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), N_2 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

70 associated reductions in fungal pathogens (14, 22). Bacterial production of secondary
71 metabolites 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN) are two potent bacterial
72 toxins known to suppress fungal pathogens in agricultural soils (14, 23, 24). In a previous study,
73 increased plant diversity was associated with enhanced soil suppressiveness measured by
74 increased DAPG and PRN producers (25). In addition, streptomycetes are a well-known group of
75 microbes to have antibiotic inhibitory effects and contribute to disease suppression in agricultural
76 soils (15, 26). However, the diversity, composition, and disease suppressive activity among
77 streptomycetes communities was similar among high and low plant diversity treatments (27).
78 Thus, the relationship between biodiversity and disease suppression in agricultural soils remains
79 unclear. By focusing on disease suppressive capacity of soil, we can evaluate how agricultural
80 land-use strategies and subsequent changes in the soil environment and resident microbes impact
81 contemporary plant growth (27, 28).

82 Heavily managed agroecosystems are ideal locations to test the influence of crop
83 diversity effects on soil microorganisms. Specifically, long-term, replicated crop diversity trials
84 provide a unique opportunity to focus on plant effects on soil microbial communities. Our
85 research addresses the following questions: (1) What is the relationship between crop diversity
86 and soil microbial community composition and disease suppressive functional potential? and (2)
87 what is the relationship between changes in soil physicochemical properties and soil microbial
88 community composition and disease suppressive functional potential in response to a crop
89 diversity gradient? We tested the hypothesis that plant and microbial biodiversity provide disease
90 suppressive functions in soils. We predicted that soil microbes established under high crop
91 diversity (i.e., long crop rotations) have relatively higher soil suppressive capacity compared to
92 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.

116 **Bacterial community sequencing**

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

134

135 **Composition and abundance of disease suppression genes**

136 We classify disease suppressive taxa as the subset of soil microorganisms possessing
137 genes that are required for the production of antifungal compounds 2,4-diacetylphloroglucinol
138 (DAPG) and pyrrolnitrin (PRN) (14, 24). We assessed the composition of disease suppressive

139 microorganisms by targeting the *phlD* gene using terminal restriction fragment length
140 polymorphism (T-RFLP) (38). For *phlD* gene amplification, the forward primer B2BF (5′-
141 ACCCACCGCAGCATCGTTTATGAGC-3′) and reverse primer FAM-BPR4 (5′-
142 CCGCCGGTATGGAAGATGAAAAAGTC-3′) to yield a 629 bp product. In each 25 μL PCR
143 reaction, we combined 5% dimethylsulfoxide, 0.8 mg ml^{-1} bovine serum albumin, 1×
144 GoTaq®Colorless Master Mix (Promega, Madison, WI), 0.2 μM of each primer and 5 μL of
145 template DNA. Reactions were cycled with initial denaturation at 94 °C for 2 min, followed by
146 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min, with a final extension carried
147 out at 72 °C for 10 min (38). The amplified PCR products were purified with QIAquick PCR
148 purification kit (Qiagen, Valencia, CA). After purification, amplicons generated from each
149 sample were digested in multiple restriction enzymes overnight in 12 μL reaction mixtures
150 containing 4 μL of PCR product, 1× enzyme buffer (38). After digestion, the enzymes were
151 inactivated for 5 min at 80 °C, and the digested products were purified according to the
152 purification kit protocol (Qiagen, Valencia, CA). For T-RFLP analysis, we combined 1.5 μL of
153 the digested product with 9 μL of HiDi formamide (Applied Biosystems, Foster City, CA) and
154 0.4 μL of internal size standard ABI GeneScan LIZ 600 (Applied Biosystems, Foster City, CA).
155 The samples were incubated for 3 min at 96 °C and then stored on ice prior to fragment analysis.
156 We determined the length and relative abundance of terminal restriction fragments (T-RFs) using
157 an ABI 3130×1 Genetic Analyzer (Applied Biosystems, Foster City, CA). Electrophoresis
158 conditions were 60 °C and 15 kV with a run time of 41 min using POP-7 polymer. The resulting
159 data was analyzed using GeneMapper Software 4.0 (Applied Biosystems, Foster City, CA). The
160 peak detection limit was set to 50 fluorescence intensity units.
161 We assessed the relative functional gene abundance of disease suppression by targeting

