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ARTICLE

Bulk soil bacterial community structure and function respond to long-term organic and conventional agricultural management

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Abstract: Understanding how soil microbiomes respond to management is essential to maximizing soil health. We contrasted microbiomes in bulk soil under long-term organic and conventional management in a grain production setting. Management category significantly impacted the relative abundances of 17% of the most abundant taxa. Both conventional and organic management favored particular taxa, but these effects were not reflected in summary richness and diversity indices. Management systems also lead to differences in soil edaphic properties, including pH and nutrient status; this may have been the mechanism by which change in the prokaryote community was enacted. Community change between years of sampling was less pronounced, with only 6 taxa differentially abundant among years. Management category also impacted the abundance of functional genes related to the production and consumption of greenhouse gases. Particulate methane monooxygenase genes were more frequent in soil under organic management, while soluble methane monooxygenase genes were more frequent in soil under conventional management in 1 of 2 years. Nitrous oxide reductase genes were significantly less abundant in soils under second-year alfalfa than in soils under corn. This work highlights the ability of agricultural management to enact broad rearrangements to the structure of bulk soil bacterial communities.

Key words: soil microbiome, amplicon sequencing, crop rotation, organic agriculture.

Résumé : Il est essentiel de comprendre comment le microbiome des sols répond au mode de culture afin de maximiser la santé de ces sols. Les auteurs ont comparé les microbiomes de sols en vrac soumis à une culture biologique ou conventionnelle à long terme dans une exploitation agricole céréalière. Le type de culture avait un impact significatif sur l'abondance relative de 17 % des taxons les plus abondants. La culture conventionnelle comme la culture biologique favorisaient des taxons en particulier, mais ces effets ne se reflétaient pas sur le plan des indices de richesse et de diversité. Le type de culture modifiait aussi les propriétés édaphiques du sol, dont le pH et l'état nutritif; il pourrait s'agir d'un mécanisme par lequel les changements dans la communauté des procaryotes sont provoqués. Les changements au sein de la communauté en fonction de l'année d'échantillonnage étaient moins prononcés, l'abondance différentielle de 6 taxons seulement étant affectée au cours des années. Le type de culture avait aussi un impact sur l'abondance de gènes fonctionnels liés à la production et à la consommation des gaz à effet de serre. Des gènes codant la méthane monooxygénase particulaire étaient plus fréquents dans le sol traité de façon biologique, alors que les gènes codant la méthane monooxygénase soluble étaient plus fréquents dans le sol traité de manière conventionnelle pendant une année sur deux. Les gènes codant la réductase d'acide nitreux étaient significativement moins abondants dans les sols cultivés pour une deuxième année avec de la luzerne comparativement à des sols cultivés avec du maïs. Ce travail met en lumière la capacité des pratiques agricoles de provoquer d'importants changements dans la structure des communautés bactériennes des sols en vrac. [Traduit par la Rédaction]

Mots-clés : microbiome du sol, séquençage d'amplicons, rotation des cultures, agriculture biologique.

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Introduction

The activities of soil microorganisms undergird many of the ecological and biogeochemical processes by which agroecosystem functioning impacts sustainability-related concerns, such as nutrient loading to surface waters or the exchange of greenhouse gases with the atmosphere. Soil microorganisms are ultimately responsible for the transformation of fresh inputs of plant residues into stabilized soil organic matter. Soil microbial communities foster carbon sequestration and actively cycle nitrogen and other nutrients that are critical for crop production. Given the large portion of the Earth's surface under active agricultural management, it is thus essential that we understand how agricultural management influences soil microbial communities, in terms of both structure (i.e., which taxa are present, and how abundant are they?) and function (i.e., what processes are these microbes able to carry out?).

Several recent reports have indicated that agricultural management can shift the functional activity of soil microbiomes. Using a microarray technique, Xue et al. (2013) demonstrated that low-input agricultural management practices can significantly increase microbial functional gene diversity related to nutrient cycling, even if rotation system is held constant. Using a microcosm experiment with soils from vegetable production systems, Bonanomi et al. (2016) showed that the soil microbiomes under long-term organic management exhibited greater functionality, measured as rate of wood decay, than under conventional management. Similarly, crop rotational diversity has been shown to impact the rate of microbial processing of fresh residues (McDaniel et al. 2014).

Among the diverse and vital functions performed by soil microbial communities in agroecosystems are many chemical transformations related to the biogeochemical cycling of greenhouse gases. Understanding the impacts of agroecosystem management decisions on microbial functions connected to greenhouse gas fluxes from soils is particularly relevant to efforts aimed at estimating the potential for agricultural lands to mitigate emissions from other sectors of the economy (Chambers et al. 2016). Beyond carbon dioxide, nitrous oxide and methane are among the principal greenhouse gasses that can be exchanged by agroecosystems. Because the mechanisms of biological transformation of nitrous oxide and methane are well-understood, it is possible to directly monitor the abundance of genes whose products are responsible for particular chemical transformations (Holmes et al. 1995; Fuse et al. 1998; Costello and Lidstrom 1999; Jones et al. 2013). Although gene abundances are removed by some distance from actual process rates, assessing functional gene abundances has proven useful, for example, in modeling biogeochemical processes (Li et al. 2017). Thus, we are able to ask whether agricultural land management shifts microbial functions that may not be the deliberate target of management, but that nonetheless carry important ramifications for sustainability.

Reductionist approaches that isolate the effects of individual land management practices on soil microbial communities are powerful tools for revealing drivers and mechanisms underlying microbial community change. However, contrasts at the scale of whole systems can provide complementary insights and ultimately are required to account for complex interactions among multiple drivers and emergent outcomes from simultaneously acting mechanistic forces. Furthermore, land managers often adopt a suite of management practices as an integrated bundle (Drinkwater 2016). For instance, many farmers who use winter cover crops also manage without tillage. Similarly, farmers aiming for certified organic products are restricted from using chemical inputs, are required to employ more complex or extended rotations, and are likely to make use of organic amendments such as compost. While comparisons made at the systems level make it difficult to tie observed differences to a precise mechanism, the systems contrast can be informative in revealing the cumulative or integrated effects of a suite of related management practices (Brandt et al. 2010).

Our objective in this work was to contrast the effects of organic and conventionally managed corn- and soybean-based cropping systems on the structure of soil prokaryote communities and on functional potential related to the cycling of greenhouse gases. We hypothesized that long-term management impacts would be measurable as differences in the composition and structure of the bulk soil microbiome and in the abundances of functional genes of interest.

Materials and methods

Field experiment

The field site is located on an Iowa State University research farm near Greenfield, Iowa, USA (lat 41.28, long 94.45). Samples were collected from a long-term experiment, managed continuously since 1998, on an approximately 7 ha ridge top field with uniform slopes of 0%–2%. The predominant soil at the site is a moderately well-drained Chernozem (U.S. soil taxonomy: Macksburg silty clay loam; fine, smectitic, mesic Aquic Argiudoll). Cropping system treatments represent typical crop rotations planted by conventional and organic farmers in the region, and consist of a conventionally managed corn (*Zea mays*) – soybean (*Glycine max*) rotation (C–S), an organically managed rotation of corn–soybean–oats (*Avena sativa*) with alfalfa (*Medicago sativa*) (C–S–O/A), and an organic corn–soybean–oats with alfalfa–alfalfa rotation (C–S–O/A–A). In the organic treatments, a rye (*Secale cereale*) cover crop is planted between the corn and soybean phase (October–April) to aid in weed management, per local organic practices. All phases of each rotation

are grown every year and all treatments are replicated 4 times. Plots (43 m × 21 m) were assigned to cropping treatments in a completely randomized design.

