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Functional Gene Differences in Soil Microbial Communities from Conventional, Low-Input, and Organic Farmlands

Kai Xue,[a] Liyou Wu,[a] Ye Deng,[a] Zhili He,[a] Joy Van Nostrand,[a] Philip G. Robertson,[b,c] Thomas M. Schmidt,[b,d] Jizhong Zhou[a]

Institute for Environmental Genomics, University of Oklahoma, Norman, Oklahoma, USA[a]; W. K. Kellogg Biological Station, Michigan State University, East Lansing, Michigan, USA[b]; Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan, USA[c]; Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, USA[d]

Various agriculture management practices may have distinct influences on soil microbial communities and their ecological functions. In this study, we utilized GeoChip, a high-throughput microarray-based technique containing approximately 28,000 probes for genes involved in nitrogen (N)/carbon (C)/sulfur (S)/phosphorus (P) cycles and other processes, to evaluate the potential functions of soil microbial communities under conventional (CT), low-input (LI), and organic (ORG) management systems at an agricultural research site in Michigan. Compared to CT, a high diversity of functional genes was observed in LI. The functional gene diversity in ORG did not differ significantly from that of either CT or LI. Abundances of genes encoding enzymes involved in C/N/P/S cycles were generally lower in CT than in LI or ORG, with the exceptions of genes in pathways for lignin degradation, methane generation/oxidation, and assimilatory N reduction, which all remained unchanged. Canonical correlation analysis showed that selected soil (bulk density, pH, cation exchange capacity, total C, C/N ratio, NO_3^-, NH_4^+, available phosphorus content, and available potassium content) and crop (seed and whole biomass) variables could explain 69.5% of the variation of soil microbial community composition. Also, significant correlations were observed between NO_3^- concentration and denitrification genes, NH_4^+ concentration and ammonification genes, and N_2O flux and denitrification genes, indicating a close linkage between soil N availability or process and associated functional genes.

Soil microbial communities are responsible for performing many critical ecosystem functions and can be important biotic indicators of soil health (1, 2). Any detrimental effects of agricultural management systems on soil microbial communities would damage the functions that they perform and hence impact the ecological services provided by soils, such as nutrient cycling and crop protection (3). Thus, it is important to gain a better mechanistic understanding of the functional diversity, composition, and dynamics of soil microbial communities under various management systems. This would help to identify the ecological consequences of various agricultural practices and the development of beneficial management strategies.

The impacts of agricultural management systems on whole soil microbial community structure have been studied intensively via phospholipid fatty acid (PLFA) analysis (4–13), fingerprinting-based techniques such as community-level physiological profiles (13, 14), denaturing gradient gel electrophoresis (10, 15), and terminal restriction fragment length polymorphism (T-RFLP) (16–18). 16S rRNA-targeted oligonucleotide probes (19), 16S rRNA gene clone libraries (8, 18), and 454 pyrosequencing (20, 21) have also been utilized to analyze microbial communities at the phylogenetic and taxonomic levels. However, information on ecological functions of the soil microbial community under various agricultural systems is generally lacking in most studies. Although specific functional groups of microbes, such as denitrifiers (22), nitrifiers (23), and methanotrophs (20); specific functional genes, such as *nirS*, *nosZ*, and *nifH* (24); and specific extracellular enzyme activities, such as nitrous oxide reductase (22), have been analyzed previously, they provide only small pieces of information on ecological functions of soil microbial communities. A more comprehensive and simultaneous evaluation of microbial communities is needed. The development of GeoChip, a high-throughput microarray-based technique that contains approximately 28,000 probes covering more than 57,000 gene variants from 292 functional gene families, allows us to investigate the ecological functions of soil microbial communities involved in nitrogen (N), carbon (C), sulfur (S), and phosphorus (P) cycles and other processes (25) comprehensively in various ecosystems, including agricultural lands (26).

In this study, we collected soil samples from three agricultural systems under conventional (CT), low-input (LI), and organic (ORG) management systems, all following a corn (*Zea mays* L.), soybean [*Glycine max* (L.) Merr.], and winter wheat (*Triticum aestivum* L.) rotation at the W. K. Kellogg Biological Station (KBS) Long Term Ecological Research (LTER) site in southwestern Michigan. The soil microbial communities were analyzed with GeoChip 3.0 technology (25) to evaluate changes of functional genes involved in important soil processes based on their diversity, composition, and abundances under various agricultural systems. The relationships between functional genes of soil microbial communities and environmental variables, including greenhouse gas fluxes, were evaluated as well. We hypothesized that the diversity, composition, and functional gene abundances of soil microbial communities were dramatically affected by agricultural systems. Moreover, we hypothesized that the functional genes of soil

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Address correspondence to Jizhong Zhou, zhou-aem@rccc.ou.edu.
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microbial communities and environmental variables were significantly correlated.

MATERIALS AND METHODS

Study site. This study was conducted at the KBS LTER site in Hickory Corners, MI (42° 24′ N, 85° 24′ W, 288 m above sea level), established in 1989. Soils at the KBS LTER site are well-drained Typic Hapludalfs developed on glacial outwash (27). The mean annual precipitation is 920 mm, and the mean annual temperature is 9.7°C (28).

Management systems. We studied plots at the KBS LTER site managed (i) conventionally with standard chemical inputs (CT), (ii) with low inputs (LI), and (iii) organically with no chemical inputs (ORG). Treatment plots were arranged in a randomized-block design with six replicate blocks, and within each block, all 1-ha plots were separated by 10-m-wide grass strips. The CT treatment has been managed as a corn-soybean-wheat rotation since 1993 and was managed as a corn-soybean rotation from 1989 to 1992. The LI and ORG treatments have been managed as corn-soybean-wheat rotation since 1988, with a red clover (*Trifolium pratense* L.) winter cover crop to supply biologically fixed N, prior to the corn and soybean phases of the rotation (29).

Synthetic N fertilizer applied in the form of NH_4NO_3 before 1995 and as 28% UAN (solution of urea and ammonium nitrate) thereafter was applied to the CT and LI treatments during corn and wheat years. In the CT treatment, corn received 123 kg N ha^{-1}, and wheat received 56 kg N ha^{-1}. The LI treatment received one-third of the fertilizer applied for the CT treatment. Lime, N, P, and K were applied as needed, according to Michigan State University (MSU) recommendations (30). The ORG system received no N fertilizer, and no compost or manure was applied to any of the treatments.

All crop varieties were herbicide susceptible. In the CT treatment, herbicides were applied at the rate recommended for the region (31). In the ORG treatment, weed management consisted of multiple passes with a cultivator and rotary hoe. For the LI treatment, the weed management was similar to that for ORG, although it also received postemergence herbicide applications banded within rows. No insecticides were applied for any of the treatments. Additional information on the agronomic practices can be found at the KBS LTER website (http://lter.kbs.msu.edu/Data/DataCatalog.html).

Field sampling and environmental variable measurements. Bulk soil samples were collected with bucket augers on 3 September 2008 when corn was planted in all three systems. Six soil cores (3.8 by 10 cm) collected across the row to achieve a sample unbiased by plant proximity were pooled for each replicate plot. Composited soil samples were sieved through a 4-mm sieve in the field and then stored overnight at 4°C. Subsamples were taken on the next day and frozen at −80°C until shipment to the University of Oklahoma for soil microbial community analysis. Other environmental variables were measured routinely at the KBS LTER site and are available online (http://lter.kbs.msu.edu/datatables), including yield, greenhouse gas fluxes, and so on.

Microbial community DNA isolation and purification. At the University of Oklahoma, soil DNA was extracted by freeze-grinding mechanical lysis, as described previously (32). The DNA was purified using a low-melting agarose gel followed by phenol extraction. DNA quality was assessed based on the ratios of absorbance at 260/280 nm and 260/230 nm by using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE), while final soil DNA concentrations were quantified by PicoGreen (33), using a FLUOstar Optima instrument (BMG Labtech, Jena, Germany).