162 the *prnD* gene using quantitative PCR (qPCR) (24). The partial *prnD* gene abundance was
163 quantified using a SYBR green assay with primers prnD-F (5′-TGCACTTCGCGTTCGAGAC-
164 3′) and prnD-R (5′-GTTGCGCGTCGTAGAAGTTCT-3′) (24). The 25 μL PCR reaction
165 contained 1× GoTaq®Colorless Master Mix (Promega, Madison, WI), 0.4 μM of each primer,
166 and 5 μL of template DNA. Cycling conditions were the following: initial cycle 95 °C, 10 min,
167 and 30 cycles of 95 °C, 15 s and 60 °C, 1 min. For the qPCR standard curve, *prnD* gene was
168 amplified from soil genomic DNA. PCR fragments were cloned to pGEM®-T Easy Vector
169 System according to the manufacturer's manual (Promega, Madison, WI). Plasmids were
170 extracted using the QIAprep® Spin Miniprep kit (Qiagen, Valencia, CA), and cloned fragments
171 were verified by PCR and agarose gel electrophoresis. Dilutions of plasmid DNA containing
172 *prnD* gene were used to generate standard curves in quantities ranging from 5.0×10^2 to $5.0 \times$
173 10^7 copies. We quantified the *prnD* gene in 25 μL reaction volumes containing about 20 ng DNA
174 template, 1×TaqMan Environmental Master Mix 2.0 (Applied Biosystems, Valencia, CA), 1×
175 SYBR green I, and 0.4 μM of each primer. Fragments were amplified with an initial denaturation
176 step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min. For each
177 sample, PCR reactions were run in triplicate. We obtained standard curves based on serial
178 dilutions of mixed PCR product amplified from soil samples. Reactions were analyzed on a BIO-
179 RAD CFX-96™ Real-Time System (Bio-Rad, Hercules, California, USA).
180

181 **Statistical analyses**

182 We used Principal Coordinates Analysis (PCoA) to visualize microbial community
183 composition based on the Bray-Curtis dissimilarity coefficient for each possible pair of samples
184 was used to visualize patterns of community composition using the R statistical package (R Core

185 Development Team 2015). To test for differences in bacterial communities among crop diversity
186 treatments, we used permutational multivariate analysis of variance (PERMANOVA) using the
187 *adonis* function in the R Statistics Package R version 3.0.2 (R Development Core Team 2015).
188 PERMANOVA was also used to assess the contribution of soil factors to the variation in
189 bacterial community composition. The R^2 value reported refers to the treatment sums of squares
190 divided by the total sums of squares for each soil factor in the model. Because the *adonis*
191 function carries out sequential tests (similar to Type I sums of squares) (39), the effect of the last
192 soil factor or soil biological activity factor of the model was included in the final PERMANOVA
193 model summary (40). We also performed multiple linear regression (gene abundance ~ crop
194 number + total soil carbon + soil moisture + soil ammonium + soil nitrate) to test the influence
195 of soil factors and crop diversity number on abundance of disease suppression/biocontrol gene
196 *prnD* using the *lm* function in the R Statistics Package R version 3.0.2 (R Core Development
197 Team 2015).
198
199 **RESULTS**
200 **Bacterial community composition along a crop diversity gradient**
201 A total of 12,539,359 sequence reads were generated, and we analyzed 24,858 OTUs for
202 bacterial community patterns. A summary of soil attributes is presented in Table S1 and reported
203 in McDaniel et al. (2014) (41). The crop diversity treatment significantly influenced bacterial
204 community composition (PERMANOVA, crop rotation: $R^2 = 0.38$, $P < 0.001$; Table 2a, Fig. 1,
205 S1). Bacterial communities from the fallow plots and the longest crop rotations (CSW, CSW-
206 1cov, CSW-2cov) were more similar to each other than the lower crop diversity treatments (C-
207 1cov, CS) (Fig. 1). The monoculture corn (C) treatment was more distinct in bacterial

208 community composition than all other crop diversity treatments (Fig. 1). Bacterial diversity was
209 significantly higher under lower crop diversity and fallow treatments (Fig. 2). In addition,
210 percent sand, a soil texture factor, was the only soil property to significantly explain bacterial
211 community variation (PERMANOVA, Sand: R^2 = 0.063, P = 0.044, Table 3a). Potentially
212 mineralizable nitrogen, which represents the biologically available nitrogen pool, explained
213 variation in bacterial community composition (PERMANOVA, PMN: R^2 = 0.085, P = 0.013,
214 Table 3b).
215
216 **Disease suppression functional potential in soils along a crop diversity**
217 Crop diversity affected the composition of disease suppression soil microorganisms. We
218 observed a significant shift in disease suppression community composition (represented by *phlD*
219 gene T-RFLP) along the crop diversity gradient (PERMANOVA; crop rotation: R^2 = 0.52, P =
220 0.037; Fig. 3, Table 2b). The *phlD* community composition in the fallow treatment was different
221 from other cropping systems (Fig. 2). The relative abundance of dominant T-RF 280 bp fragment
222 group accounted for about 70% of the disease suppressive community under fallow conditions.
223 In addition, the disease suppressive functional group T-RF 582 bp was a dominant group,
224 representing about 31-97% relative abundance across all crop diversity treatments. In addition,
225 *prnD* gene abundances in cropping systems were higher than under fallow conditions (Fig. 3). In
226 cropping systems, the *prnD* gene in CSW-2cov treatment was the most abundant, and the gene
227 abundance was significantly higher than in CSW and fallow treatments (Fig. 3). The *prnD* gene
228 abundance results suggest CSW2cov>CSW1cov>CSW and C1cov>C, which indicated that the
229 treatments with cover crop tended to increase the *prnD* gene abundance (Fig. 3). When crop
230 input is removed, such as in the fallow treatment, *prnD* gene abundance was significantly