Crop varieties are identical between conventional and organic systems and are selected each year based on input from an Advisory Committee regarding the most useful traits for high yields and pest resistance. Planting dates are identical for organic and conventional plots, and farm-sized equipment is used for plot management. Conventional management followed Iowa State University recommendations for fertilization (32% urea and ammonium nitrate applied at 160 kg N/ha) and pre- and post-planting herbicide application. Organic management practices are certified as such by the Iowa Department of Agriculture and Land Stewardship Organic Program. Organic fertilization used composted chicken manure, supplying similar nitrogen input rates as in the conventional system. Weed management via rotary hoeing and cultivation averages 4 cultivation passes per season in the organic system. Additional details on this long-term experiment have been reported previously (Delate et al. 2013).

Sample collection and processing

We collected soil samples from the field experiment in each of 2 successive years (21 October 2014 and 21 October 2015). Soil samples were collected at the end of the growing season to minimize variation in soil properties associated with management activities. Corn, oats, and soybeans were senescent, while alfalfa remained alive. For each soil collection, 5 soil cores (32 mm diameter, 15 cm depth) were collected from inter-row locations across the plot, combined to produce 1 composite soil sample per plot, transported on ice to the laboratory, and stored at 4 °C prior to processing. As described by Delate et al. (2013), standard methods for soil analysis were utilized to measure aggregate stability, bulk density, pH, and the content of organic carbon, nitrogen (total, inorganic, NH_4, NO_3, potentially mineralizable), potassium, phosphorus, microbial biomass carbon and nitrogen, particulate organic matter, electrical conductivity, calcium, and magnesium.

On the same dates, a second sample set was collected for microbiome profiling (collected separately to allow for more rapid freezing of samples). For this purpose, surface residue was cleared from a location midway between the crop row and the center of the inter-row and an 18-mm-diameter soil core was collected to a 15 cm depth. Three subsamples were collected per plot and were retained individually (9 crop-by-rotation combinations × 4 replicate plots × 3 subsamples per plot = 108 soil cores per year). Soil cores were bagged and transported on ice to the laboratory, where they were stored at –20 °C and were processed for DNA extraction within 8 weeks. Prior to DNA extraction, soil samples were mixed individually by hand to homogenize. The PowerLyzer PowerSoil DNA Isolation kit (MO BIO) was used to isolate DNA from soil. Duplicate extractions, each using 250 mg of soil, were processed per soil core and pooled.

Amplicon library preparation and sequencing

We used polymerase chain reaction (PCR) to generate amplicons of ribosomal RNA genes from prokaryotes for high-throughput sequencing. We used primers F515 and R806 (Caporaso et al. 2011) to amplify the V4 hypervariable region of the 16S rRNA gene. Primers were modified with 5′ overhangs for compatibility with the MiSeq sequencer and to provide sample-specific barcode indices, as in Kozich et al. (2013).

Components of the PCR solution included 1.25 U of *Taq* polymerase with associated reaction buffer (Invitrogen), dNTPs at 200 nmol/L final concentration, forward and reverse primers (1 μmol/L each), 2 μL of template DNA, and nuclease-free water to a total volume of 50 μL per reaction. Thermocycling consisted of 95 °C for 2 min; 31 cycles of 95 °C for 20 s, 60 °C for 15 s, and 72 °C for 1 min; and final extension at 72 °C for 10 min. Successful amplification was confirmed by agarose gel electrophoresis and visualization with ethidium bromide staining. Clean up of PCR products and normalization of per sample contribution to the pooled library was performed simultaneously, using the SequalPrep Normalization kit (Invitrogen). Amplicon concentration in the pooled libraries was assessed using a qPCR Library Quantification kit (KAPA Biosciences). Determination of average fragment size and final library quality assessment was performed using the TapeStation instrument (Agilent Technologies).

A bacterial mock community control sample (Allen et al. 2016) was processed in triplicate from the PCR stage. Negative control samples were processed from the DNA extraction step forward (i.e., as DNA extractions performed in the absence of soil). Negative controls are useful for assessing whether contaminated laboratory reagents may have contributed observations of taxa not found in the biological samples.

Amplicon sequencing was performed on the MiSeq instrument using version 3 reagent kits (Illumina), at the National Animal Disease Center of the USDA Agricultural Research Service (Ames, Iowa). PhiX phage DNA was included with each library to increase sequence diversity as required for best performance by the MiSeq instrument. Separate runs were performed for the samples from 2014 and for the samples from 2015. Reads were split by index barcode using default settings on the MiSeq instrument, and data was exported in .fastq file format. Amplicon sequence data have been deposited at the NCBI sequence read archive as study SRP097709.

Processing of amplicon sequences

Sequence data processing was performed using the software Mothur version 1.39.5 (Schloss et al. 2009), VSearch version 2.7.1 (Rognes et al. 2016), and R version 3.2.3 (R Core Team 2015).

Because of the relatively short length of our amplicon, we were able to use fully overlapping sequences to form contigs, which significantly improves data quality compared with contigs that do not fully overlap (Kozich et al. 2013). Contigs were culled if they contained any ambiguous bases or homopolymers longer than 15 nt or if they were shorter than 100 nt in length. Reads passing these criteria were aligned to the Silva reference alignment (version 132; Quast et al. 2013). Poorly aligning reads were culled, reads were screened to a consistent amplicon, and reads differing by up to 2 bp were preclustered. Chimeras were detected using VSearch, with the reference set sequentially to self and then to the classification database, and were removed.

Remaining reads were classified against the Silva nonredundant database (version 132) using the Bayesian classifier (Wang et al. 2007) implemented in Mothur, with a confidence threshold of 80%. Reads identified as mitochondria or chloroplast or that could not be assigned below the rank of *Bacteria* were culled. Reads were binned into operational taxonomic units (OTUs) using abundance-based greedy clustering (VSearch), at a cutoff dissimilarity of 0.03. Singletons (OTUs consisting of only 1 read cumulatively across all samples) were culled as unreliable observations. The mock community and negative technical control samples were clustered and classified together with the biological samples.

A complete record of commands issued during data processing is provided (Supplementary Material – Data Processing[1]).

Quantitative PCR

From the same DNA extracts that were used for amplicon sequencing, we used qPCR to assess the abundance of several microbial genes that play a role in the cycling of methane and nitrogen — genes for particulate and soluble methane monooxygenase enzymes (pMMO, sMMO), and genes for nitrous oxide reductase, clade II (NosZII). To be able to scale measures of functional gene abundance by total prokaryote community density and to avoid the confounding effects of variable DNA extraction efficiency among samples, we also measured total 16S ribosomal RNA (rRNA) gene copy number. Primers and thermocycling conditions for each assay are provided in Supplementary Table S1[1].

