-01	N/A	**5.00E-07**	N/A
Conventional tillage - Biological based	**1.77E-04**	1.42E-01	N/A	N/A	N/A	1.46E-01	N/A	**2.62E-02**	N/A
No tillage - Reduced input	9.11E-01	1.42E-01	N/A	N/A	N/A	1.46E-01	N/A	**0.00E+00**	N/A
No tillage - Biological based	6.29E-02	1.75E-01	N/A	N/A	N/A	1.63E-01	N/A	**0.00E+00**	N/A
Reduced input - Biological based	6.35E-01	**5.27E-05**	N/A	N/A	N/A	1.00E+00	N/A	**3.18E-04**	N/A

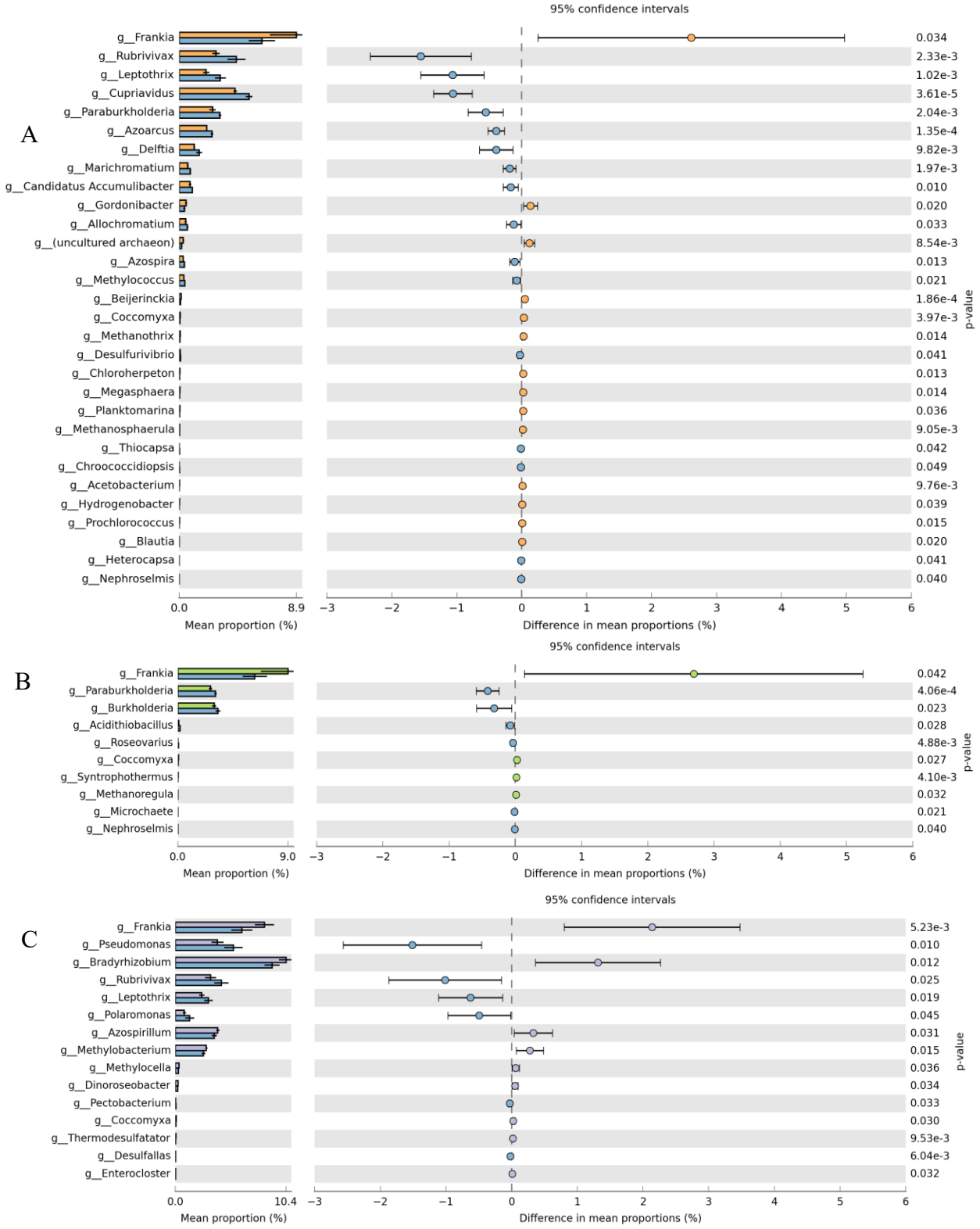
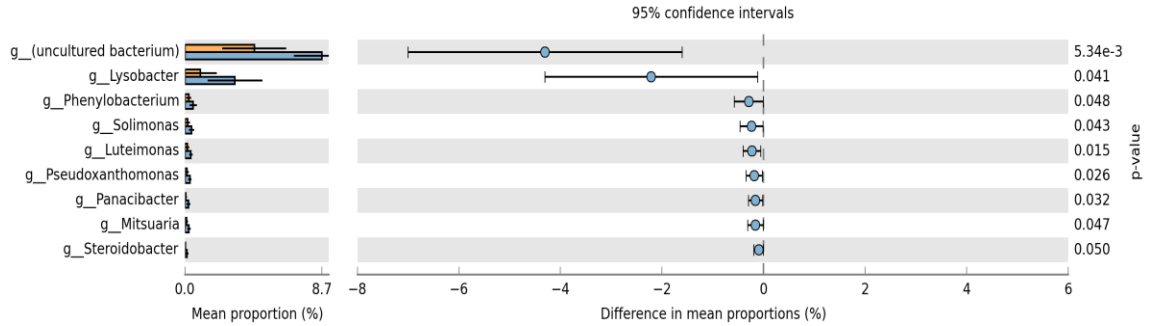
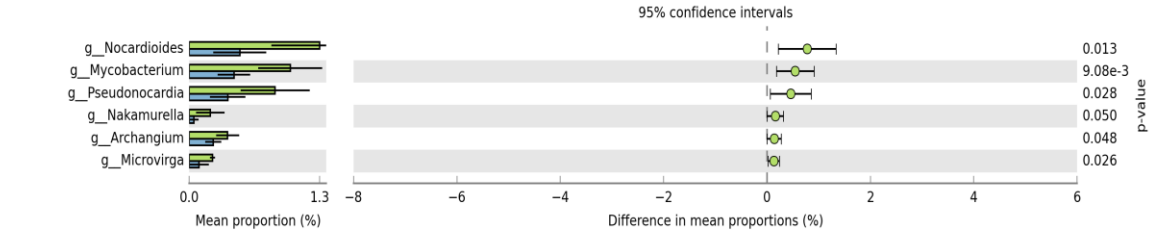