231 decreased (Fig. 4). Based on multiple linear regression analysis, plant and soil factors
232 significantly influenced *prnD* abundance (Adjusted R^2 = 0.398, F = 4.571, P = 0.005). Crop
233 species number (P = 0.003), soil carbon (P = 0.002), and soil moisture (P = 0.0005) significantly
234 influenced *prnD* gene abundance (Table 4).
235
236 **DISCUSSION**
237 **Crop diversity and disease suppression function relationship**
238 Crop rotation history impacts bacterial diversity and disease suppression potential in
239 soils. Our findings support the hypothesis that crop diversity influences bacterial community
240 composition and disease suppression potential in soils. Plants provide carbon to the soil
241 environment through root exudation of recently assimilated photosynthate, composed of soluble,
242 low molecular weight organic compounds (42). As a consequence, the increased C flow from
243 root exudates can stimulate soil microbial activity. Changes in plant inputs through variation in
244 root exudation are related to the crop species in the rotation. Changes in root exudates have been
245 observed to shift microbial community composition and stimulate a diverse microbial
246 community (18-21). Specifically, our results revealed that taxonomic diversity decreased while
247 functional diversity increased in response to increasing crop diversity. The addition of cover
248 crops in rotation increased disease suppressive potential. Crop diversity influenced the subset of
249 microbes associated with disease suppression of soil-borne pathogens. In previous studies,
250 interactions among the total microbial community and soil-borne pathogens in the plant
251 rhizosphere have influenced both plant growth and productivity (27, 43). Our results suggest that
252 plant diversity can enhance protection against soil-borne pathogens by fostering antagonistic soil
253 bacterial communities (25).

254

255 **Crop rotation effects on DAPG and PRN producers**

256 The gene abundance results suggest that incorporation of cover crop in rotations increases
257 *prnD* gene abundance. Specifically, cover crop species may have important effects on the *prnD*
258 gene abundance and disease suppressive functional potential in soils. The *prnD* gene abundance
259 in cropping systems is higher than in fallow treatment. In addition, there are no reports linking
260 the functional group T-RF 280 to a specific genotype of laboratory strains (38). In our study, this
261 bacterial group is capable of producing 2,4-diacetylphloroglucinol (DAPG) and affects DAPG
262 community composition (Fig. 3). The T-RF 582 bp was also reported to be a major 2,4-
263 diacetylphloroglucinol-producing functional *Pseudomonas kilonensis* in a previous study (38).
264 The abundance of DAPG and PRN producers increasing with plant diversity has been previously
265 observed (25). Compared to agricultural soils, the PRN producers were more frequently detected
266 in grassland or grassland-derived plots (23, 24). The PRN gene abundance increased in the
267 presence of grasses, but the legume species tended to decrease the DAPG and PRN producer
268 abundance (25). Together, our findings combined with previous studies suggest that the land-use
269 regime, plant diversity, and plant species are involved in structuring disease suppressive
270 microbial communities.

271

272 **Testing the biodiversity-ecosystem function relationship**

273 A framework to investigate biodiversity and ecosystem functioning includes four main
274 components: species composition, species abundance, functional traits possessed by each
275 species, and ecosystem function to measure in response to changes in biodiversity (44).
276 Microbial systems are ideal for addressing biodiversity and ecosystem functioning relationships