All qPCR reactions consisted of iQ SYBR Green Supermix (BioRad), primers at a final concentration of 1 μmol/L each, 2 μL of template DNA, and nuclease-free water to a total volume of 25 μL. Each qPCR run included a standard curve of known quantity, spanning 5 orders of magnitude and run in triplicate. For the pMMO and sMMO assays, standard curves of known copy number were prepared from synthesized DNA (matching GenBank accession No. AF150785.1, and positions 353–715 of AJ458523.1; Invitrogen GeneArt Strings). For the NosZII assay, a standard curve was prepared from plasmid DNA containing the amplicon of interest from *Geobacillus thermodenitrificans* ATCC 29492. Specifically, the NosZII amplicon was generated by PCR and cloned into chemically competent *Escherichia coli* using the TOPO TA cloning kit (Invitrogen). Plasmid DNA was isolated from an overnight culture of the resulting clone, and copy number was calculated from concentration, based on the size of the plasmid and the size of the insert. For the 16S rRNA gene assay, genomic DNA was extracted from 10^9 cells of an overnight culture of *E. coli* and serially diluted. Seven copies of the 16S rRNA gene per *E. coli* cell was assumed (Stoddard et al. 2015).

Each qPCR run also included controls with no template. In the 16S rRNA gene assay, the no template controls did eventually amplify, apparently due to trace amounts of background contamination. However, C_t values for the no template controls were always >10 cycles after the sample with the lowest concentration, and so this amplification was disregarded as negligible.

Statistical analyses

In processing OTU tables, we subtracted the number of reads observed for each OTU in the negative control (per year, means across three technical replicates, rounded up) from each biological sample. Sampling effort was standardized to 1000 reads per sample (13 samples with fewer than 1000 reads were dropped) via rarefying observations. Finally, OTU abundances were averaged across soil cores drawn from the same plot and means were rounded to the nearest integer. OTU abundance data that are presented here always represent mean values across subsamples (i.e., microbiome profiles at the plot scale).

In assessing OTU diversity, the exponential of the Shannon diversity metric was used to estimate α-diversities as effective species numbers ("Hill's index"; Hill et al. 2003). For assessments of phylogenetic diversity and UniFrac distances (Lozupone and Knight 2005), we used Clearcut (Sheneman et al. 2006) to generate a rough phylogenetic tree, using 1 representative sequence per OTU (selected based on abundance of sequence variants within each OTU) and the mean abundance of each OTU at the plot scale.

For visualization and contrasts of taxon relative abundance among management categories, we first dropped OTUs present at a mean relative abundance of <0.1% and transformed the OTU abundance tables using the centred log-ratio (CLR) method (Gloor and Reid 2016). CLR-transformed values express the abundance of a given taxon relative to all others in the data set (as is appropriate for compositional data) and were used to

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjm-2018-0134.

generate principal component (function *prcomp*) bi-plots for visualization. Taxa differing significantly in relative abundance between categories of land management were identified via ALDEx (ANOVA-Like Differential Expression; Fernandes et al. 2013). Only those OTUs differing significantly between land management categories were displayed on the bi-plots (ALDEx with either the Welch or the Wilcox test; corrected P value < 0.05). We also used the *envfit* function of the Vegan package for R (Oksanen et al. 2016) to test relationships between soil edaphic characteristics and the principal component ordinations derived from prokaryote community structure, via permutation test.

The *betadisper* function (Vegan) was used to perform a principal coordinates ordination on Bray–Curtis dissimilarities, to estimate the mean distance-to-centroid by grouping factor, and to assess significance via permutation test. To assess whether differences between management categories could be attributed to common or to uncommon taxa, we used the variably weighted Odum dissimilarity index, which is related to the Bray–Curtis index but can be tuned to increase or decrease taxon weights, according to abundance, in assessing pairwise community similarities (Manter and Bakker 2015). Permutational multivariate analysis of variance (ANOVA) (function *adonis* in Vegan) was used to assess the impacts of year and management category on prokaryote community structure. The R package ggplot2 (Wickham 2009) and the hierarchical data visualization tool Krona (Ondov et al. 2011) were used in preparation of figures.

For qPCR data, fluorescence values were converted to copy numbers based on the standard curve for each assay. For each functional gene target, copy numbers were expressed per 10^9 copies 16S rRNA and then log-transformed. Differences in functional gene abundances were assessed with ANOVA, using additive models including either year and management category, or year and rotation system. The Tukey method was used for post-hoc contrasts.

Results

Profiling of bulk soil microbiomes

Our sequencing effort yielded 10.6 million reads passing quality screening, divided among 27 461 OTUs. Of these, 26 OTUs were found only in the mock community samples, and 6 OTUs were found only in the negative controls. Subtracting observations in negative control samples from the biological samples resulted in the loss of 26 OTUs; 19 124 OTUs were lost with rarefication, and 5689 OTUs were lost when mean values across subsamples were rounded down to zero. Of the remaining 2590 OTUs, the majority were very rare; just 157 OTUs were present at a mean relative abundance of >0.1% of sequence reads.

Inclusion of bacterial mock community control samples demonstrated a low error rate and accurate classification of OTUs (Supplementary Material — Mock Community[1]). Based on the known 16S rRNA gene sequences for the members of the mock community, the per nucleotide error rate in our processed amplicon sequence data was 0.014%.

With slight variations in order of relative abundance between years, the dominant phyla present at our field site were *Acidobacteria*, *Verrucomicrobia*, *Proteobacteria*, *Thaumarchaeota*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*, *Armatimonadetes*, *Firmicutes*, *Gemmatimonadetes*, and *Latescibacteria* (Fig. 1). Additional phyla were detected at low relative abundances; a complete list of observed prokaryote OTUs is provided (Supplementary Table S2[1]).

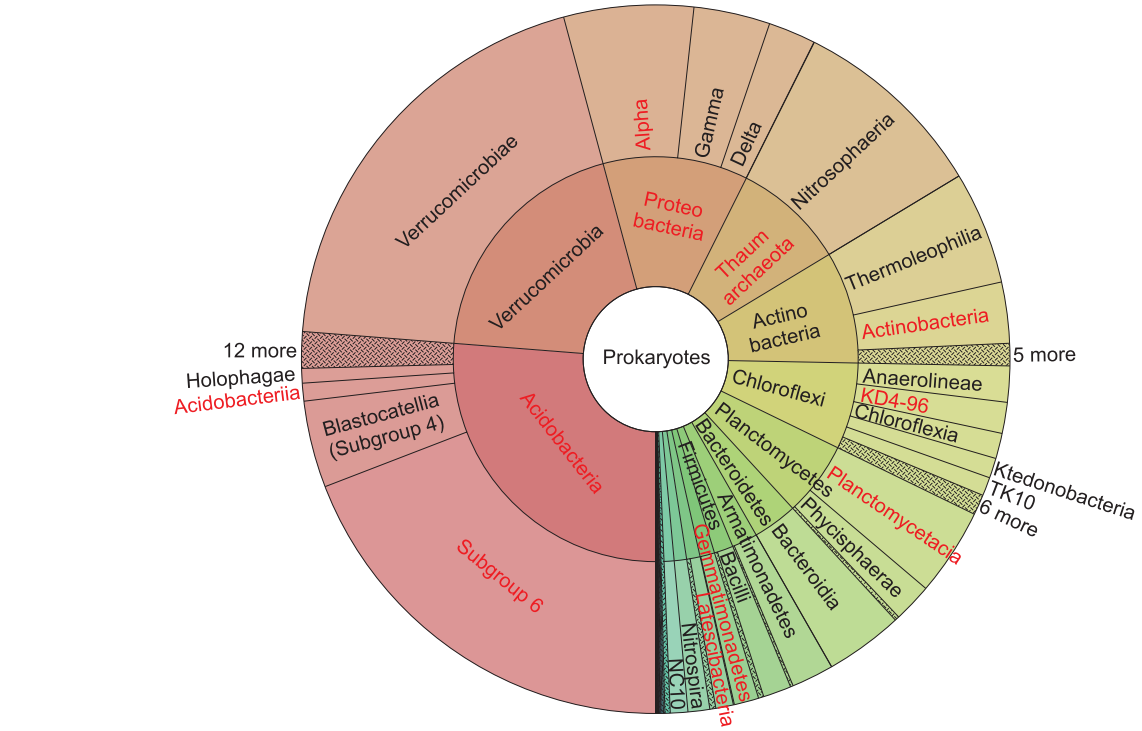
Year of sampling discriminated poorly among observed community profiles, and the relative abundances of just 6 OTUs differed significantly among years (Supplementary Fig. S1[1]). At coarser taxonomic ranks, 3 phyla (*Gemmatimonadetes*, *Proteobacteria*, and *Thaumarchaeota*) and 3 classes (*Acidobacteria* subgroup 6, *Actinobacteria*, and *Alphaproteobacteria*) differed in relative abundance between years (Fig. 1).

