Crop diversity enhances disease suppressive potential in soils

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ABSTRACT

Agricultural management can have lasting impacts on microbial community structure and function through changes in biotic (i.e., plant inputs) and abiotic (i.e., soil fertility) soil properties. Enhancing biodiversity is expected to increase ecosystem functions, and in an agricultural context, such functions include enhanced plant production and pest suppression. For example, disease suppressive soils are characterized by the biocontrol properties provided by resident soil microorganisms capable of producing antifungal or antibacterial compounds known to suppress the growth of soil-borne pathogens. In this study, we investigated the impact of long-term crop diversity on microbial communities and disease suppressive functional potential in soils. In 2012, we collected soil samples from the Biodiversity Gradient Experiment (established in 2000) at the Kellogg Biological Station Long-Term Ecological Research (KBS-LTER) site. We sampled 7 treatments along the crop diversity gradient (monoculture to 5 crop species) and a spring fallow treatment to examine the influence of crop diversity on total bacterial community composition (16S rRNA amplicon sequencing) and a subset of microorganisms capable of producing antifungal compounds (2,4-diacetylphloroglucinol: phlD gene fingerprint analysis; pyrrolnitrin: prnD gene quantitative PCR). Our study revealed that crop diversity significantly influenced bacterial community composition and abundance of disease suppressive functional groups. Variation in plant inputs to soil organic matter pools may be a possible mechanism driving shifts in microbial community patterns and disease suppressive functional potential.
INTRODUCTION

Intensive agricultural management leads to reduced biodiversity and declines in ecosystem functions (1). However, crop diversification within agricultural landscapes is known to restore ecosystem functions such as carbon sequestration, pest control, and nutrient cycling (2, 3). Restoring ecosystem functions remains challenging to achieve given the loss of complex biological, chemical, and physical functions that natural ecosystems provide and humans value (i.e., ecosystem services). Understanding the effect of crop diversity on microbial contributions to plant health can provide us with tools to enhance plant productivity beyond additions of chemical inputs (4-7). Enhancing aboveground biodiversity (i.e., incorporating cover crops, perennial crops) could enhance ecological functions in the context of agricultural production and environmental quality. By managing soils to support more complex soil food webs, ecosystem services such as nutrient cycling regulation and natural pest suppression can be restored (7-9).

Soil microorganisms influence plant growth and reproduction through solubilization of mineral nutrients in soil (10), N₂ fixation (11), plant growth promotion (12), and plant pathogen suppression (13-15). Plant-microbe interactions directly influence community composition above and belowground through plant-soil feedback mechanisms. The exchange of resources between plants and microorganisms can positively or negatively impact plant growth rates. The plant influences microbial community composition, and this change can affect plant growth rates (13, 16, 17). In addition, plants contribute root exudates with different qualities providing a variety of carbon substrates to soil microbes (18-21).

A subset of soil microorganisms is known to exhibit antimicrobial properties, which ultimately provide plants with protection from soil-borne plant pathogens. This disease suppressive capacity has been associated with the abundance of antagonistic bacteria and
associated reductions in fungal pathogens (14, 22). Bacterial production of secondary metabolites 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN) are two potent bacterial toxins known to suppress fungal pathogens in agricultural soils (14, 23, 24). In a previous study, increased plant diversity was associated with enhanced soil suppressiveness measured by increased DAPG and PRN producers (25). In addition, streptomycetes are a well-known group of microbes to have antibiotic inhibitory effects and contribute to disease suppression in agricultural soils (15, 26). However, the diversity, composition, and disease suppressive activity among streptomycetes communities was similar among high and low plant diversity treatments (27). Thus, the relationship between biodiversity and disease suppression in agricultural soils remains unclear. By focusing on disease suppressive capacity of soil, we can evaluate how agricultural land-use strategies and subsequent changes in the soil environment and resident microbes impact contemporary plant growth (27, 28).