GeoChip analysis. GeoChip 3.0 was used in this study, as described previously (34), with the following steps.

(i) Template labeling. For template labeling, 3 μg extracted DNA was labeled with the fluorescent dye Cy5 by random priming. First, DNA was mixed with 20 μl random primers, denatured at 99.9°C for 5 min, and then immediately chilled on ice. Labeling master mix containing 2.5 μl deoxynucleoside triphosphate (dNTP) (5 mM dATP/dGTP/dCTP, 2.5 mM dTTP), 1 μl Cy5 dUTP (Amersham, Piscataway, NJ), 80 U of the large Klenow fragment (Invitrogen, Carlsbad, CA), and 2.5 μl water was added to denatured DNA and incubated at 37°C for 3 h, followed by heating at 95°C for 3 min to stop labeling. The labeled DNA was purified by use of the QIAquick purification kit (Qiagen, Valencia, CA) based on the manufacturer's instructions, measured by using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE) to check the label efficiency, and dried in a SpeedVac (ThermoSavant, Milford, MA) at 45°C for 45 min.

(ii) Hybridization and imaging processing. For hybridization and imaging processing, the labeled DNA was resuspended in 50 μl hybridization solution (40% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 5 μg of unlabeled herring sperm DNA [Promega, Madison, WI], and 0.1% SDS) and 2 μl universal standard DNA (0.2 pmol μl^{-1}) labeled with the fluorescent dye Cy3 (35), denatured at 95°C for 5 min, and maintained at 50°C until loaded onto microarray slides. Arrays were hybridized on a MAUI Hybridization Station (Roche, South San Francisco, CA) for 12 h at 42°C. After washing and drying, the microarrays were scanned by using a ScanArray Express microarray scanner (PerkinElmer, Boston, MA) at 633 nm using a laser power of 90% and a photomultiplier tube (PMT) gain of 75%. The obtained images were analyzed by using ImaGene, version 6.0 (Biodiscovery, El Segundo, CA), to determine the intensity of each spot and identify poor-quality spots.

(iii) Data preprocessing. For data preprocessing, raw data from ImaGene were submitted to the laboratory's Microarray Data Manager System (http://ieg.ou.edu/microarray/) and analyzed by the following major steps. (i) The spots flagged as 1 or 3 by ImaGene and with a signal-to-noise ratio (SNR) of less than 2.0 (36) were removed as poor-quality spots. (ii) After the poor-quality spots were removed, a two-step normalization method was used. First, the mean Cy3 intensity of the universal standards in each subgrid was used to normalize the Cy5 intensity for samples in the same subgrid. Second, the Cy5 intensity after the first normalization was normalized by the mean Cy3 intensity of universal standards in all slides. (iii) The probes that appeared in only one of six replicates in each treatment were removed as noise. Afterwards, the relative abundance in each sample was calculated by dividing the individual signal intensity of each probe by the sum of the original signal intensity for all detected probes in that sample. The relative abundance was then multiplied by the mean value for the sums of the original signal intensity in all samples. A natural logarithm transformation was performed for the amplified relative abundance plus 1.

Statistical analysis. To calculate the gene overlap at the treatment level, the detected genes in six replicates of each treatment were composited as one pool. The gene overlap between treatments was calculated by the number of genes detected in both treatments. The proportion of overlapped genes was calculated by dividing the number of overlapped genes between treatments by the number of all genes detected in both treatments. The proportion of unique genes in each treatment was calculated by dividing the number of genes that were not detected in any other treatments by the number of all genes detected in this treatment.

Detrended correspondence analysis (DCA), three nonparametric tests (multiple-response permutation procedure [MRPP], permutational multivariate analysis of variance [Adonis], and analysis of similarity [ANOSIM]), canonical correlation analysis (CCA), Mantel test, and analysis of variance (ANOVA) were performed by using R, version 2.9.1 (R Foundation for Statistical Computing, Vienna, Austria). Differences between treatments were compared by *post hoc* Fisher's least-significant-difference (LSD) test with Holm-Bonferroni adjustment, which is more powerful and less conservative than the Bonferroni procedure (37). The significant differences were defined as a P value of <0.05, or with listed P values.

RESULTS

Yield, greenhouse gas emission, and other environmental variables. Various management systems had significant effects on

TABLE 1 Gene overlap, uniqueness, and diversity indices[a]

Parameter	Value for treatment[d]		
	CT	LI	ORG
No. (%) of genes			
CT	**310 (13.6)**	*1,832 (53.3)*	*1,788 (51.1)*
LI		**514 (17.16)**	*2,303 (62.2)*
ORG			**576 (19.1)**
No. of genes in treatments	2,276	2,995	3,013
Mean richness (no. of probes) ± SE	1,300 ± 173 (b)	1,806 ± 220 (a)	1,728 ± 217 (ab)
Mean H^b ± SE	7.11 ± 0.16 (a)	7.45 ± 0.15 (a)	7.41 ± 0.12 (a)
Mean invsimpson (1/*D*)[c] ± SE	1,293.06 ± 171.58 (b)	1,795.43 ± 218.21 (a)	1,717.15 ± 214.30 (ab)

[a] Gene probes were regarded as "species," and their abundances were represented by the normalized signal intensities.
[b] Shannon-Weaver index is defined as $H = -\sum p_i \times \ln(p_i)$, where p_i is the proportional abundance of species *i*.
[c] Simpson's index is based on $D = \sum p_i^2$ and invsimpson returns (1/*D*).
[d] Different letters in parentheses indicate statistical differences at a *P* value of <0.05 among treatments by least-significant-difference (LSD) tests. Italic type indicates gene overlap, and boldface type indicates uniqueness.

TABLE 2 Nonparametric analyses to test dissimilarity of communities between any two treatments of CT, LI, and ORG[e]

Treatment	Adonis[a]		ANOSIM[b]		MRPP[c]	
	F	P^d	*R*	P^d	δ	P^d
CT vs LI	0.189	0.01	0.328	0.01	0.401	0.02
CT vs ORG	0.180	0.01	0.320	0.01	0.415	0.01
LI vs ORG	0.125	0.18	0.085	0.18	0.399	0.13

[a] Permutational multivariate analysis of variance using distance matrices. Significance tests were performed by *F* tests based on sequential sums of squares from permutations of the raw data.
[b] ANOSIM, analysis of similarities. The statistic *R* is based on the difference of mean ranks between groups and within groups. The significance of observed *R* is assessed by permuting the grouping vector to obtain the empirical distribution of *R* under the null model.
[c] MRPP, multiresponse permutation procedure. The statistic δ is the overall weighted mean of within-group means of the pairwise dissimilarities among sampling units. The significance test is the fraction of permuted deltas that are less than the observed delta.
[d] *P* value of the corresponding significance test.
[e] All three tests are multivariate analyses based on the Bray-Curtis index.

crop yield in 2008 ($P = 0.01$). The yields (mean ± standard error) were 5,276.7 (±504.2) kg ha^{-1} in CT, 5,079.7 (±144.4) kg ha^{-1} in LI, and 3,717.5 (±291.8) kg ha^{-1} in ORG. Based on LSD tests, the yield in ORG was significantly lower than those in both CT and LI treatments by 29.5 and 26.8%, respectively. However, there was no significant difference in yield between CT and LI.