Contrasting effects of conventional and organic management

Prokaryote communities could be readily distinguished by management category along axis 1 of a principal component ordination (Fig. 2), which explained 14.3% of the variance. Seven OTUs were significantly enriched in bulk soils under conventional management, while 19 OTUs were significantly enriched in bulk soils under organic management (Table 1; Fig. 2; ALDEx; P < 0.05). The OTUs that differed significantly in relative abundance tended to belong to phyla that are typically considered free-living bulk soil inhabitants and not phyla that are commonly found as plant-associated. For instance, one quarter of the abundant OTUs within the phylum *Acidobacteria* differed significantly in relative abundance among management categories (9 of 36), while only 1 of the 21 common *Actinobacteria* OTUs differed between management categories (Table 1). It is notable that in some cases OTUs with the same classification responded oppositely to management; for instance, 1 OTU within each of *Acidobacteria* subgroup 6, *Chitinophagaceae*, and *Sphingomonas* were enriched by conventional management, while different OTUs within the same taxa were enriched by organic management (Fig. 2). Aggregated at broader taxonomic ranks, the relative abundances of 1 phylum and of 4 classes differed significantly in relative abundance between conventional and organic management (Fig. 1).

A number of soil edaphic characteristics showed significant relationships with prokaryote community structure. In general, prokaryote communities of soils under organic management experienced higher nutrient status (potassium, phosphorus, potentially mineralizable nitrogen) and higher pH, compared with soils under conventional management (Fig. 2). Notably, inorganic

Fig. 1. Taxonomic summary of bulk soil prokaryote communities at a field site in Iowa, USA. Shown is the simple proportion of reads in each phylum (inner ring) and class (outer ring), across years and management categories. Phyla and classes for which relative abundance differed significantly between years or between management categories (ALDEx, $P < 0.05$) are labeled in red, and corresponding box plots are shown below. Boxplots indicate median, first, and third quartiles. Whiskers extend up to 1.5 times the interquartile range. Con, conventional agricultural management; Org, organic agricultural management.



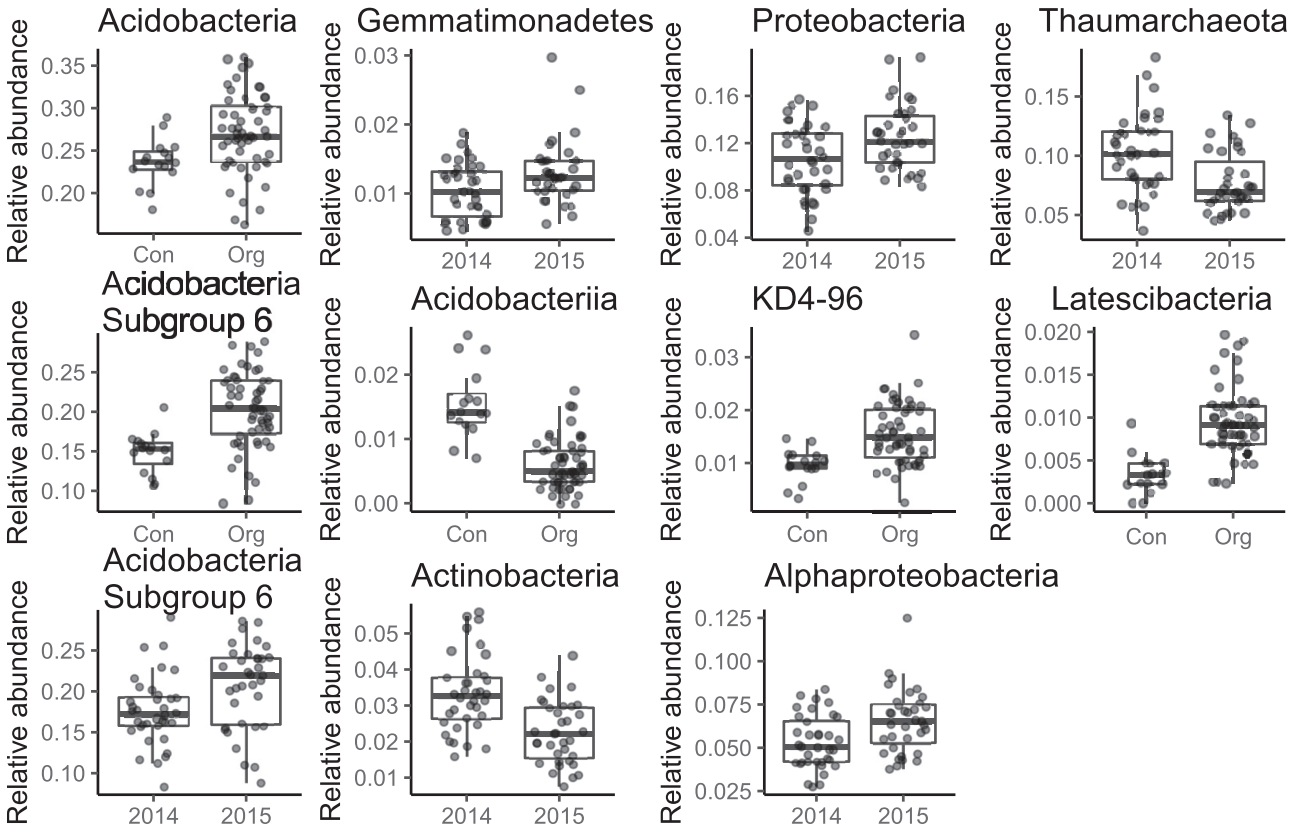


Fig. 2. A principal component ordination of soil prokaryote community structure, with samples colour-coded by management category. Underlying data are the transformed counts (centred log-ratio method) of partial 16S rRNA gene sequences for operational taxonomic units (OTUs) present at a mean relative abundance across samples of >0.1%. Taxon names and blue vectors are shown only for those OTUs whose relative abundances were significantly impacted by management (ALDEx; $P < 0.05$). Black vectors reflect soil edaphic parameters with significant fit to the ordination (permutation test; $P < 0.05$). BD, bulk density (g/cm^3); Ca, calcium content (mg/kg); EC, electrical conductivity (μS/cm); InorgN, inorganic nitrogen content (mg/kg); K, potassium content (mg/kg); MBC, microbial biomass carbon (mg/kg); Mg, magnesium content (mg/kg); NO3, nitrate content (mg/kg); P, phosphorus content (mg/kg); pH, potential hydrogen; PMINN, potentially mineralizable nitrogen (mg/kg); SOC, soil organic carbon (μg/g); TN, total nitrogen (μg/g).