Heavily managed agroecosystems are ideal locations to test the influence of crop diversity effects on soil microorganisms. Specifically, long-term, replicated crop diversity trials provide a unique opportunity to focus on plant effects on soil microbial communities. Our research addresses the following questions: (1) What is the relationship between crop diversity and soil microbial community composition and disease suppressive functional potential? and (2) what is the relationship between changes in soil physicochemical properties and soil microbial community composition and disease suppressive functional potential in response to a crop diversity gradient? We tested the hypothesis that plant and microbial biodiversity provide disease suppressive functions in soils. We predicted that soil microbes established under high crop diversity (i.e., long crop rotations) have relatively higher soil suppressive capacity compared to soil microbes developed under low crop diversity (i.e., short crop rotation).
MATERIALS AND METHODS

Experimental design

We collected at the Biodiversity Gradient Experiment plots at W.K. Kellogg Biological Station Long-Term Ecological Research (KBS LTER) site in southwest, Michigan, USA. These soils are Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapluadalfs formed under glacial outwash (29). We sampled six diversity treatments including monoculture, two-crop rotation, and three-crop rotation with two or three cover crops and a spring fallow treatment (Table 1). Cover crops included in this experiment were red clover (Trifolium pretense L.), crimson clover (Trifolium incarnatum L.) and cereal rye (Secale cereal L.). Plantings of cover crop were dependent on the main crop in rotation (30). A complete set of biodiversity plots is replicated four times. No additional fertilizer is applied to these plots.

Field sampling

We sampled 7 treatments (Table 1) all in the corn phase of the rotation along the biodiversity gradient and a spring fallow treatment. At each of the four replicate 10 x 20 m plots, we combined five soil cores (5 cm diameter) to a 10 cm depth. A subsample from each composite sample was sieved through 4 mm in the field, flash frozen in the field in liquid nitrogen, and stored at -80 °C prior to molecular-based microbial analyses. On a subsample of field-collected soil, we analyzed inorganic nitrogen (N) (KCl extractable ammonium and nitrate) and gravimetric soil moisture. The remaining soil was air-dried and ground for analysis of soil pH and total C and N based on elemental combustion analysis.
Bacterial community sequencing

We extracted DNA using the MoBio PowerSoil® DNA Isolation Kit Soil (MO BIO Laboratories, Inc., Carlsbad, CA). DNA concentration was adjusted to a standard concentration of 20 ng µl$^{-1}$ and used as template in PCR reactions. To characterize bacterial taxonomic diversity, we used barcoded primers (515f/806r primer set) developed by the Earth Microbiome Project to target the V4-V5 region of the bacterial 16S subunit of the ribosomal RNA gene (rDNA) (31). For each sample, PCR product combined from three 50 µl reactions, concentration quantified, and PCR product from each soil sample was combined in equimolar concentrations for paired-end 250×250 sequencing using the Illumina MiSeq platform according to details in M. Muscarella, et al. (32). Briefly, we assembled the paired-end 16S rRNA sequence reads using the Needleman algorithm (33). All sequences were subjected to systematic checks to reduce sequencing and PCR errors. High quality sequences (i.e., >200 bp in length, quality score of >25, exact match to barcode and primer, and contained no ambiguous characters) were retained. In addition, we identified and removed chimeric sequence using the UCHIME algorithm (34). We aligned our sequence data set with the bacterial SILVA-based bacterial reference database (35). During data analysis, operational taxonomic units (OTUs) were binned at 97% sequence identity and phylogenetic classifications of bacterial sequences performed. Sequences were processed using the software package mothur v.1.35.1 (36, 37).

Composition and abundance of disease suppression genes

We classify disease suppressive taxa as the subset of soil microorganisms possessing genes that are required for the production of antifungal compounds 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN) (14, 24). We assessed the composition of disease suppressive
microorganisms by targeting the \textit{phlD} gene using terminal restriction fragment length polymorphism (T-RFLP) (38). For \textit{phlD} gene amplification, the forward primer B2BF (5′-ACCCACCGCAGCATCGTTTATGAGC-3′) and reverse primer FAM-BPR4 (5′-CCGCCGGTATGGAAGATGAAAAAGTC-3′) to yield a 629 bp product. In each 25 µL PCR reaction, we combined 5% dimethylsulfoxide, 0.8 mg ml$^{-1}$ bovine serum albumin, 1× GoTaq®Colorless Master Mix (Promega, Madison, WI), 0.2 µM of each primer and 5 µL of template DNA. Reactions were cycled with initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min, with a final extension carried out at 72 °C for 10 min (38). The amplified PCR products were purified with QIAquick PCR purification kit (Qiagen, Valencia, CA). After purification, amplicons generated from each sample were digested in multiple restriction enzymes overnight in 12 µL reaction mixtures containing 4 µL of PCR product, 1× enzyme buffer (38). After digestion, the enzymes were inactivated for 5 min at 80 °C, and the digested products were purified according to the purification kit protocol (Qiagen, Valencia, CA). For T-RFLP analysis, we combined 1.5 µL of the digested product with 9 µL of HiDi formamide (Applied Biosystems, Foster City, CA) and 0.4 µL of internal size standard ABI GeneScan LIZ 600 (Applied Biosystems, Foster City, CA). The samples were incubated for 3 min at 96 °C and then stored on ice prior to fragment analysis.