The field gas fluxes of CO_2, CH_4, and N_2O were measured in 2008 during the growing season at eight time points: 8 April, 23 April, 20 May, 3 June, 8 July, 23 July, 20 August, and 16 September. The CO_2 flux included both heterotrophic and autotrophic respiration. The measured values for CH_4 flux at most time points were negative, reflecting that the process of CH_4 oxidation overwhelmed CH_4 production. By repeated-measure ANOVA, management systems had significant effects only on CO_2 flux ($P = 0.003$) but not on the flux of CH_4 or N_2O. At single time points, CT treatment consistently had a lower CO_2 flux than LI or ORG except on 20 August and 16 September (see Fig. S1A in the supplemental material). The CO_2 flux in LI and ORG treatments did not differ significantly at any single time point. There was no significant effect of management systems on CH_4 flux for all single time points (see Fig. S1B in the supplemental material). For N_2O flux, the only significant difference was observed on 23 July ($P = 0.02$), when N_2O flux in CT was significantly higher than that in LI but not that in ORG (see Fig. S1C in the supplemental material).

The soil total C and C/N ratio measured in 2001 were significantly affected by management systems ($P < 0.05$), which were both significantly lower in CT than in LI and ORG treatments (see Table S1 in the supplemental material). The effects of management systems on soil nitrate content were significant as well ($P = 0.01$), but not on ammonium content, all measured in 2007 (see Table S1 in the supplemental material). The soil nitrate content was significantly higher in CT than in LI and ORG, while there was no significant difference between LI and ORG.

Functional diversity of soil microbial communities. Based on the detected functional genes, community diversity was assessed by richness (detected number of probes in each sample), Shannon-Weaver (*H*), and Simpson reciprocal (1/*D*) indexes (Table 1). Results showed that richness was significantly lower in CT than in LI, while there was no significant difference between LI and ORG or CT and ORG, based on LSD tests. Similarly, CT had a significantly lower Simpson reciprocal index value than did LI, representing a significantly lower diversity in CT, while the difference was not significant between LI and ORG or CT and ORG. No significant difference was observed for Shannon-Weaver index values.

Gene overlap was calculated at the whole-treatment level (Table 1). The proportions of overlapped genes between treatments ranged from 51.1% between CT and ORG to 62.2% between ORG and LI. To understand the heterogeneity of microbial populations in these samples, the proportions of unique genes detected in each treatment were calculated as well. The CT treatment had the lowest proportion of unique genes, at 13.6%, while ORG had the highest proportion, at 19.1%.

Functional compositions of soil microbial communities. Three nonparametric tests (MRPP, Adonis, and ANOSIM) were performed by utilizing the Bray-Curtis index and consistently showed that the communities in CT were significantly different from those in LI or ORG ($P < 0.02$), while the communities in LI and ORG did not differ (Table 2). The DCA profile illustrated that CT was separated clearly from LI and ORG along DCA1, while LI and ORG were distinct along DCA2 (Fig. 1).

Abundance of functional genes in carbon/nitrogen/phosphorus/sulfur cycles. The geochemistry cycles of carbon (C), nitrogen (N), phosphorus (P), and sulfur (S), important in sustaining ecosystem functions, including providing nutrients for crop growth, are of our particular interests. The functional genes involved in these processes were investigated, although more comprehensive functional genes involved in other functional catalogs, like metal resistance, were also included in GeoChip (25).

The abundances of the genes involved in active C degradation were significantly lower in CT than in LI ($P < 0.05$), including those encoding α-amylase (*amyA*) and pullulanase (*amyX*) for starch decomposition, arabinofuranosidase (*ara* for fungi) and xylanase (*xylA*) for hemicellulose decomposition, cellobiase for cellulose decomposition, endochitinase for chitin decomposition, and isocitrate lyase (*aceA*), malate synthase (*aceB*), limonene-1,2-

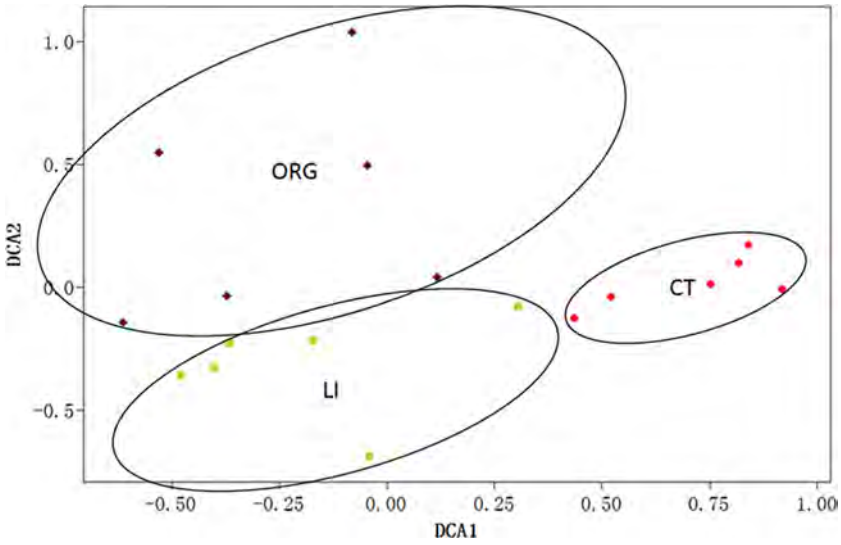


FIG 1 Detrended correspondence analysis (DCA) of GeoChip data showing that treatments of conventional (CT), low-input (LI), and no-input (ORG) systems had substantial influences on functional compositions of soil microbial communities.

epoxide hydrolase (*limEH*), and vanillin dehydrogenase (*vdh*) for decomposition of aromatic substances (Fig. 2). The *amyX* gene was not detected in CT, but it was detected in LI and ORG treatments. For the *amyA*, endochitinase, and *aceB* genes, abundances were significantly lower in CT than in ORG ($P < 0.05$). There were no significant differences between CT and ORG for other genes involved in C degradation. The abundances of most C degradation genes in LI and ORG treatments did not differ significantly, except those of the *ara* (fungi), *xylA*, and cellobiase genes, which were significantly lower in ORG than in LI ($P < 0.05$). Meanwhile, the abundances of genes involved in lignin degradation did not differ significantly among the three treatments.

The abundances of all genes involved in the function of C fixation (*aclB*, encoding ATP citrate lyase, and the CODH [carbon monoxide dehydrogenase], Pcc [propionyl-CoA carboxylase], and Rubisco [ribulose-1,5-bisphosphate carboxylase/oxygenase] genes) were significantly lower in CT than in LI ($P < 0.05$) (see Fig. S2 in the supplemental material). Among them, the *aclB* gene was not detected in CT but was detected in LI and ORG. The abundances of the Pcc and Rubisco genes were significantly lower in CT than in ORG as well ($P < 0.05$). There were no significant differences between LI and ORG for the abundances of all genes involved in C fixation. For genes involved in methane oxidation and production, no significant treatment effects were observed.