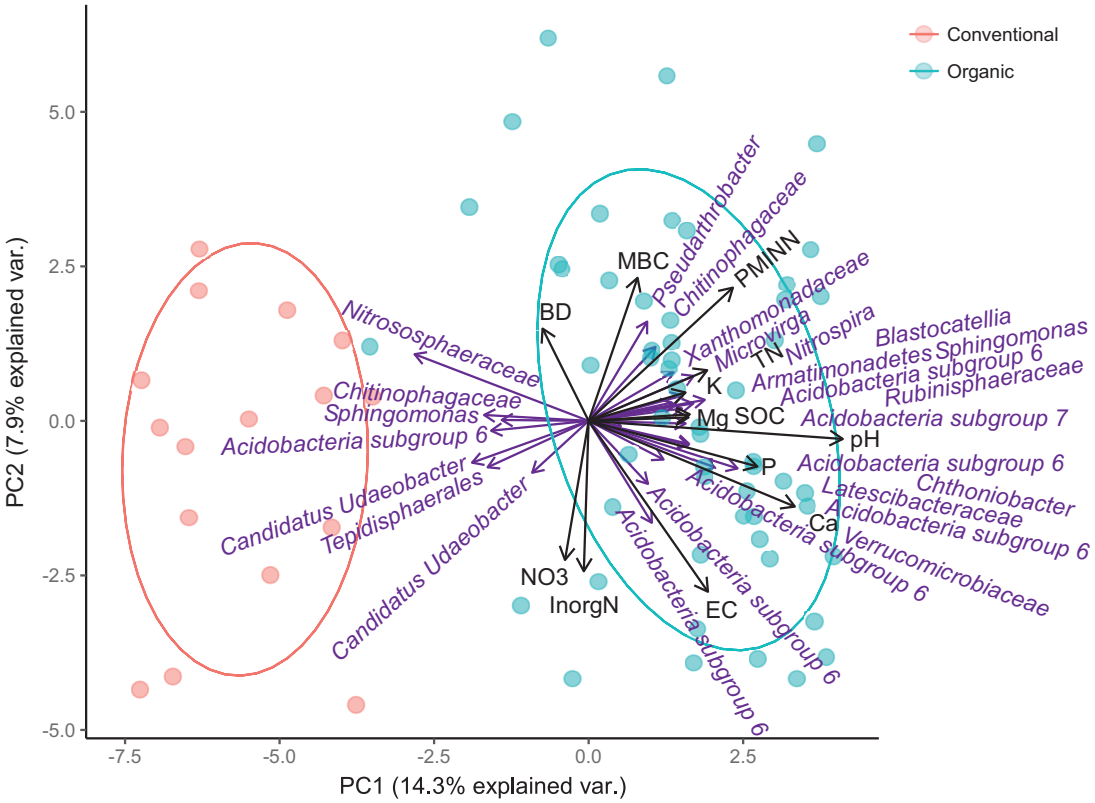


Table 1. Abundant operational taxonomic units (OTUs) (mean relative abundance > 0.1%, across treatments and years) whose relative abundance was significantly impacted by agricultural management system, summarized at the rank of phylum.

			No. enriched in:	
Division	Phylum	No. of abundant OTUs	Conventional	Organic
Archaea	*Thaumarchaeota*	6	1	0
Bacteria	*Acidobacteria*	36	1	8
	Actinobacteria	21	0	1
	Armatimonadetes	5	0	1
	Bacteroidetes	10	1	1
	BRC1	1	0	0
	Chloroflexi	15	0	0
	Firmicutes	2	0	0
	Gemmatimonadetes	2	0	0
	Latescibacteria	1	0	1
	Nitrospirae	3	0	1
	Planctomycetes	12	1	1
	Proteobacteria	21	1	3
	Rokubacteria	2	0	0
	Verrucomicrobia	20	2	2
	Sum	157	7	19

nitrogen availability differed primarily among years (i.e., fitting with the second principal component axis; Supplementary Fig. S1[1]).

Despite the ready ability to distinguish soil microbiomes exposed to conventional vs. organic management, via ordination on community profiles, summary measures such as richness and diversity indices did not capture this difference; neither year of sampling nor management category were significant factors in explaining prokaryote OTU richness or diversity (data not shown; *t* tests, $P > 0.05$). Neither did β-diversity (variability in community structure among samples) differ between management categories (data not shown; permutation test, $P = 0.46$ for Bray–Curtis dissimilarities; $P = 0.60$ for weighted UniFrac distances).

Increasing the influence of abundant taxa on an adjustably weighted dissimilarity index decreased the significance of both year of sampling and of management category as explanatory factors for variability among samples (Fig. 3). Thus, impacts on less common taxa were an important component of the changes brought about to bulk soil microbiomes by contrasting agricultural management practices.

Frequencies of genes related to methane oxidation and nitrous oxide reduction

pMMO genes were present at higher frequencies (relative to overall 16S rRNA gene copy number) in soils under organic management than under conventional management (Fig. 4; *t* tests, $P < 0.001$ in each year). This difference remained when contrasts were limited to a given crop species (i.e., organic vs. conventional corn; organic vs. conventional soybeans; *t* tests, $P < 0.0025$). Within individual rotation systems, the pMMO gene frequencies sometimes differed among crop species. Specifically, pMMO gene frequencies were significantly lower for oats with alfalfa than for corn or soybean in the 3-year organic rotation in 2014, and were lower for alfalfa than for corn in the 4-year organic rotation in 2015 (ANOVA with Tukey contrasts, $P < 0.05$; data not shown). This makes the overall contrast between management systems even more notable; the organic system includes rotation crops that tend to lower pMMO gene frequencies, yet maintains an overall average higher pMMO gene frequency compared with the conventional system.

Soluble methane monooxygenase gene (sMMO) frequencies were less sensitive to management impacts. In 2014, conventional management supported higher sMMO gene frequencies compared with organic management (Fig. 4; *t* test, $P = 0.015$). However, within individual crop species, this difference held only for soybean ($P = 0.024$) but not for corn ($P = 0.41$). Management systems did not differ in sMMO gene frequencies in 2015 (Fig. 4; $P = 0.48$) and crop species within individual rotation systems did not differ for sMMO gene frequencies in either year (data not shown).