We determined the length and relative abundance of terminal restriction fragments (T-RFs) using an ABI 3130×1 Genetic Analyzer (Applied Biosystems, Foster City, CA). Electrophoresis conditions were 60 °C and 15 kV with a run time of 41 min using POP-7 polymer. The resulting data was analyzed using GeneMapper Software 4.0 (Applied Biosystems, Foster City, CA). The peak detection limit was set to 50 fluorescence intensity units.

We assessed the relative functional gene abundance of disease suppression by targeting
the prnD gene using quantitative PCR (qPCR) (24). The partial prnD gene abundance was quantified using a SYBR green assay with primers prnD-F (5′-TGCACTTCGCTCGTCAGAC-3′) and prnD-R (5′-GTTGCGCTCGTAGAAGTTCT-3′) (24). The 25 µL PCR reaction contained 1× GoTaq® Colorless Master Mix (Promega, Madison, WI), 0.4 µM of each primer, and 5 µL of template DNA. Cycling conditions were the following: initial cycle 95 °C, 10 min, and 30 cycles of 95 °C, 15 s and 60 °C, 1 min. For the qPCR standard curve, prnD gene was amplified from soil genomic DNA. PCR fragments were cloned to pGEM®-T Easy Vector System according to the manufacturer’s manual (Promega, Madison, WI). Plasmids were extracted using the QIAprep® Spin Miniprep kit (Qiagen, Valencia, CA), and cloned fragments were verified by PCR and agarose gel electrophoresis. Dilutions of plasmid DNA containing prnD gene were used to generate standard curves in quantities ranging from 5.0 × 10^2 to 5.0 × 10^7 copies. We quantified the prnD gene in 25 µL reaction volumes containing about 20 ng DNA template, 1×TaqMan Environmental Master Mix 2.0 (Applied Biosystems, Valencia, CA), 1× SYBR green I, and 0.4 µM of each primer. Fragments were amplified with an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min. For each sample, PCR reactions were run in triplicate. We obtained standard curves based on serial dilutions of mixed PCR product amplified from soil samples. Reactions were analyzed on a BIO-RAD CFX-96™ Real-Time System (Bio-Rad, Hercules, California, USA).

**Statistical analyses**

We used Principal Coordinates Analysis (PCoA) to visualize microbial community composition based on the Bray-Curtis dissimilarity coefficient for each possible pair of samples was used to visualize patterns of community composition using the R statistical package (R Core
Development Team 2015). To test for differences in bacterial communities among crop diversity treatments, we used permutational multivariate analysis of variance (PERMANOVA) using the \textit{adonis} function in the R Statistics Package R version 3.0.2 (R Development Core Team 2015). PERMANOVA was also used to assess the contribution of soil factors to the variation in bacterial community composition. The $R^2$ value reported refers to the treatment sums of squares divided by the total sums of squares for each soil factor in the model. Because the \textit{adonis} function carries out sequential tests (similar to Type I sums of squares) (39), the effect of the last soil factor or soil biological activity factor of the model was included in the final PERMANOVA model summary (40). We also performed multiple linear regression (gene abundance $\sim$ crop number + total soil carbon + soil moisture $+$ soil ammonium + soil nitrate) to test the influence of soil factors and crop diversity number on abundance of disease suppression/biocontrol gene \textit{prnD} using the \textit{lm} function in the R Statistics Package R version 3.0.2 (R Core Development Team 2015).