277 within this framework (45). In our study, higher crop diversity but lower soil bacterial diversity
278 supported disease suppression functional potential in soils. We posit that rotation has a filtering
279 effect on soil microbes, whereby crop diversity selects for antagonistic microbes with disease
280 suppressive potential. Our study further supports the hypothesis that plant diversity can support
281 biocontrol functional potential by enhancing antagonistic properties of resident soil microbes
282 against soil-borne pathogens. The soil microbial community composition may be more important
283 than soil microbial diversity to soil ecological function. Increasing evidence supports that crop
284 species and soil type have been shown to shape the soil microbial composition and function (2,
285 3); thus, land management can have a large effect on soil microbial processes and microbial
286 community composition (46-48).
287
288 **Conclusion**
289 Here, we demonstrate that crop diversity influenced bacterial community composition
290 and disease suppressive functional potential in soils. Higher disease suppressive potential is
291 supported when crop rotations are longer and more complex. Plants and microbes drive nutrient
292 cycling in this long-term cropping diversity since no other synthetic or organic amendments are
293 applied. In our study, once crop species were removed (fallow), disease suppressive potential
294 dramatically decreased. Managing for enhanced disease suppressive soils remains challenging to
295 implement given the complex plant-soil-microbial interactions occurring in agricultural soils.
296 However, the legacy effects associated with crop diversity have potential to be diluted by
297 changes in management when fields are not actively in agricultural production (e.g., fallow plots
298 no longer planted with crops).
299

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453 management and sample year on abundance of soil bacterial communities in organic and
454 conventional cropping systems. J Appl Microbiol.
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459 **TABLES**

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

465

Crop diversity treatment description	Number of crop species
(1) Continuous monoculture (C)	1
(2) Continuous monoculture, one cover crop (C-1cov)	2
(3) Two-crop rotation (CS)	2
(4) Three-crop rotation (CSW)	3
(5) Three-crop rotation, one cover crop (CSW-1cov)	4
(6) Three-crop rotation, two cover crops (CSW-2cov)	5
(7) Spring Fallow/early successional field (fallow)	10

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474 Table 2. Effects of crop rotation on the composition of bacterial community composition and
475 disease suppressive community composition. Summary of the contribution of crop rotation (i.e.,
476 crop diversity) on (A) bacterial community variation based on 16S rRNA amplicon sequencing
477 or (b) disease suppressive community variation based on *phlD* gene T-RFLP at the KBS
478 Biodiversity Gradient Experimental Plots based on permutational MANOVA (PERMANOVA)
479 results. Rotation effect was considered to significantly contribute to community variation at $P <$
480 0.05.
481

(A) 16S rRNA

	df	SS	MS	F	R^2	P-value
Rotation	6	0.321	0.054	2.135	0.379	<0.001
Residuals	21	0.527	0.025	0.621		
Total	27	0.848	1.000			

(B) *phlD* gene

	df	SS	MS	F	R^2	P-value
Rotation	8	3.024	0.378	1.748	0.518	0.037
Residuals	13	2.811	0.216	0.482		
Total	21	5.835	1.000			

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487 Table 3. Summary of the contribution of (A) soil factors and (B) soil biological activity on
488 bacterial community variation at the KBS Biodiversity Gradient Experimental Plots based on
489 permutational MANOVA (PERMANOVA). Soil factor effects were considered to significantly
490 contribute to community variation at $P < 0.05$.

491 (A) Soil Factors

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

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498 (B) Soil Biological Activity

Effect	df	SS	MS	F	R^2	P-value
PMN	1	0.072	0.072	2.485	0.085	0.013
PMC	1	0.038	0.038	1.314	0.045	0.211
POXC	1	0.050	0.050	1.715	0.059	0.104
Residuals	24	0.698	0.029		0.823	
Total	27	0.848			1.000	

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514 Table 4. Summary of multiple linear regression to test the influence of disease suppressive

515 functional potential (*prnD* gene abundance) on soil factors and crop diversity.

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Factor	Estimate	Std error	t-value	*p*-value
Intercept	7.444	0.420	17.728	< 0.001
Crop_number	-0.085	0.025	-3.355	0.003
Carbon	0.180	0.050	3.618	0.002
Moisture	-11.564	2.817	-4.105	< 0.001
Ammonium	-0.701	0.948	-0.739	0.468
Nitrate	0.093	0.136	0.684	0.501

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530 **FIGURES**

531

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

537

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

542

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

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547 Figure 4. Abundance of *prn*D gene (PRN producers) in response to crop diversity treatment
548 analyzed using quantitative PCR and expressed as log copy number of *prn*D gene. Different
549 letters above points reflect significant differences in Different letters above boxplots considered
550 significantly different in gene abundance at $P < 0.05$ (Tukey's HSD *post-hoc* analysis).