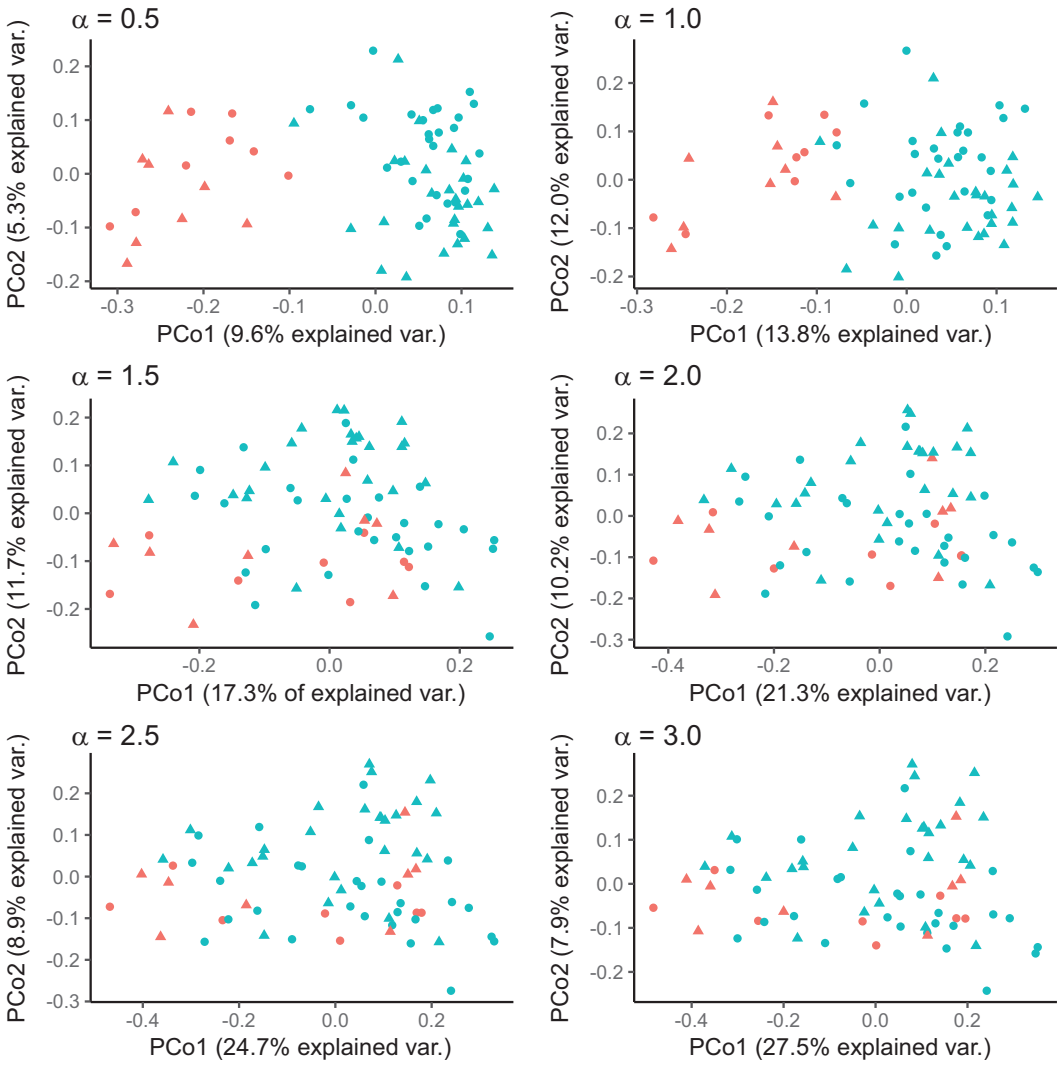
Nitrous oxide reductase gene frequencies did not differ significantly between land management categories ($P > 0.1$; data not shown), but did vary with plant cover; in 2014, NosZII genes were significantly less abundant in plots growing second-year alfalfa, compared with plots growing corn (Fig. 5; ANOVA with Tukey contrasts, $P = 0.046$) or, with weaker support, soybean ($P = 0.067$). However, NosZII gene densities were highly variable among the 4 plots in second-year alfalfa, suggesting that these differences should be more robustly tested. Relationships between NosZII gene frequencies and soil nitrate concentrations were not significant (2014, $R^2 = 0.094$, $P = 0.068$; 2015, $R^2 = 0.0042$, $P = 0.71$), and soil nitrate concentrations were not at their lowest under second-year alfalfa, being still lower under the previous oats–alfalfa bi-culture phase of the rotation (in 2014, mean soil nitrate concentrations were 1.32 ± 0.13 mg NO_3/kg soil under oats–alfalfa, 2.22 ± 0.31 for alfalfa, 3.86 ± 0.28 for corn, 3.85 ± 0.31 for soybean).

Discussion

We have demonstrated that agricultural management can significantly impact the relative abundances of a substantial portion of the dominant members of bulk soil bacterial communities, leading in turn to alterations in functional potential. Our results highlight the potential for land management decisions to influence processes and microbial ecosystem services that are not likely to be among the intended outcomes of management decisions. For instance, in our long-term field site, organic management consistently and significantly enriched the frequency of particulate methane monooxygenase genes among soil bacteria. We note that these functional gene frequencies may not translate directly into process rates or net fluxes. Nevertheless, with sufficient understanding, agricultural management may adopt explicit aims related to shifting the structure of soil microbiomes in advantageous directions (Bakker et al. 2012; Hartman et al. 2018) or fostering beneficial ecosystem services such as the net consumption of atmospheric methane.

Our experimental system could not address the mechanisms by which management systems influence methanotroph frequency. In aquatic systems, with methane generation occurring in anoxic sediments and oxygen diffusing from above, methanotrophs have been shown to preferentially colonize substrates at the oxic–anoxic boundary (Reim et al. 2012), which may suggest connections to soil bulk density or aggregate structure in our system. However, these patterns may differ in environments, such as nonsaturated soils, in which both methane and oxygen originate primarily from the same atmospheric source. It is possible that manure inputs to the organic system represented an on-going immigration or input of methanotrophic taxa, as methanotrophs are known constituents of manure and rumen environments (Mitsumori et al. 2002; Hoefman et al. 2014). The

Fig. 3. Principal coordinate ordinations and statistical tests of the significance of year of sampling and of management category in differentiating bulk soil prokaryote communities. The underlying index of dissimilarity is the variably weighted Odum score, which can be tuned with parameter α to give increasing influence to abundant or to rare taxa. At $\alpha = 1$, this dissimilarity index is equivalent to the commonly used Bray–Curtis index. Statistical testing used a permutational multivariate ANOVA using dissimilarity matrices.



wOdum	Year			Management		
α	$F_{1,68}$	R^2	P	$F_{1,68}$	R^2	P
0.5	2.70	0.035	< 0.001	6.46	0.084	< 0.001
1.0	3.89	0.048	< 0.001	8.66	0.11	< 0.001
1.5	4.80	0.059	< 0.001	8.28	0.10	< 0.001
2.0	4.37	0.056	0.008	6.31	0.080	0.002
2.5	3.59	0.047	0.029	5.18	0.067	0.011
3.0	2.94	0.039	0.061	4.62	0.061	0.016