**RESULTS**

**Bacterial community composition along a crop diversity gradient**

A total of 12,539,359 sequence reads were generated, and we analyzed 24,858 OTUs for bacterial community patterns. A summary of soil attributes is presented in Table 2. The crop diversity treatment significantly influenced bacterial community composition (PERMANOVA, crop rotation: $R^2 = 0.38$, $P < 0.001$; Table 3a, Fig. 1, S1). Bacterial communities from the fallow plots and the longest crop rotations (CSW, CSW-1cov, CSW-2cov) were more similar to each other than the lower crop diversity treatments (C-1cov, CS) (Fig. 1). The monoculture corn (C) treatment was more distinct in bacterial community composition than all other crop diversity
treatments (Fig. 1). Bacterial diversity was significantly higher under lower crop diversity and fallow treatments (Fig. 2). In addition, percent sand, a soil texture factor, was the only soil property to significantly explain bacterial community variation (PERMANOVA, Sand: $R^2 = 0.063$, $P = 0.044$, Table 4a). Potentially mineralizable nitrogen, which represents the biologically available nitrogen pool, explained variation in bacterial community composition (PERMANOVA, PMN: $R^2 = 0.085$, $P = 0.013$, Table 4b).

Disease suppression functional potential in soils along a crop diversity

Crop diversity affected the composition of disease suppression soil microorganisms. We observed a significant shift in disease suppression community composition (represented by $phlD$ gene T-RFLP) along the crop diversity gradient (PERMANOVA; crop rotation: $R^2 = 0.52$, $P = 0.037$; Fig. 3, Table 3b). The $phlD$ community composition in the fallow treatment was different from other cropping systems (Fig. 2). The relative abundance of dominant T-RF 280 bp fragment group accounted for about 70% of the disease suppressive community under fallow conditions. In addition, the disease suppressive functional group T-RF 582 bp was a dominant group, representing about 31-97% relative abundance across all crop diversity treatments. In addition, $prnD$ gene abundances in cropping systems were higher than under fallow conditions (Fig. 3). In cropping systems, the $prnD$ gene in CSW-2cov treatment was the most abundant, and the gene abundance was significantly higher than in CSW and fallow treatments (Fig. 3). The $prnD$ gene abundance results suggest CSW2cov>CSW1cov>CSW and C1cov>C, which indicated that the treatments with cover crop tended to increase the $prnD$ gene abundance (Fig. 3). When crop input is removed, such as in the fallow treatment, $prnD$ gene abundance was significantly decreased (Fig. 4). Based on multiple linear regression analysis, plant and soil factors
significantly influenced \( prnD \) abundance (Adjusted \( R^2 = 0.398, F = 4.571, P = 0.005 \)). Crop species number (\( P = 0.003 \)), soil carbon (\( P = 0.002 \)), and soil moisture (\( P = 0.0005 \)) significantly influenced \( prnD \) gene abundance (Table 5).

DISCUSSION

**Crop diversity and disease suppression function relationship**

Crop rotation history impacts bacterial diversity and disease suppression potential in soils. Our findings support the hypothesis that crop diversity influences bacterial community composition and disease suppression potential in soils. Plants provide carbon to the soil environment through root exudation of recently assimilated photosynthate, composed of soluble, low molecular weight organic compounds (41). As a consequence, the increased C flow from root exudates can stimulate soil microbial activity. Changes in plant inputs through variation in root exudation are related to the crop species in the rotation. Changes in root exudates have been observed to shift microbial community composition and stimulate a diverse microbial community (18-21). Specifically, our results revealed that taxonomic diversity decreased while functional diversity increased in response to increasing crop diversity. The addition of cover crops in rotation increased disease suppressive potential. Crop diversity influenced the subset of microbes associated with disease suppression of soil-borne pathogens. In previous studies, interactions among the total microbial community and soil-borne pathogens in the plant rhizosphere have influenced both plant growth and productivity (27, 42). Our results suggest that plant diversity can enhance protection against soil-borne pathogens by fostering antagonistic soil bacterial communities (25).
The gene abundance results suggest that incorporation of cover crop in rotations increases prnD gene abundance. Specifically, cover crop species may have important effects on the prnD gene abundance and disease suppressive functional potential in soils. The prnD gene abundance in cropping systems is higher than in fallow treatment. In addition, there are no reports linking the functional group T-RF 280 to a specific genotype of laboratory strains (38). In our study, this bacterial group is capable of producing 2,4-diacetylphloroglucinol (DAPG) and affects DAPG community composition (Fig. 3). The T-RF 582 bp was also reported to be a major 2,4-diacetylphloroglucinol-producing functional Pseudomonas kilonensis in a previous study (38). The abundance of DAPG and PRN producers increasing with plant diversity has been previously observed (25). Compared to agricultural soils, the PRN producers were more frequently detected in grassland or grassland-derived plots (23, 24). The PRN gene abundance increased in the presence of grasses, but the legume species tended to decrease the DAPG and PRN producer abundance (25). Together, our findings combined with previous studies suggest that the land-use regime, plant diversity, and plant species are involved in structuring disease suppressive microbial communities.