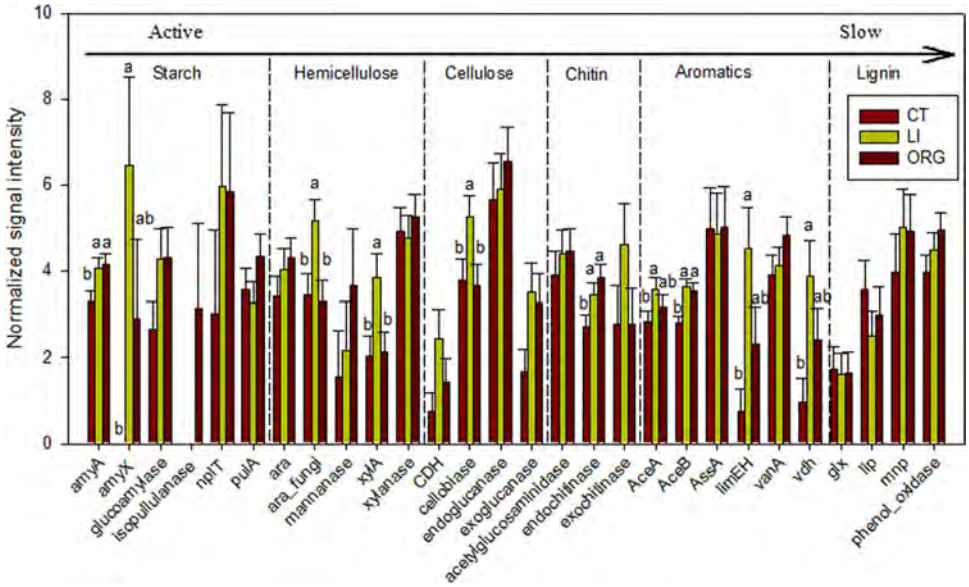


FIG 2 Normalized signal intensity of detected genes in carbon (C) degradation under conventional (CT), low-input (LI), and organic (ORG) management systems. The complexity of carbon is presented in order from labile to recalcitrant. Error bars represent standard errors. Different letters indicate statistical differences at a *P* value of <0.05 among treatments by least-significant-difference (LSD) tests. No letter was labeled if there was no treatment effect. CDH, cellobiose dehydrogenase.

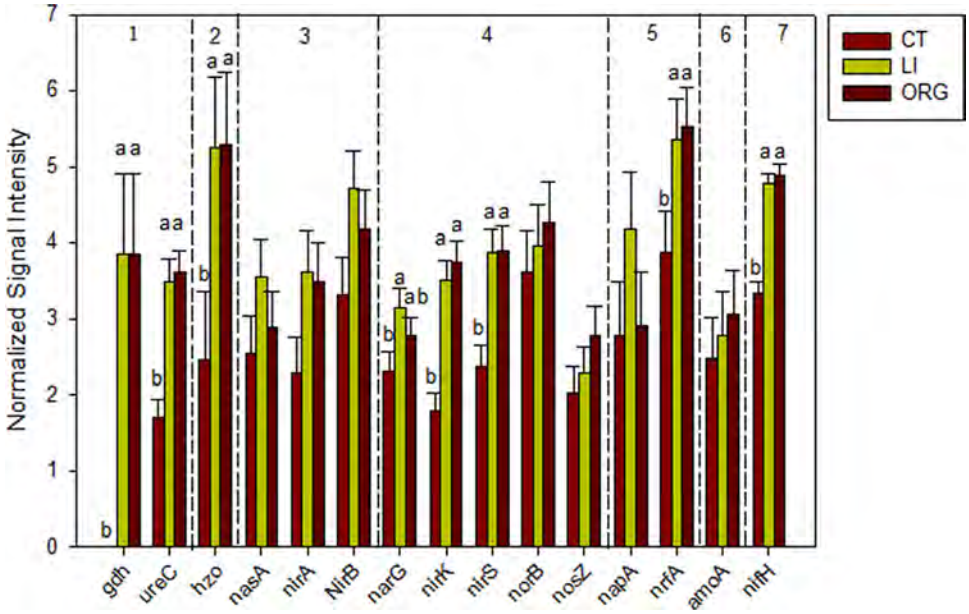


FIG 3 Normalized signal intensity of detected genes in nitrogen cycles under conventional (CT), low-input (LI), and organic (ORG) management systems. The processes involved in N cycles include ammonification (1), anammox (2), assimilatory N reduction (3), denitrification (4), dissimilatory N reduction (5), nitrification (6), and N fixation (7). Error bars represent standard errors. Different letters indicate statistical differences at a *P* value of <0.05 among treatments by LSD tests. No letter was labeled if there was no treatment effect.

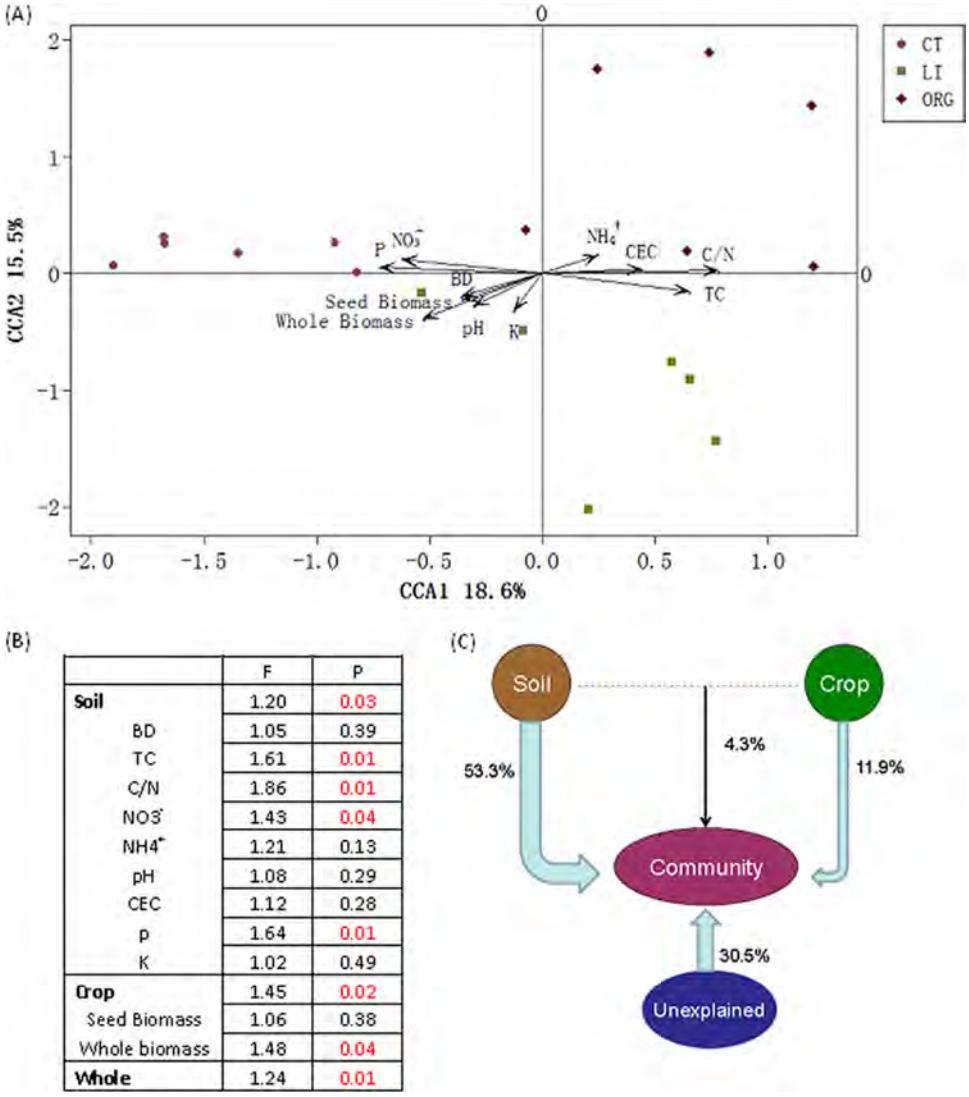
For the N cycle processes, compared to LI or ORG, CT had significantly low abundances of *gdh*, encoding glutamate dehydrogenase; *ureC*, encoding urease subunit α for ammonification; *hzo*, encoding hydrazine oxidase for anammox; *nirK*, encoding copper-containing nitrite reductase; *nirS*, encoding a cytochrome cd_1 nitrite reductase precursor for denitrification; *nrfA*, encoding *c*-type cytochrome nitrite reductase for dissimilatory N reduction; and *nifH*, encoding nitrogenase reductase for N fixation ($P < 0.05$) (Fig. 3). The abundance of *narG*, encoding the α subunit of nitrate reductase, was significantly lower in CT than in LI ($P < 0.05$) but did not differ from that in ORG. There were no significant differences between LI and ORG for all genes in the N cycle. The gene abundances in functional processes of assimilatory N reduction and nitrification were not affected by management treatments. For example, the abundances of *amoA*, encoding ammonia monooxygenase, did not differ significantly by treatments, no matter whether the genes were from bacteria ($P = 0.77$), archaea ($P = 0.50$), or both groups ($P = 0.63$).