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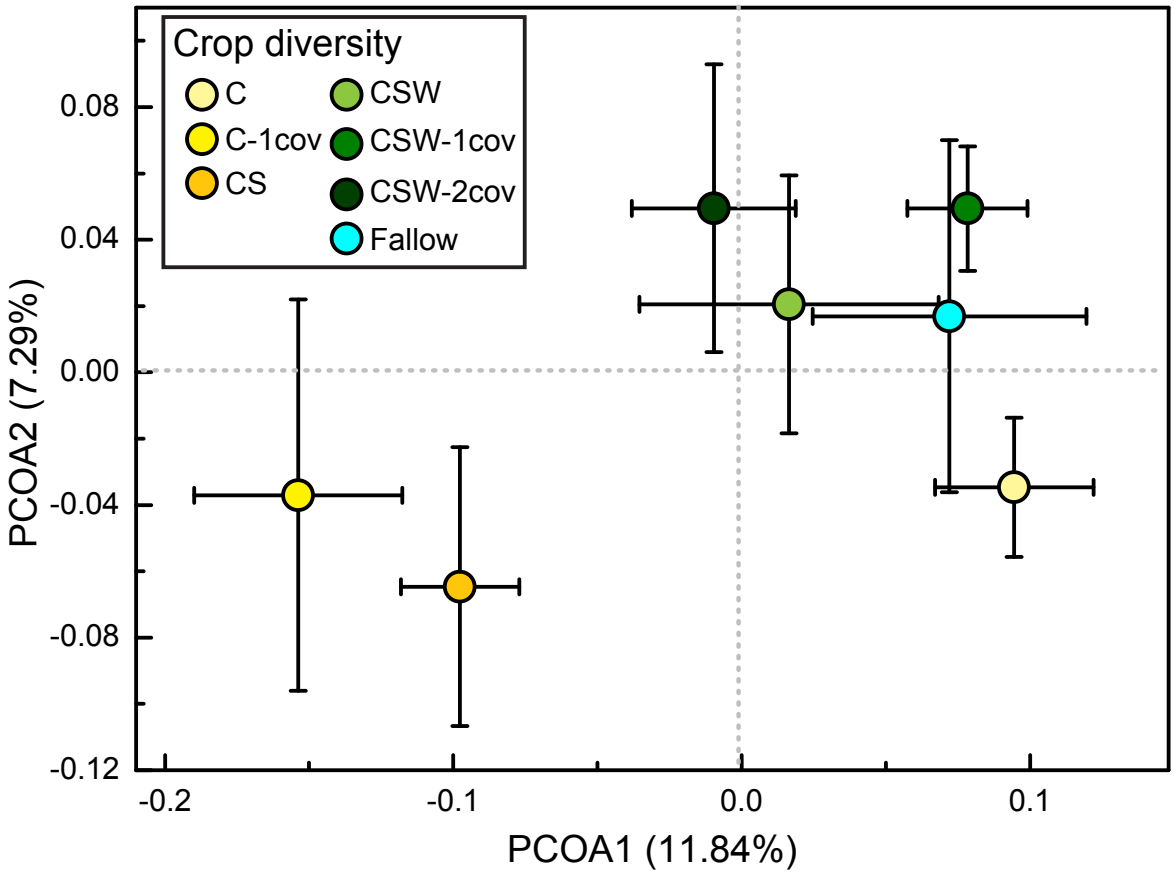
553 Figure 1



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558 CSW-1cov=corn/soy/wheat/1 cover crop; CSW-2cov=corn/soy/wheat/2 cover crops;
559 fallow=spring fallow, tilled annually).