Testing the biodiversity-ecosystem function relationship

A framework to investigate biodiversity and ecosystem functioning includes four main components: species composition, species abundance, functional traits possessed by each species, and ecosystem function to measure in response to changes in biodiversity (43). Microbial systems are ideal for addressing biodiversity and ecosystem functioning relationships within this framework (44). In our study, higher crop diversity but lower soil bacterial diversity
supported disease suppression functional potential in soils. We posit that rotation has a filtering
effect on soil microbes, whereby crop diversity selects for antagonistic microbes with disease
suppressive potential. Our study further supports the hypothesis that plant diversity can support
biocontrol functional potential by enhancing antagonistic properties of resident soil microbes
against soil-borne pathogens. The soil microbial community composition may be more important
than soil microbial diversity to soil ecological function. Increasing evidence supports that crop
species and soil type have been shown to shape the soil microbial composition and function (2,
3); thus, land management can have a large effect on soil microbial processes and microbial
community composition (45-47).

Conclusion

Here, we demonstrate that crop diversity influenced bacterial community composition
and disease suppressive functional potential in soils. Higher disease suppressive potential is
supported when crop rotations are longer and more complex. Plants and microbes drive nutrient
cycling in this long-term cropping diversity since no other synthetic or organic amendments are
applied. In our study, once crop species were removed (fallow), disease suppressive potential
dramatically decreased. Managing for enhanced disease suppressive soils remains challenging to
implement given the complex plant-soil-microbial interactions occurring in agricultural soils.
However, the legacy effects associated with crop diversity have potential to be diluted by
changes in management when fields are not actively in agricultural production (e.g., fallow plots
no longer planted with crops).
ACKNOWLEDGEMENTS

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REFERENCES


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Table 1. Cropping diversity treatments at the Kellogg Biological Station Long-term Ecological Research (KBS LTER) Biodiversity Gradient Experiment Plots. Plant treatments were established in 2000. Treatments were composed of monoculture, two-crop rotation, three-crop rotation +/- cover crops, and fallow plots (early successional) and soil collected during the corn phase of the rotation. Treatment abbreviations are in parentheses.