Regarding phosphorus (P) and sulfur (S) cycles, the abundances of *ppx*, encoding exopolyphosphatase for P utilization; *aprA*, encoding adenylylsulfate reductase subunit α; *dsrA*, encoding sulfite reductase subunit α for sulfite reduction; and *sox*, for sulfur oxidation, were significantly lower in CT than in LI and ORG ($P < 0.05$) (see Fig. S3 in the supplemental material). Phytase genes for P utilization were detected only in LI and not in CT and ORG. There were no significant differences between LI and ORG treatments for all detected genes involved in P and S cycles.

Environmental variables shaping the whole microbial community. To test which environmental variables shape soil microbial community structure, CCA was performed based on all detected functional genes and measured soil and crop properties (Fig. 4A), including bulk density (BD), total C content (TC), C/N ratio (C/N), nitrate (NO_3^-), ammonium (NH_4^+), pH, cation exchange capacity (CEC), available phosphorus content (P), and available potassium content (K) for soil and seed biomass and whole biomass for crop (Fig. 4B). The CCA results revealed that the functional gene composition of microbial communities was significantly correlated with all selected variables in the adopted CCA model ($P = 0.01$) by a Monte Carlo test, with a sum of total eigenvalues of 1.359. The variance inflation factors (VIF) varied from 2.0 for soil nitrate content to 20.9 for TC. For each individual variable, significant correlation was found between microbial communities and soil TC ($P = 0.01$), C/N ratio ($P = 0.01$), NO_3^- ($P = 0.040$), P ($P = 0.01$), and whole-crop biomass ($P = 0.01$).

In the CCA profile (Fig. 4A), the first two canonical axes explained 18.6 and 15.5% of the constrained variations in microbial communities, respectively. The communities in CT separated clearly from those in LI and ORG along the first canonical axis (CCA1), while communities in LI and ORG separated along the second canonical axis (CCA2). The projection of environmental variables demonstrated that LI and ORG samples were positively correlated with several variables, including soil NH_4^+, CEC, C/N, and TC, while CT samples were negatively correlated with them. Other variables, including BD, pH, NO_3^-, P, K^+, whole-crop biomass, and seed biomass, were negatively correlated with CT samples but positively correlated with LI and ORG samples. Indicated by the length of arrows in the CCA profile (Fig. 4A), the impacts of the variables TC, C/N ratio, NO_3^-, P, and crop whole biomass on microbial communities were greater than those of other variables.

Both soil and crop variables were significantly correlated with microbial communities at *P* values of 0.031 and 0.015, respectively. Variation partitioning analysis (VPA) was conducted to examine the contribution of each catalog in influencing the soil microbial communities in the CCA analysis (Fig. 4C). The soil variables explained 53.3% of the variation in microbial commu-



	F	P
Soil	1.20	0.03
BD	1.05	0.39
TC	1.61	0.01
C/N	1.86	0.01
NO3-	1.43	0.04
NH4+	1.21	0.13
pH	1.08	0.29
CEC	1.12	0.28
p	1.64	0.01
K	1.02	0.49
Crop	1.45	0.02
Seed Biomass	1.06	0.38
Whole biomass	1.48	0.04
Whole	1.24	0.01

FIG 4 Canonical correspondence analysis (CCA) of GeoChip data under conventional (CT), low-input (LI), and organic (ORG) management systems with selected environmental variables (A), model significances (B), and partial CCA-based variation partitioning analysis (VPA) (C). The selected environmental variables include bulk density (BD), total C content (TC), C/N ratio (C/N), nitrite (NO_3^-), ammonium (NH_4^+), pH, cation exchange capacity (CEC), available phosphorus (P), and available potassium (K) for soil, and seed biomass and whole biomass for crop.

nities, and the crop variables explained 11.9%, while their interaction explained 4.3%, leaving 30.5% unexplained.

Linkage between greenhouse gas fluxes or N availability and functional genes. To examine the linkage between the field flux of CH_4 (mean of all measurements during the growing season in 2008 [http://lter.kbs.msu.edu/datatables/28]) and the detected functional genes in methane cycling, both a Mantel test and CCA were performed, but none of them showed a significant correlation (see Table S2 in the supplemental material). For field flux of N_2O (mean of all measurements during the growing season in 2008 [http://lter.kbs.msu.edu/datatables/28]), it was significantly correlated with the whole genes for denitrification ($P = 0.04$) by CCA. Other than *narG*, encoding the α subunit of nitrate reductase, all other individual denitrification genes were significantly correlated with N_2O flux by CCA at a *P* value 0.02 for *norB* or $0.05 < P < 0.10$ for *nirK*, *nirS*, and *nosZ*. The only significant correlation captured by the Mantel test was between *nosZ* and N_2O flux ($P = 0.03$) (see Table S2 in the supplemental material).

The linkage between soil N availability (NO_3^- and NH_4^+) and functional genes in N cycles was analyzed by a Mantel test and CCA as well (see Table S3 in the supplemental material). The Mantel test captured only a correlation between *gdh*, encoding glutamate dehydrogenase, in the ammonification process and soil NH_4^+ content at a *P* value of 0.08. The CCA was relatively more sensitive than the Mantel test by capturing more significant correlations between N availability and N functional genes. By CCA, a correlation between the whole genes for N cycling and soil NO_3^- content, but not NH_4^+, was observed at a *P* value of 0.09. The whole genes for ammonification, especially *ureC*, were significantly correlated with soil NH_4^+ content ($P = 0.05$) rather than NO_3^-, while the whole genes for denitrification, driven mainly by *narG*, were significantly correlated with soil NO_3^- content ($P =$

0.05) but not NH_4^+. Moreover, the *nirA* gene, involved in assimilatory N reduction, and the *norB* gene, involved in denitrification, had correlations with soil NH_4^+ content at *P* values of 0.01 and 0.08, respectively.

DISCUSSION

Based on the Simpson reciprocal index, the functional gene diversity of soil microbial communities in the CT treatment was significantly lower than that in the LI treatment. Many differences existed between the CT and LI management systems in this study. The LI treatment used winter cover crop (red clover) to provide supplemental N for plant growth, while the CT treatment did not. The fertilizer and herbicide applied in the LI treatment were one-third and two-thirds of those in the CT treatment, respectively. Moreover, the total C concentration in A/Ap horizon of the LI soil was 6.7% higher than that of the CT soil (38). All these differences may contribute to the distinction of functional gene diversity in soil microbial communities between the CT and LI treatments. For example, higher soil C storage in the LI treatment may provide a more benign dwelling for soil microbes, as it has beneficial effects on soil drainage, structure, water-holding capacity, and other important soil properties to promote plant growth (39) and supply more substrates. Moreover, high doses of N fertilizer in the CT treatment may have a suppressing effect on the soil microbial community. It has been reported that chemical N fertilizer decreased the soil microbial diversity at the genotype level measured by random amplified polymorphic DNA analysis (40) or at the metabolism level by Biolog (41). The mechanisms for the suppressing effect of N fertilizer on the diversity of the soil microbial community in the CT treatment may be similar to that for plant communities (42–46), like intensified resource preemption and competitive exclusion (47–49).