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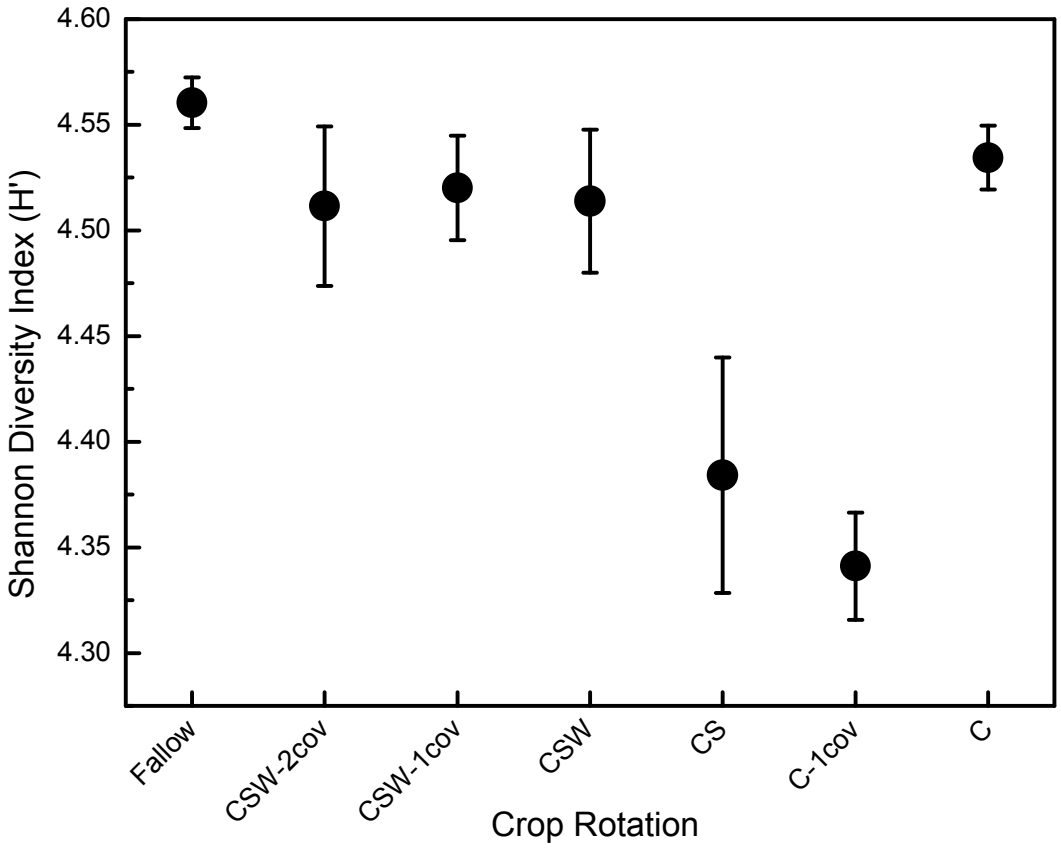
565 Figure 2



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567 Figure 2. The bacterial diversity (mean ± SEM based on Shannon Diversity Index H') in

568 response to long-term crop diversity treatment. Different letters above points reflect significant

569 differences in gene abundance along crop diversity gradient at $P < 0.05$ (Tukey's HSD *post-hoc*

570 analysis).

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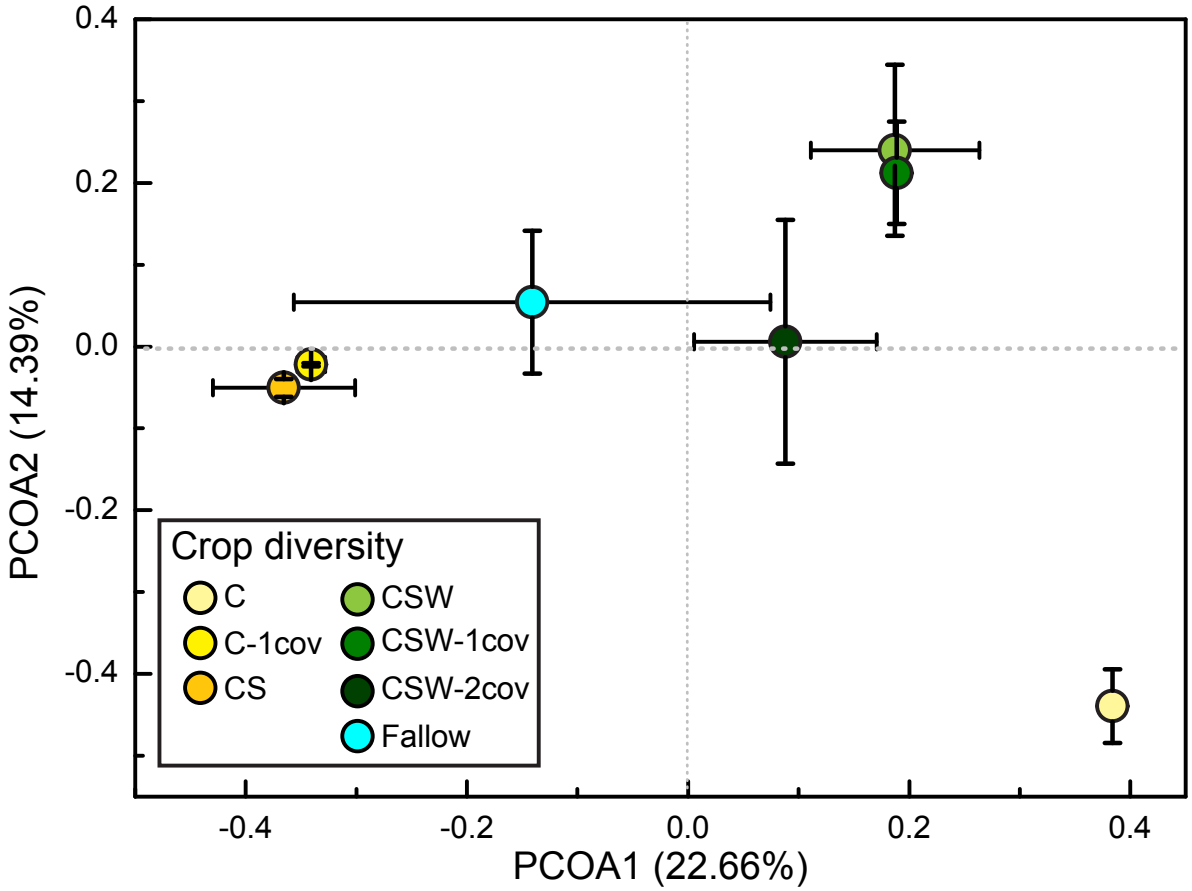
577 Figure 3