Increasing influence of the most abundant taxa on dissimilarity scores

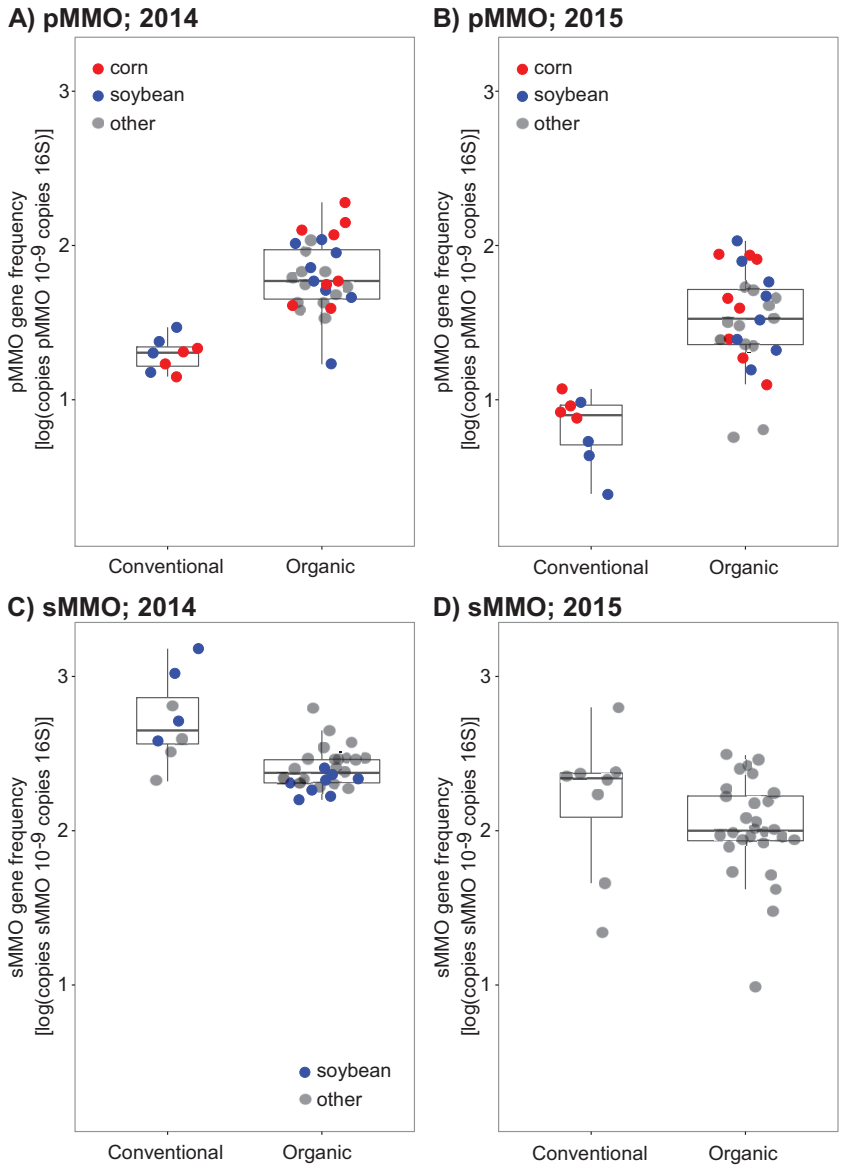
Year: 2014, 2015

Management: Conventional, Organic

complexity of the soil environment offers abundant opportunity for niche differentiation, which may be important to the co-existence of methanotrophic taxa once they are brought into a common environment (Reim et al. 2012; Bodelier et al. 2013; Ho et al. 2013).

Reduction of nitrous oxide is an important microbial function from the perspective of climate change and heat-trapping atmospheric gases (Melillo et al. 2014). In this regard, our assessment of NosZII gene frequencies demonstrates that agricultural management has the potential to impact off-target microbial functions related to environmental quality and agro-ecosystem sustainability. However, reduction of nitrous oxide is only 1 component of much larger nitrogen cycling pathways. More focused investigations, including additional functional genes as well as measuring actual process rates, will be required to deepen our understanding of how divergent management practices have altered nitrogen cycling in

Fig. 4. Assessment via qPCR of the frequency of particulate methane monooxygenase (pMMO) genes in (A) 2014 and (B) 2015; and of soluble methane monooxygenase (sMMO) genes in (C) 2014 and (D) 2015. Boxplots indicate median, first and third quartiles. Whiskers extend up to 1.5 times the interquartile range.

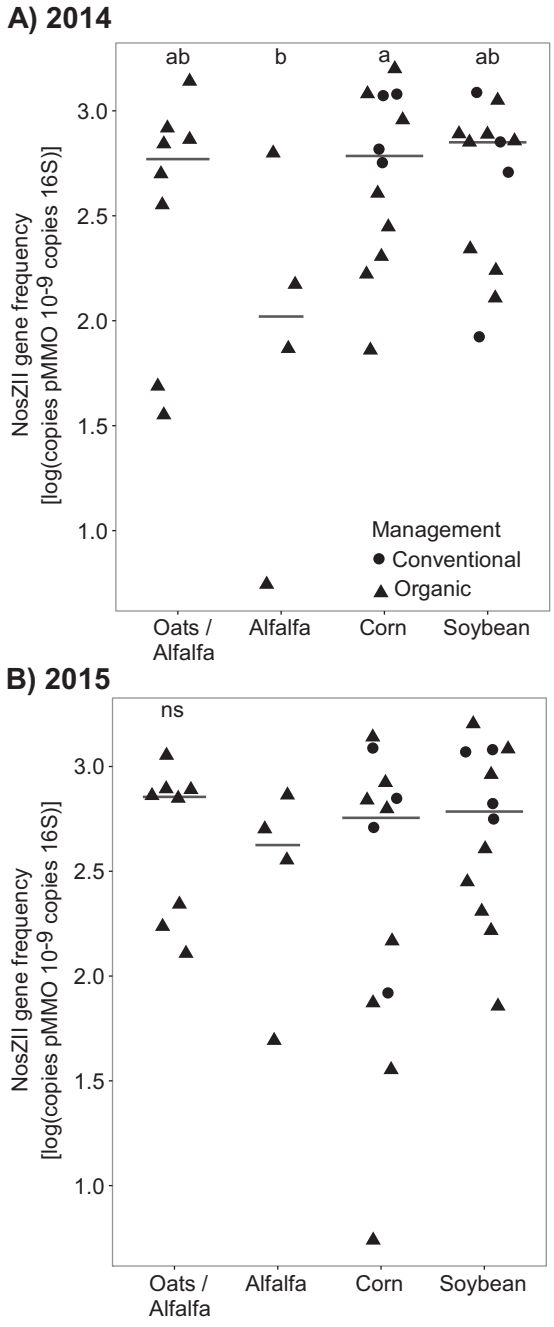


this field experiment. For instance, measured NosZII gene frequencies did not clearly relate to soil nitrogen status. There are several possible explanations for this. We measured soil nitrate concentrations and NosZII gene frequencies on the same date, while a time lag between the two might be expected if NosZII gene frequencies respond to nitrogen availability. Furthermore, analyses of NosZII gene frequencies and of soil nitrogen status were performed on separate samples, which may have contributed to the decoupling of these variables. Finally, our NosZII assay did not provide comprehensive coverage of all nitrous oxide reductase genes present. Attempts at measuring NosZ clade I gene frequencies were stymied by poor qPCR assay performance (data not shown).