Although the total C concentration in the A/Ap horizon of the ORG soil was 17.3% higher than that of the CT soil (38), and no chemicals were applied, the functional gene diversity of soil microbial communities in the ORG treatment did not differ significantly from that of the LI or CT treatment. In this study, none of the treatments applied compost or manure, which is commonly adopted in many other organic systems and has been reported to increase the soil microbial diversity (8, 13, 50, 51). Similar to the LI treatment, the ORG treatment also used winter cover crop to supply N. However, the relative deficiency of most easy-access substrates in the ORG treatment, which could be provided by mineral fertilizer, may limit the ability of soil microbes to utilize nutrients in cover crop, lead to C accumulation, and affect the functional gene diversity. Compared to the ORG treatment, the mineral fertilizer applied to the LI treatment, though limited, may have a priming effect (52, 53) on soil microbes by stimulating their activities in decomposing cover crop residues, releasing nutrients and causing higher-function gene diversity.

While the diversity metrics represent the variability among species (gene probes) in a community (54), the community structure or composition refers to the pattern of species distribution (the different kinds of species and their abundances) (55). The community composition of soil microbes based on functional genes detected by GeoChip (functional gene composition) in the CT treatment was significantly different from those in the LI or ORG treatment by both DCA and nonparametric tests, while the communities in the LI and ORG treatments did not differ from one another, as measured by nonparametric tests, but were readily distinguishable in the DCA profile. The impacts of different management systems on soil microbial community composition, but not functional gene composition as measured by GeoChip, were also observed previously in other studies (4, 5, 56, 57). At the same experiment site, KBS LTER, by using T-RFLP, the compositions of microbial communities in various soil fractions were previously reported to be significantly different among different agricultural systems with conventionally and organically managed corn and continuous alfalfa (16, 17). Buckley and Schmidt (19) also included conventionally and organically managed corn fields at the KBS LTER site in their study but found that the soil microbial community structures in these treatments were remarkably similar by utilizing 16S rRNA-targeted oligonucleotide probes and 16S rRNA T-RFLP techniques, which is possibly explained by the low number of replicates (three) analyzed and high natural variability in the microbial communities, as mentioned by those authors. The other possible reasons for the conflicting results may include the use of different techniques (17) and temporal variability of the soil microbial community.

Temporal variability is important for the soil microbial community, over either a seasonal or a successional scale. The shifts of climate factors, such as temperature and precipitation, during seasons and over years are often the strongest factors influencing biological populations (58, 59). The interaction between treatments and time dynamics may also occur for soil microbial communities (58). However, in this study, samples were analyzed at only a single time point for soil microbial community, and its temporal variability was not investigated. Future studies are needed to test whether the treatment effects on functional gene diversity and composition of soil microbial community were consistent over both short and long time scales.

Well-established linkages between environmental variables or processes and functional genes of soil microbial communities were observed. The entire functional gene composition was significantly correlated with selected soil and crop variables. Significant correlations were also observed between soil NO_3^- content and denitrification genes and between soil NH_4^+ content and ammonification genes, implying a close relationship between soil N availability and associated functional genes. Although correlations between N_2O fluxes and denitrification genes were significant, both unchanged N_2O fluxes and lower abundances of some denitrification genes in CT were observed. In this study, we investigated only three annual crop treatments. By including more treatments, especially those for perennial, successional systems with native vegetation and forest, well-established linkages between greenhouse gas emissions and soil microbial communities were observed in previous studies at the same experimental site. For example, the gene abundances of *nirS* minus *nosZ* were assessed as a proxy for determining soil N_2O emissions, and a strong correlation between them was reported (24) when all treatments at the KBS LTER site were included.

Conclusion. Compared to the conventional farming system, low-input systems had a high functional gene diversity of soil microbial communities. However, the functional gene diversity did not differ between the LI and ORG systems. The abundances of genes involved in C/N/P/S cycles were consistently higher in the low-input or organic systems than in the conventional system. These results demonstrated that low-input systems may help in sustaining higher soil microbial community diversity and potentially enhance functions mediated by soil microbes in nutrient

cycling. Moreover, the soil microbial community composition was significantly correlated with selected soil (bulk density, pH, cation exchange capacity, total C, C/N ratio, NO_3^-, NH_4^+, available phosphorus content, and available potassium content) and crop (seed and whole biomass) variables, which could explain 69.5% of the variation. Significant correlations between soil NO_3^- content and denitrification genes, soil NH_4^+ content and ammonification genes, and N_2O flux and denitrification genes were also observed, implying a close linkage between soil N availability or process and associated functional genes.