<table>
<thead>
<tr>
<th>Crop diversity treatment description</th>
<th>Number of crop species</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Continuous monoculture (C)</td>
<td>1</td>
</tr>
<tr>
<td>(2) Continuous monoculture, one cover crop (C-1cov)</td>
<td>2</td>
</tr>
<tr>
<td>(3) Two-crop rotation (CS)</td>
<td>2</td>
</tr>
<tr>
<td>(4) Three-crop rotation (CSW)</td>
<td>3</td>
</tr>
<tr>
<td>(5) Three-crop rotation, one cover crop (CSW-1cov)</td>
<td>4</td>
</tr>
<tr>
<td>(6) Three-crop rotation, two cover crops (CSW-2cov)</td>
<td>5</td>
</tr>
<tr>
<td>(7) Spring Fallow/early successional field (fallow)</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 2. Soil properties measured along the KBS cropping diversity gradient averaged over four replicate blocks. We acknowledge M.D. McDaniel and A.S. Grandy for these results.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total C (g C kg⁻¹ soil)</th>
<th>Total N (g N kg⁻¹ soil)</th>
<th>NH₄⁺ (mg N kg⁻¹ soil)</th>
<th>NO₃⁻ (mg N kg⁻¹ soil)</th>
<th>pH</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Sand (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fallow</td>
<td>8.74 ± 2.41</td>
<td>0.77 ± 0.16</td>
<td>0.07 ± 0.04</td>
<td>1.50 ± 0.31</td>
<td>6.70 ± 0.28</td>
<td>25 ± 9</td>
<td>39 ± 19</td>
<td>36 ± 11</td>
</tr>
<tr>
<td>CSW-2cov</td>
<td>8.98 ± 1.81</td>
<td>0.91 ± 0.12</td>
<td>0.09 ± 0.04</td>
<td>1.24 ± 0.50</td>
<td>6.25 ± 0.11</td>
<td>21 ± 10</td>
<td>51 ± 22</td>
<td>29 ± 12</td>
</tr>
<tr>
<td>CSW-1cov</td>
<td>9.63 ± 1.29</td>
<td>0.91 ± 0.04</td>
<td>0.06 ± 0.02</td>
<td>1.09 ± 0.51</td>
<td>6.37 ± 0.25</td>
<td>24 ± 10</td>
<td>46 ± 17</td>
<td>31 ± 7</td>
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<tr>
<td>CSW</td>
<td>7.43 ± 1.58</td>
<td>0.70 ± 0.10</td>
<td>0.07 ± 0.03</td>
<td>1.26 ± 0.62</td>
<td>6.54 ± 0.21</td>
<td>21 ± 10</td>
<td>45 ± 21</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>CS</td>
<td>7.70 ± 2.11</td>
<td>0.73 ± 0.26</td>
<td>0.10 ± 0.09</td>
<td>1.16 ± 0.74</td>
<td>6.66 ± 0.12</td>
<td>23 ± 11</td>
<td>41 ± 24</td>
<td>36 ± 14</td>
</tr>
<tr>
<td>C-1cov</td>
<td>9.09 ± 1.86</td>
<td>0.93 ± 0.16</td>
<td>0.06 ± 0.02</td>
<td>1.50 ± 0.65</td>
<td>6.46 ± 0.25</td>
<td>27 ± 8</td>
<td>36 ± 19</td>
<td>38 ± 12</td>
</tr>
<tr>
<td>C</td>
<td>8.09 ± 1.24</td>
<td>0.71 ± 0.15</td>
<td>0.16 ± 0.16</td>
<td>1.31 ± 0.36</td>
<td>6.58 ± 0.38</td>
<td>25 ± 9</td>
<td>36 ± 19</td>
<td>38 ± 11</td>
</tr>
</tbody>
</table>
Table 3. Effects of crop rotation on the composition of bacterial community composition and disease suppressive community composition. Summary of the contribution of crop rotation (i.e., crop diversity) on (A) bacterial community variation based on 16S rRNA amplicon sequencing or (b) disease suppressive community variation based on \textit{phlD} gene T-RFLP at the KBS Biodiversity Gradient Experimental Plots based on permutational MANOVA (PERMANOVA) results. Rotation effect was considered to significantly contribute to community variation at $P < 0.05$.

(A) 16S rRNA

<table>
<thead>
<tr>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>$R^2$</th>
<th>$P$-value</th>
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</thead>
<tbody>
<tr>
<td>Rotation</td>
<td>6</td>
<td>0.321</td>
<td>0.054</td>
<td>2.135</td>
<td>0.379</td>
</tr>
<tr>
<td>Residuals</td>
<td>21</td>
<td>0.527</td>
<td>0.025</td>
<td>0.621</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>0.848</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(B) \textit{phlD} gene

<table>
<thead>
<tr>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>$R^2$</th>
<th>$P$-value</th>
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</thead>
<tbody>
<tr>
<td>Rotation</td>
<td>8</td>
<td>3.024</td>
<td>0.378</td>
<td>1.748</td>
<td>0.518</td>
</tr>
<tr>
<td>Residuals</td>
<td>13</td>
<td>2.811</td>
<td>0.216</td>
<td>0.482</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>5.835</td>
<td>1.000</td>
<td></td>
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</table>
Table 4. Summary of the contribution of (A) soil factors and (B) soil biological activity on bacterial community variation at the KBS Biodiversity Gradient Experimental Plots based on permutational MANOVA (PERMANOVA). Soil factor effects were considered to significantly contribute to community variation at $P < 0.05$.