It is interesting to note that pathways for methane conversion and for nitrogen cycling may involve shared enzymes. In particular, the pMMO gene is homologous to the ammonia monooxygenase gene (Holmes et al. 1995), and in some cases the same enzyme may be able to oxidize either methane or ammonia (reviewed in Hooper et al. 1997). Such interconnectedness between functional gene pathways for conversion of disparate compounds highlights the importance of striving toward more comprehensive profiling of the functional attributes of soil microbial communities.

Soil microbial diversity appears to be positively related to soil functioning (Thiele-Bruhn et al. 2012; van der Heijden and Wagg 2013) and may be an important support to agricultural productivity. A number of studies

Fig. 5. Assessment via qPCR of the frequency of nitrous oxide reductase (clade II; NosZII) genes in (A) 2014 and (B) 2015. Horizontal lines indicate medians. Different letters indicate that means differ significantly (ANOVA with Tukey contrasts; $P < 0.05$). ns, no significant differences.



have demonstrated increased density (Henneron et al. 2015) or diversity in soil bacteria (Esperschütz et al. 2007) and fungi (Oehl et al. 2004; Sugiyama et al. 2010; Verbruggen et al. 2010) in organically managed soils compared with conventional systems. In our results, measurements of taxon richness and diversity did not differ between management systems. Another study found that soil microbial β-diversity, or variability in community structure across locations, was greater for organic than conventionally managed soils (Lupatini et al. 2017). Again, this effect was not evident in our system.

These contrasts with other findings highlight the importance of continued study of how management practices influence soil microbial community structure and functioning. Our results, in a commodity grain production system in the upper midwestern region of the United States, may not reflect findings from other regions or agricultural production systems. Given the breadth of soil types, climatic conditions, and cropping systems that agriculture encompasses, it remains important to assess the impacts of management practices on soil microbiology in diverse locations. For instance, even organic amendments of different chemical composition vary in their effects on soil microbiomes (Heijboer et al. 2016). Descriptors of management systems, such as "organic" and "conventional", similarly encompass a wide range of practices. The aggregate outcomes of these broad management categories may best be revealed by repeated contrasts in diverse geographic locations and cropping systems, an effort to which we contribute here.

In some cases, particular component practices within larger management systems have been linked to changes in soil microbiomes. For instance, application of composted manure in organic management systems has been highlighted as a factor that may explain much of the difference in soil microbial community structure between organic and conventional management systems (Esperschütz et al. 2007; Hartmann et al. 2015). Fungi associated with composted manure inputs have been shown to remain detectible in soil (Sun et al. 2016). A study in Canada suggested that crop rotation was less discriminative than soil pH changes in contrasts between organic and conventional management (Li et al. 2012). In extensive grain production systems with limited external inputs, organic management may lead to reduced microbial biomass, likely as a function of lower primary productivity (Arcand et al. 2016). A study in Brazil suggested that tillage had a larger impact on soil microbiomes than other aspects of crop management (Souza et al. 2013). Other studies have tested extreme manipulations that while mechanistically instructive, are drastic departures from agronomic production systems. For instance, 2 groups have recently demonstrated enrichment of oligotrophic bacterial taxa in systems that have withheld all external fertilizer inputs from agronomic plots for over a century (Francioli et al. 2016; Soman et al. 2017). The form and rate of fertilizer application are also known to have measurable impacts on soil microbiomes (Zhou et al. 2015; Francioli et al. 2016; Ding et al. 2017).

Our goal was not to tie changes in the soil microbiome to particular mechanistic causes, but to test whether management systems, each comprised of diverse particular decisions, result in measurably different bulk soil microbiomes. Soil edaphic characteristics were signif-

icantly related to prokaryote community structure, which is consistent with the hypothesis that impacts of agricultural management on soil microbiomes are mediated through changes to soil chemical and physical properties. These inter-relationships reinforce the importance of understanding the integrated impacts of whole systems; soil microbiomes shift in response to management practices but do so in concert with a suite of soil properties, ranging from bulk density to pH and organic carbon content. However, these interactions between microbiomes and soil properties should not be conceived as 1-directional; microbes are both impacted by soil edaphic properties (e.g., Lauber et al. 2009) and are also capable of shifting many of those properties (e.g., Fließbach and Mäder 2000).

The bulk soil prokaryote community profiles that we generated contribute to our understanding of the natural history of many poorly understood taxa. Exploring different weightings in estimating community dissimilarities revealed that treatment effects were not evident when the most abundant taxa were heavily weighted in calculating pairwise dissimilarity scores (Fig. 3). This indicates that management-driven changes to soil bacterial communities did not primarily occur in the dominant taxa, and highlights the need for further characterization of soil microbiota, including those taxa that are present at moderate and low abundances.

It will be particularly powerful to begin to develop linkages between changes in microbiome composition or structure and microbiome functional activities. However, this long-standing challenge is not easily overcome. For instance, we were not able to explore relationships between methane monooxygenase gene frequencies and the relative abundances of methanotrophic taxa, because putative methanotrophs were observed at very low frequencies in the amplicon sequence data (1 to 8 reads per sample; data not shown).

One of the difficulties in moving toward a predictive understanding of how soil microbiome functional potential will respond to management is the often broad distribution of important microbial functions across phylogenetic lineages. For example, the capacities for both reducing nitrous oxide and oxidizing methane are found in diverse taxa. This allows for substantial changes to bacterial community structure without a corresponding change in functional gene frequency. For instance, we observed significant differences in bacterial community structure but not in NosZII gene frequencies between plots under organic or under conventional management. Conversely, NosZII gene frequencies differed with contemporary plant cover, without a corresponding shift evident in overall bacterial community profile. This may be explained by divergent rates of spatial variation in the abundance of particular taxa compared with broad-scale community change.

It is challenging to devise selective strategies for enriching beneficial functions if the carriers of those functions possess divergent physiologies, habitat preferences, or competitive abilities (Bakker et al. 2012). Nevertheless, it is known that legacy effects of microbial community structure can have implications for functional outcomes that can persist for years (Martiny et al. 2017) and that rhizosphere community development is sensitive to initial bulk soil community structure (Bakker et al. 2015).

Our ability to link microbiome structure and function is further constrained by the necessarily limited scope of our estimates of functional potential, which were based on frequencies of particular genes encoding enzymes with known activities; we could only target an infinitesimal portion of the overall functional potential of the soil microbiome. There are many additional functions of great importance that are performed by soil microorganisms. These include antagonizing pathogens, enhancing access to soil nutrient pools, contributing to the development of physical soil structure, processing of organic materials, biotransforming toxic and xenobiotic compounds, and many others.

Conclusions

Using a long-term field experiment in the Midwest region of the United States, we demonstrate that bulk soil microbiomes change significantly in response to suites of agricultural management in a commodity crop production setting. Nearly 20% of the most abundant bacterial taxa were significantly impacted by assignment of plots to either organic or conventional management. These changes accompanied broader changes to soil edaphic properties. Changes in the abundance of functional genes related to the cycling of greenhouse gases were also evident. This work supports the validity of efforts to use agricultural management to enhance beneficial microbial functions.

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