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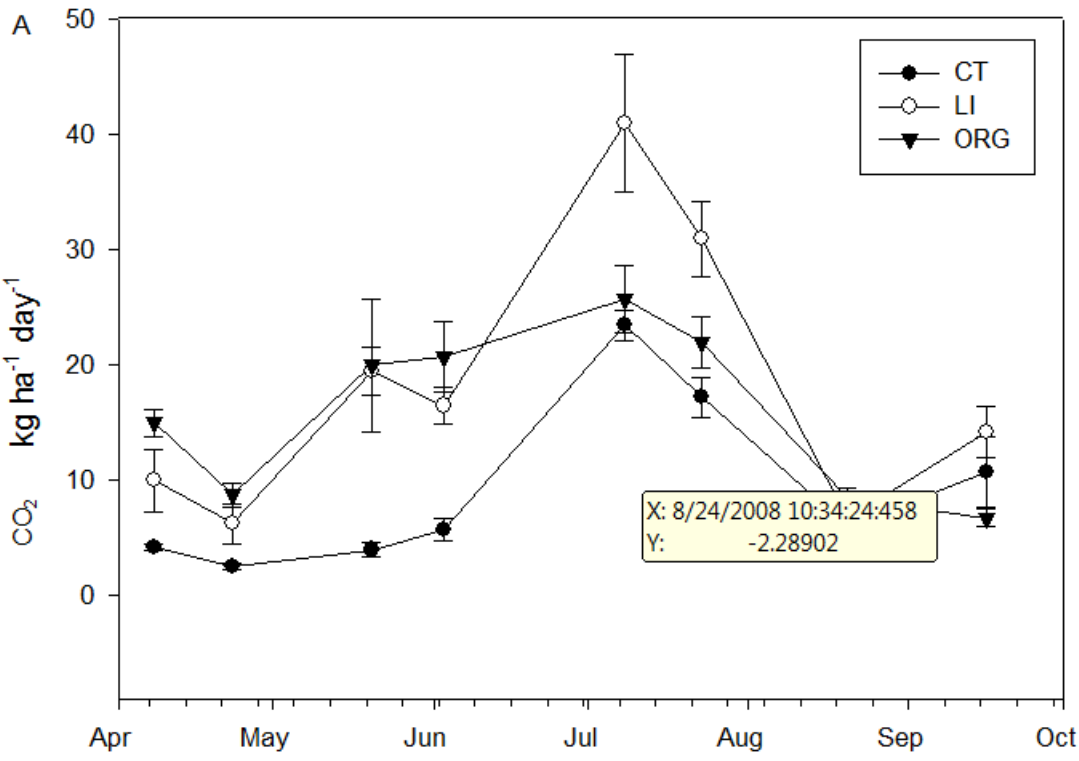
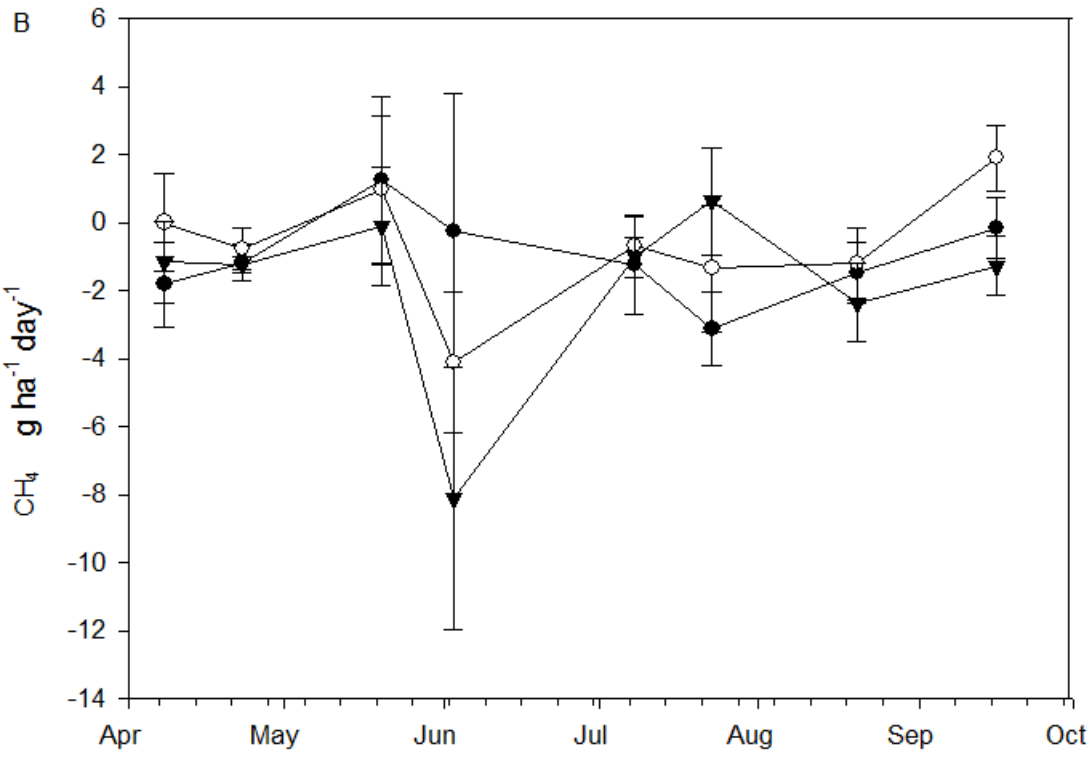
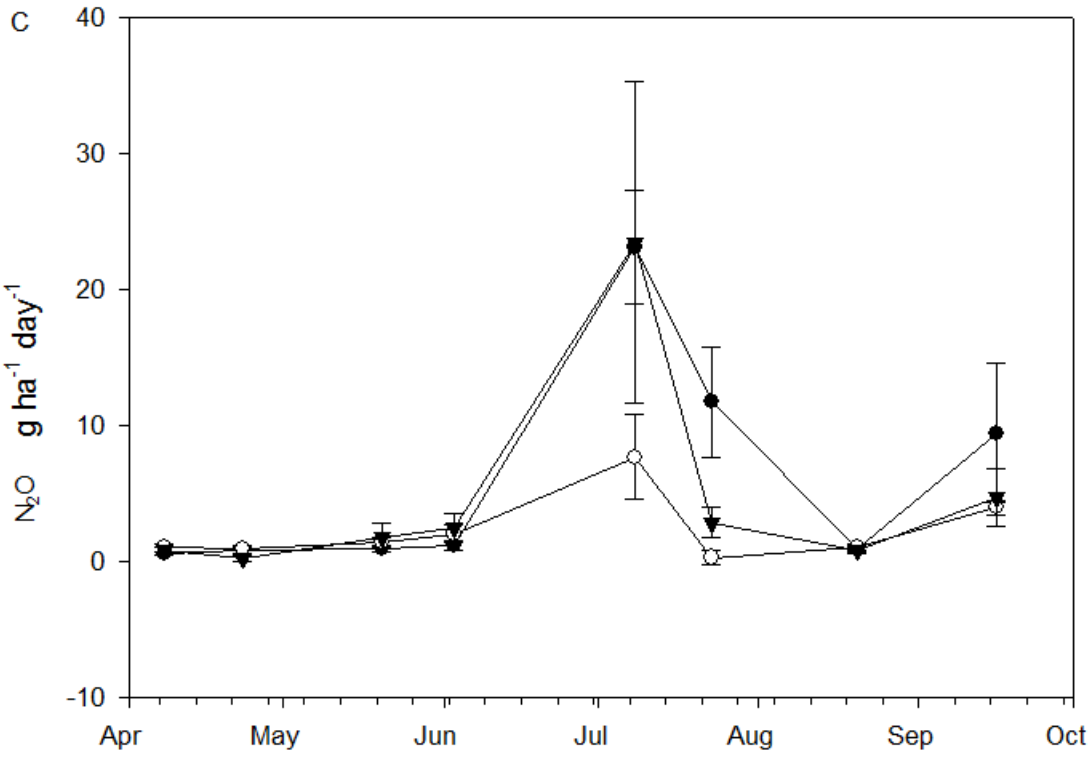


	4/8	4/23	5/20	6/3	7/8	7/23	8/20	9/16
CT	b	b	b	b	b	b	a	a
LI	ab	ab	a	a	a	a	a	a
ORG	a	a	a	a	ab	ab	a	a



	4/8	4/23	5/20	6/3	7/8	7/23	8/20	9/16
CT	a	a	a	a	a	a	a	a
LI	a	a	a	a	a	a	a	a
ORG	a	a	a	a	a	a	a	a



	4/8	4/23	5/20	6/3	7/8	7/23	8/20	9/16
CT	a	a	a	a	a	a	a	a
LI	a	a	a	a	a	b	a	a
ORG	a	a	a	a	a	ab	a	a

Fig.S1. Field greenhouse gas fluxes of CO_2 (A), CH_4 (B) and N_2O(C) measured in 2008 during the growing season at various time points under conventional (CT), low-input (LI) and organic (ORG) management systems. Different letters in the table indicate statistical differences at $P<0.05$ among treatments by least significant distance (LSD) tests in each single time point.