(A) Soil Factors

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>$R^2$</th>
<th>$P$-value</th>
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<tbody>
<tr>
<td>Sand</td>
<td>1</td>
<td>0.053</td>
<td>0.053</td>
<td>2.042</td>
<td>0.063</td>
<td>0.044</td>
</tr>
<tr>
<td>Silt</td>
<td>1</td>
<td>0.052</td>
<td>0.052</td>
<td>2.003</td>
<td>0.061</td>
<td>0.056</td>
</tr>
<tr>
<td>Clay</td>
<td>1</td>
<td>0.051</td>
<td>0.051</td>
<td>1.961</td>
<td>0.060</td>
<td>0.064</td>
</tr>
<tr>
<td>pH</td>
<td>1</td>
<td>0.024</td>
<td>0.024</td>
<td>0.915</td>
<td>0.028</td>
<td>0.513</td>
</tr>
<tr>
<td>Nitrate</td>
<td>1</td>
<td>0.010</td>
<td>0.010</td>
<td>0.385</td>
<td>0.012</td>
<td>0.982</td>
</tr>
<tr>
<td>Ammonium</td>
<td>1</td>
<td>0.009</td>
<td>0.009</td>
<td>0.345</td>
<td>0.011</td>
<td>0.981</td>
</tr>
<tr>
<td>Nitrogen</td>
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<td>0.028</td>
<td>1.061</td>
<td>0.033</td>
<td>0.369</td>
</tr>
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<td>Carbon</td>
<td>1</td>
<td>0.013</td>
<td>0.013</td>
<td>0.501</td>
<td>0.015</td>
<td>0.902</td>
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<tr>
<td>Moisture</td>
<td>1</td>
<td>0.039</td>
<td>0.039</td>
<td>1.481</td>
<td>0.045</td>
<td>0.145</td>
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<tr>
<td>Residuals</td>
<td>18</td>
<td>0.469</td>
<td>0.026</td>
<td></td>
<td>0.553</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>0.848</td>
<td></td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(B) Soil Biological Activity

<table>
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<tr>
<th>Effect</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>$R^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN</td>
<td>1</td>
<td>0.072</td>
<td>0.072</td>
<td>2.485</td>
<td>0.085</td>
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<tr>
<td>PMC</td>
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<td>0.038</td>
<td>1.314</td>
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<td>0.211</td>
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<tr>
<td>POXC</td>
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<td>0.050</td>
<td>0.050</td>
<td>1.715</td>
<td>0.059</td>
<td>0.104</td>
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<tr>
<td>Residuals</td>
<td>24</td>
<td>0.698</td>
<td>0.029</td>
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<td>0.823</td>
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<tr>
<td>Total</td>
<td>27</td>
<td>0.848</td>
<td></td>
<td></td>
<td>1.000</td>
<td></td>
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</table>
Table 5. Summary of multiple linear regression to test the influence of disease suppressive functional potential \((prnD\) gene abundance) on soil factors and crop diversity.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Estimate</th>
<th>Std error</th>
<th>t-value</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.444</td>
<td>0.420</td>
<td>17.728</td>
<td>&lt; 0.001</td>
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<tr>
<td>Crop_number</td>
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<td>0.025</td>
<td>-3.355</td>
<td>0.003</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.180</td>
<td>0.050</td>
<td>3.618</td>
<td>0.002</td>
</tr>
<tr>
<td>Moisture</td>
<td>-11.564</td>
<td>2.817</td>
<td>-4.105</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ammonium</td>
<td>-0.701</td>
<td>0.948</td>
<td>-0.739</td>
<td>0.468</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.093</td>
<td>0.136</td>
<td>0.684</td>
<td>0.501</td>
</tr>
</tbody>
</table>
FIGURES

Figure 1. Ordination from Principal Coordinates Analysis depicting soil bacterial communities along a cropping diversity gradient. Symbols are colored according to cropping diversity treatment (C=continuous corn; C-1cov=corn/1 cover crop; CS=corn/soy; CSW=corn/soy/wheat; CSW-1cov=corn/soy/wheat/1 cover crop; CSW-2cov=corn/soy/wheat/2 cover crops; fallow=spring fallow, tilled annually).

Figure 2. The bacterial diversity (mean ± SEM based on Shannon Diversity Index H’) in response to long-term crop diversity treatment. Different letters above points reflect significant differences in gene abundance along crop diversity gradient at $P < 0.05$ (Tukey’s HSD post-hoc analysis).

Figure 3. Ordination from Principal Coordinates Analysis of disease suppressive community based on T-RFLP of phlD gene (DAPG producers) T-RF relative abundance data obtained from different cropping system treatments.

Figure 4. Abundance of prnD gene (PRN producers) in response to crop diversity treatment analyzed using quantitative PCR and expressed as log copy number of prnD gene. Different letters above points reflect significant differences in gene abundance at $P < 0.05$ (Tukey’s HSD post-hoc analysis).
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Figure S1. Principal coordinates analysis plot of classified only 16S rRNA sequences based on relative abundance of classified OTUs at the phylum, order, and genus levels.