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579 Figure 3. Ordination from Principal Coordinates Analysis of disease suppressive community

580 based on T-RFLP of *phl*D gene (DAPG producers) T-RF relative abundance data obtained from

581 different cropping system treatments.

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589 Figure 4

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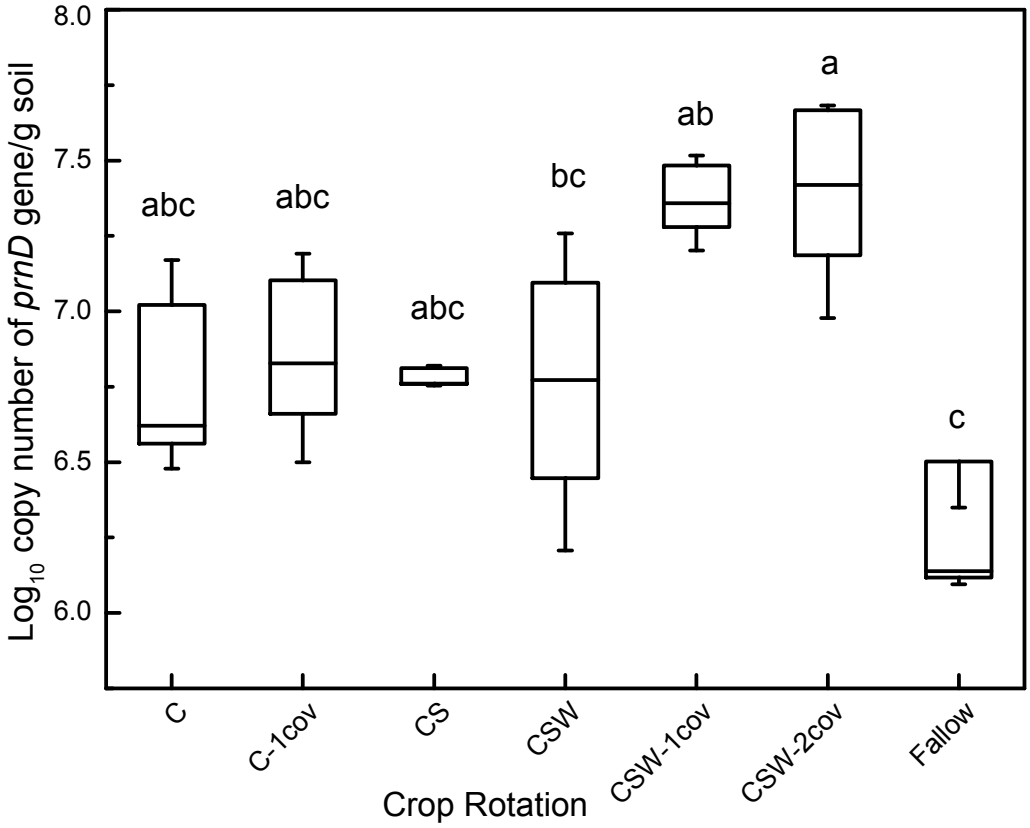

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592 Figure 4. Abundance of *prn*D gene (PRN producers) in response to crop diversity treatment

593 analyzed using quantitative PCR and expressed as log copy number of *prn*D gene. Different

594 letters above points reflect significant differences in Different letters above boxplots considered

595 significantly different in gene abundance at $P < 0.05$ (Tukey's HSD *post-hoc* analysis).