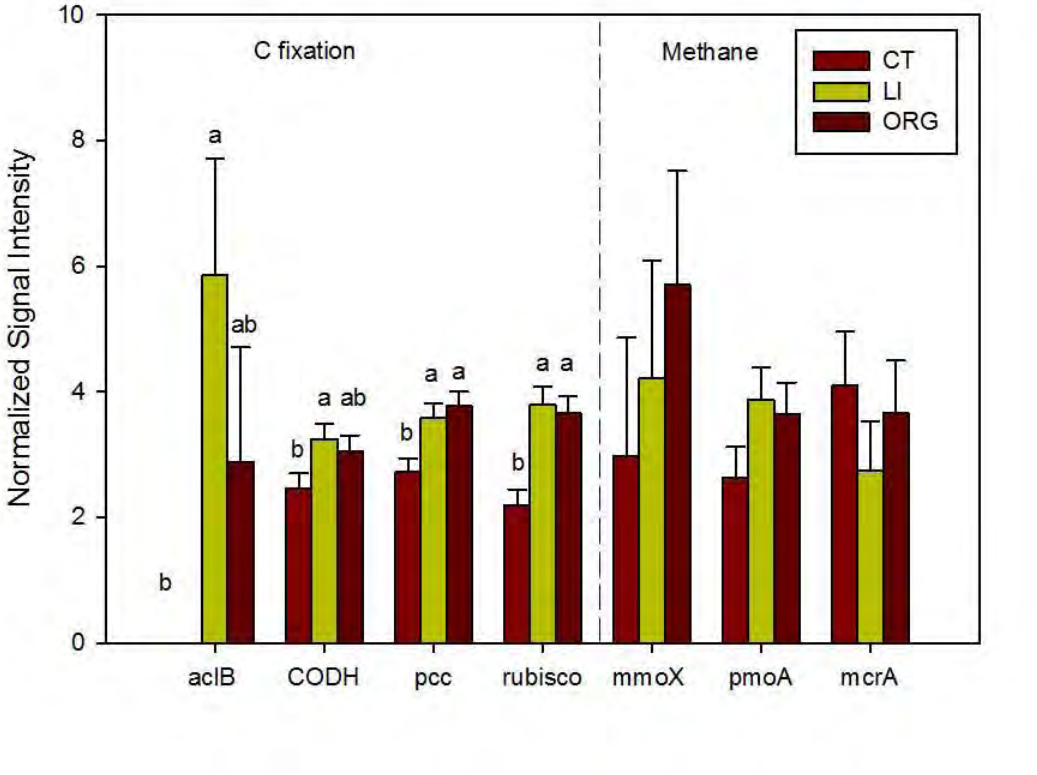


Fig.S2. The normalized signal intensity of detected genes involved in C fixation and methane oxidation/production functions in treatments of high (HI), low (LOW) and no (NO) chemical input. Error bars represent standard error. Different letters indicate statistical differences at $P<0.05$ among treatments by least significant distance (LSD) tests. No letter was labeled if there was no treatment effect.

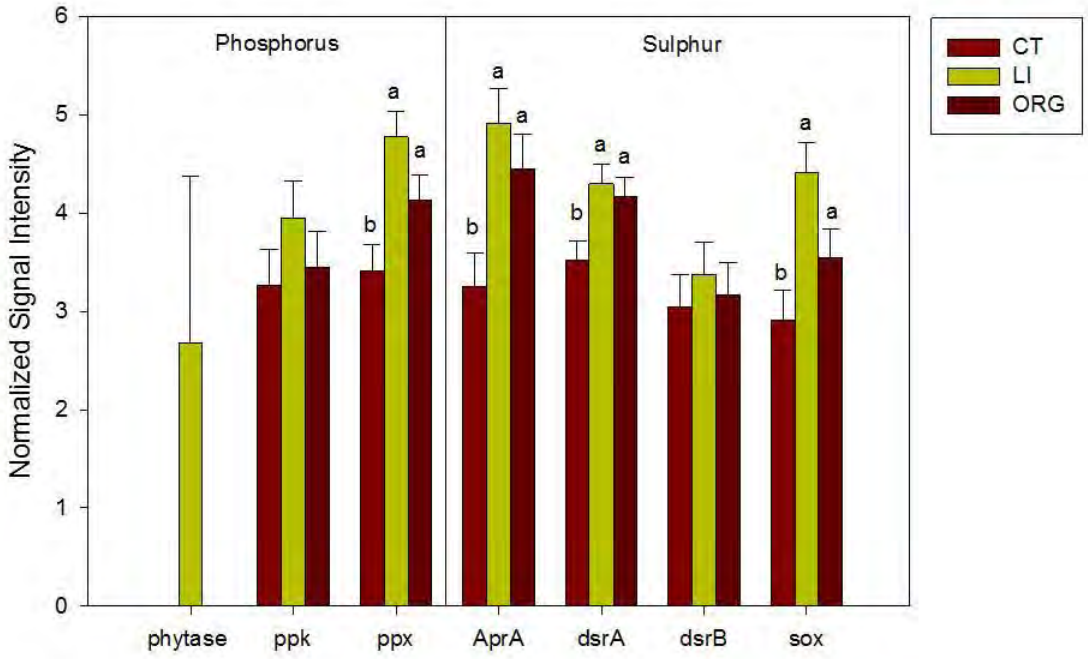


Fig.S3. The normalized signal intensity of detected genes involved in phosphorus and sulphur cycles in treatments of high (HI), low (LOW) and no (NO) chemical input. Error bars represent standard error. Different letters indicate statistical differences at $P<0.05$ among treatments by least significant distance (LSD) tests. No letter was labeled if there was no treatment effect.

Table S1. One-way analysis of variance (ANOVA) for soil and crop variables (mean±standard error) under conventional (CT), low-input (LI) and organic (ORG) management systems. Different letters indicate statistical differences at P<0.05 among treatments by least significant distance (LSD) tests with Holm–Bonferroni adjustment, following ANOVA. No letter was labeled if there was no treatment effect.

		ANOVA		Mean±Standard error		
	Variables	F value	Pr(>F)	CT	LI	ORG
Soil	Bulk density (g cm^{-2})	1.35	0.29	1.49±0.04	1.47±0.03	1.41±0.03
	Total C (%)	3.74	**0.05**	0.77±0.08 **b**	0.95±0.04 **a**	0.98±0.05 **a**
	C:N	14.65	**<0.01**	8.28±0.18 **b**	9.17±0.14 **a**	9.42±0.14 **a**
	Nitrate (µg g^{-1})	6.98	**0.01**	4.63±1.26 **a**	1.16±0.10 **b**	1.37±0.15 **b**
	Ammonium (µg g^{-1})	3.43	0.06	1.82±0.11	2.09±0.19	2.63±0.32
	pH	1.48	0.26	6.15±0.07	6.18±0.08	6.02±0.07
	CEC (meq $100g^{-1}$)	0.52	0.60	6.33±0.62	6.57±0.22	6.90±0.19
	Phosphorus (µg g^{-1})	2.58	0.11	39.00±6.67	29.17±5.34	22.33±2.91
	Potassium (µg g^{-1})	1.40	0.28	107.33±15.45	100.33±5.73	81.50±10.51
Crop	Seed Biomass (g m^{-2})	1.56	0.24	537.10±91.33	496.56±60.78	381.11±24.54
	Whole biomass (g m^{-2})	4.93	**0.02**	1173.48±132.32 **a**	1081.89±70.59 **ab**	792.43±39.81 **b**

Table S2. Significance of correlations between greenhouse gas fluxes (CH_4 and N_2O) and individual or all genes involved in methane cycling and denitrification process.

Gas flux	Gene	Mantel test	CCA
CH_4	**Methane (all genes)**	ns[1]	ns
	pmoA	ns	ns
	mcrA	ns	ns
N_2O	**Denitrification (all genes)**	ns	******[2]
	narG	ns	ns
	nirK	ns	*[3]
	nirS	ns	*
	norB	ns	**
	nosZ	**	*

1. ns: no significant;
2. **: Significant at the level of $p<0.05$;
3. *: Significant at the level of $0.05<p<0.10$.

Table S3. Significance of correlations between soil N availability and individual or all genes involved various N processes.

	Mantel test		CCA	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+
All genes in N cycling	ns[1]	ns	*[2]	ns
Ammonification (all genes)	ns	ns	ns	**[3]
gdH	ns	*	-[4]	-
ureC	ns	ns	ns	**
Assimilatory N reduction (all genes)	ns	ns	ns	ns
nasA	ns	ns	ns	ns
nirA	ns	ns	ns	**
NirB	ns	ns	ns	ns
Denitrification (all genes)	ns	ns	**	ns
narG	ns	ns	**	ns
nirK	ns	ns	ns	ns
nirS	ns	ns	ns	ns
norB	ns	ns	ns	*
nosZ	ns	ns	ns	ns
Dssimilartory N reduction (all genes)	ns	ns	ns	ns
napA	ns	ns	-	-
nrfA	ns	ns	ns	ns
Nitrification:amoA	ns	ns	ns	ns
N fixation: nifH	ns	ns	ns	ns

1. ns: no significant;
2. *: Significant at the level of $0.05<p<0.10$;
3. **: Significant at the level of $p<0.05$;
4. -: Not applicable for CCA analysis as it requires that the sum of abundances of detected genes in each sample must be > 0.