Figure S1. Genera with *nifH* genes significantly different between conventional tillage soil (in blue) and the other soils from the assembled contigs. Those enriched in no tillage soil compared to conventional tillage soil are shown in yellow (A), those enriched in reduced input soil compared to conventional tillage soil are shown in green (B) and those enriched in biologically based soil compared to conventional tillage soil are shown in purple (C). The data (generated in Megan, six metagenomes for each soil) were analyzed using STAMP with the two group analysis option (each soil compared to conventional tillage soil) and Welch's two sided t-test (p<0.05).

A



B



C

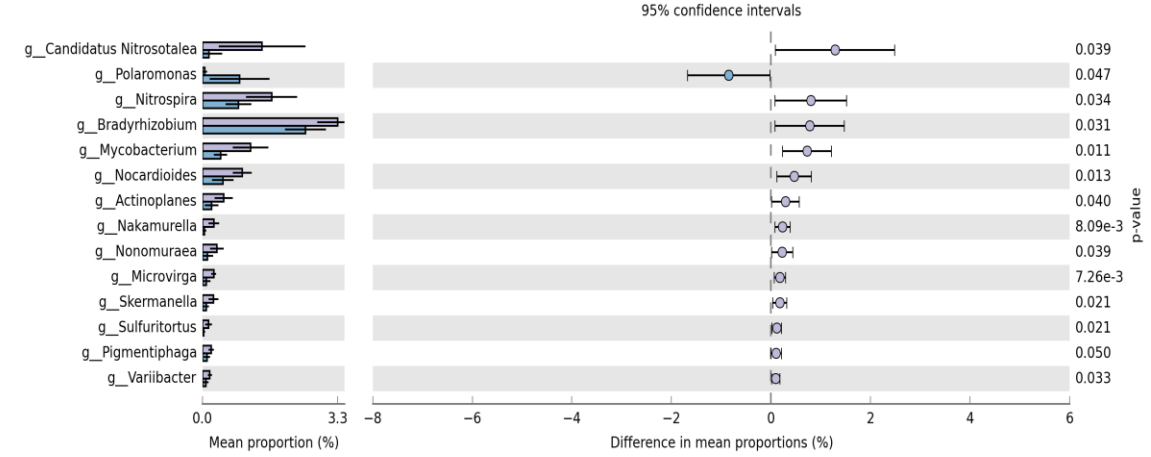


Figure S2. Genera significantly different between conventional tillage (in blue) and the other three soils from the assembled contigs. Those enriched in soil 1 compared to no tillage soil are shown in blue (no genera were enriched in no tillage soil compared to conventional tillage soil) (A), those enriched in reduced input soil compared to conventional tillage soil are shown in green (B) and those enriched in biologically based soil compared to conventional tillage soil are shown in purple (C). The data (generated in Megan, six metagenomes for each soil) were analyzed using STAMP with the two group analysis option (each soil compared to conventional tillage soil) and Welch's two sided t-test (p<0.05).