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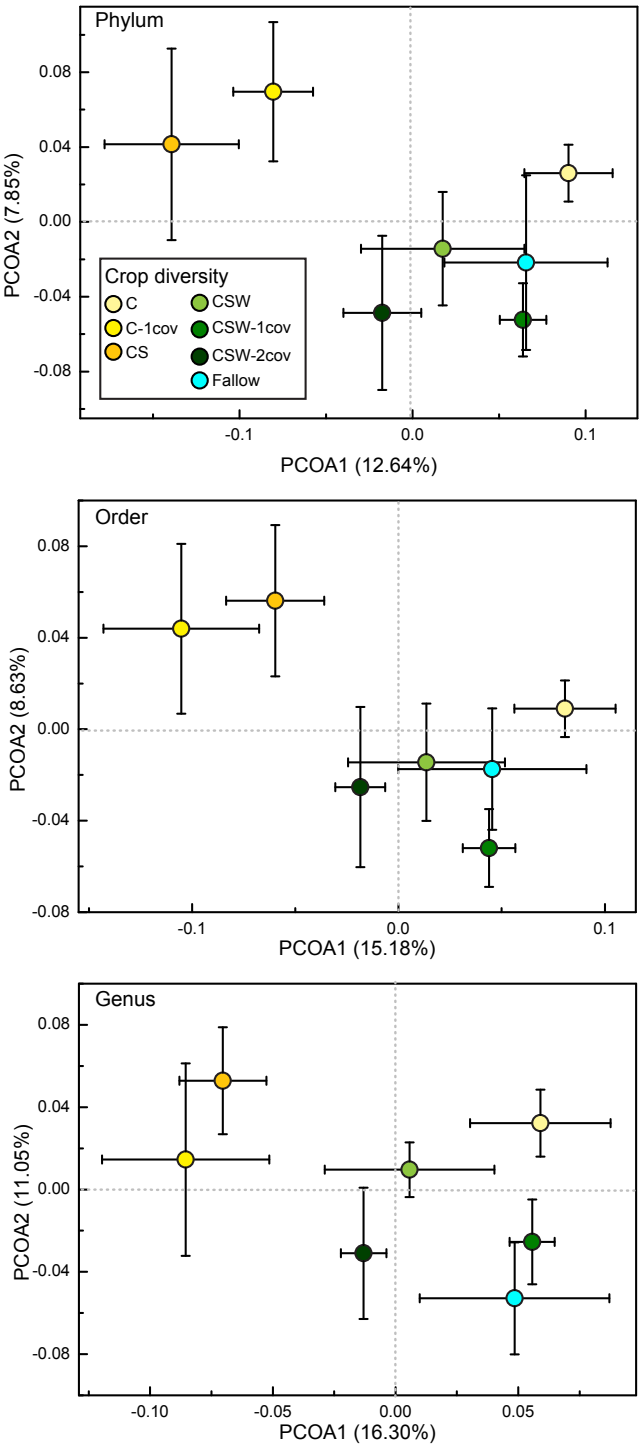
601 **SUPPLEMENTAL**



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603 Figure S1. Principal coordinates analysis plot of classified only 16S rRNA sequences based on

604 relative abundance of classified OTUs at the phylum, order, and genus levels.

603 **SUPPLEMENTAL**

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605 Table S1. Soil properties measured along the KBS cropping diversity gradient averaged over four replicate blocks. Comparison of soil

606 properties and biological activity results are summarized in M.D. McDaniel et al. (2014) (41).

607

Treatment	Total C (g C kg^{-1} soil)	Total N (g N kg^{-1} soil)	NH_4^+ (mg N kg^{-1} soil)	NO_3^- (mg N kg^{-1} soil)	pH	Clay (%)	Silt (%)	Sand (%)
Fallow	8.74 ± 2.41	0.77 ± 0.16	0.07 ± 0.04	1.50 ± 0.31	6.70 ± 0.28	25 ± 9	39 ± 19	36 ± 11
CSW-2cov	8.98 ± 1.81	0.91 ± 0.12	0.09 ± 0.04	1.24 ± 0.50	6.25 ± 0.11	21 ± 10	51 ± 22	29 ± 12
CSW-1cov	9.63 ± 1.29	0.91 ± 0.04	0.06 ± 0.02	1.09 ± 0.51	6.37 ± 0.25	24 ± 10	46 ± 17	31 ± 7
CSW	7.43 ± 1.58	0.70 ± 0.10	0.07 ± 0.03	1.26 ± 0.62	6.54 ± 0.21	21 ± 10	45 ± 21	34 ± 12
CS	7.70 ± 2.11	0.73 ± 0.26	0.10 ± 0.09	1.16 ± 0.74	6.66 ± 0.12	23 ± 11	41 ± 24	36 ± 14
C-1cov	9.09 ± 1.86	0.93 ± 0.16	0.06 ± 0.02	1.50 ± 0.65	6.46 ± 0.25	27 ± 8	36 ± 19	38 ± 12
C	8.09 ± 1.24	0.71 ± 0.15	0.16 ± 0.16	1.31 ± 0.36	6.58 ± 0.38	25 ± 9	36 ± 19	38 ± 11

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