Crop diversity enhances disease suppressive potential in soils

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ABSTRACT

Biodiversity is thought to regulate a wide range of agroecosystem processes including plant production and disease suppression. Farmers have used crop rotations, a form of biodiversity, for thousands of years and this may be due, in part, to early observations of "disease prevention" in the form of increased yield. However, the evidence for a mechanistic link between crop rotations and disease suppression has not yet been elucidated. Disease suppressive soils are characterized by the biocontrol properties provided by resident soil microorganisms. Biocontrol properties include antibiosis via production of 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 (via rotation) on microbial communities and disease suppressive functional potential in soils. We hypothesized that plant and microbial biodiversity provide disease suppressive functions in soils. To address these hypotheses, we collected soil samples from a 12-year crop rotation experiment at the Kellogg Biological Station Long-Term Ecological Research (KBS LTER) site. We sampled seven treatments along a crop diversity gradient (monoculture to five crop species) and a spring fallow (naturally regenerating plants) treatment to examine the influence of crop diversity on total bacterial community composition (16S rRNA gene 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 crop rotations decreased bacterial diversity by 4% on average compared to monocultures. Crop rotations did, however, increase disease suppressive functional group *prnD* gene abundance in the more diverse rotation (corn-soybean-wheat + cover crops) by about 9% compared to monocultures. Variation in plant inputs to soil organic matter pools may be a

47 possible mechanism driving shifts in microbial community patterns and disease suppressive
48 functional potential.
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50 Keywords
51 Crop rotation; disease suppression; microbial diversity; structure-function relationships
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53 Abbreviations
54 2,4-diacetylphloroglucinol (DAPG); plant growth promoting rhizobacteria (PGPR); plant
55 pathogen suppression (PPS); pyrrolnitrin (PRN)
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1. Introduction

Agricultural intensification has led to declines in biodiversity and in associated ecosystem functions (Tilman et al., 2002). However, crop diversification within agricultural landscapes is a strategy used to alleviate this loss in biodiversity. Increases in crop biodiversity on the farm can take many forms, such as crop rotations, cover crops, inter-cropping, and cover crop mixtures. From a management or conservation perspective, crop rotations are not the traditional form of increasing biodiversity. Instead of managing species in space, crop rotations increase diversity through time. This is because at any given time the species richness on a farm using crop rotations is often one (same as monocultures), but there is a diverse suite of biochemical inputs from crops planted at different times to soil microorganisms. There is mounting evidence that this form of 'temporal biodiversity' may provide some of the same beneficial ecosystem functions as traditional spatial biodiversity, such as carbon sequestration, pest control, and nutrient cycling (Ball et al., 2005; McDaniel et al., 2014b; Tiemann et al., 2015; Venter et al., 2016).

Different mechanisms confer plant pathogen suppression (PPS) in the soil. First, crops and the soil microbial community are linked by the plant inputs of carbon and nutrients to the soil. Studies have shown the quantity and quality, and likely even the diversity of crop inputs (residues and rhizodeposits) to soil can alter the microbial community and functioning (Zak et al., 2003; Hättenschwiler et al., 2005; Dijkstra et al., 2010; van der Putten et al., 2016). Second, each crop will affect the physical characteristics of the soil environment whether it is with differences in water use (Tilman et al., 2002), shading (Liebman and Dyck, 1993), aggregation (Tiemann et al., 2015), root morphology affecting porosity (Smucker, 1993), or all of the above. Both of these chemical and physical mechanisms of crop influence on soil microbial

93 communities might alter the functions important to crops, such as nutrient mineralization from
94 soil organic matter (Barness et al., 1991), N_2 fixation (Reed et al., 2010), plant growth promotion
95 (Lugtenberg and Kamilova, 2009), and plant pathogen suppression (Bever et al., 1997; Haas and
96 Defago, 2005; Perez et al., 2008). The stimulation of these functions is what likely controls
97 plant-soil feedbacks in agroecosystems (Bever et al., 1997; Kulmatiski et al., 2008; van der
98 Putten et al., 2016).

99 Greater plant pathogen suppression has been associated with soil microbial communities
100 known to exhibit antimicrobial properties, which ultimately provide plants with protection from
101 soil-borne plant pathogens. Greater soil microbial diversity can provide more opportunity for
102 PPS potential microorganisms to be maintained although may not be required. Maintenance of
103 these PPS microorganisms in the community are thought to occur due to competition for iron,
104 antibiosis, lytic enzymes, and induction of system resistance with host plant (Doornbos et al.,
105 2012). Specifically, antibiosis has been linked to disease suppressive capacity, whereby the
106 abundance of antagonistic bacteria has been associated reductions in fungal pathogens through
107 competitive inhibition (Weller et al., 2002; Haas and Defago, 2005). Bacterial production of
108 secondary metabolites 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN) are two potent
109 toxins known to suppress fungal pathogens in agricultural soils (Garbeva et al., 2004a; Garbeva
110 et al., 2004b; Haas and Defago, 2005). In a previous study, increased plant diversity was
111 associated with enhanced soil disease suppressiveness measured by increased DAPG and PRN
112 producers (Latz et al., 2012). In other studies, streptomycetes (a well-known group of bacteria
113 possessing antibiotic inhibitory effects) were found to contribute to disease suppression in
114 agricultural soils (Wiggins and Kinkel, 2005; Perez et al., 2008). However, the diversity,
115 composition, and disease suppressive activity among streptomycetes communities has also been

116 found to be unrelated to plant diversity treatments (Bakker et al., 2010). Thus, the relationship
117 between biodiversity and disease suppression in agricultural soils remains unclear. By focusing
118 on the disease suppressive capacity of soil, we can evaluate how agricultural land-use strategies
119 and subsequent changes in the soil environment and resident microorganisms impact plant
120 growth (Bakker et al., 2010; Kulmatiski and Beard, 2011).

121 Given the unknown effect of crop diversity, via rotations on microbial communities and
122 plant pathogen suppression, we used a long-term (12 y) crop rotation study at the Kellogg
123 Biological Station LTER to test the effect of crop diversity on soil bacterial biodiversity and PPS
124 potential. Specifically, our research addresses the following questions: (1) what is the
125 relationship between crop diversity and soil microbial community composition and disease
126 suppressive functional potential? and (2) what is the relationship between changes in soil
127 physicochemical properties, soil microbial community composition, and disease suppressive
128 functional potential in response to a crop diversity gradient? We tested the hypothesis that plant
129 and microbial biodiversity provide disease suppressive functions in soils. We predicted that soils
130 from high crop diversity (i.e., long crop rotations) would have greater soil bacterial diversity and
131 have greater PPS capacity compared to soil microbial communities developed under low crop
132 diversity (i.e., monoculture and short crop rotations like corn-soybean).
133
134 **2. Methods**
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136 *2.1. Site description & experimental design*
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138 We collected soils from the Biodiversity Gradient Experiment

(http://lter.kbs.msu.edu/research/long-term-experiments/biodiversity-gradient/) at W.K. Kellogg Biological Station Long-Term Ecological Research (KBS LTER) site in southwest, Michigan, USA. Mean annual temperature is 9.7 °C and mean annual precipitation is 890 mm. The soils are Kalamazoo (fine-loamy) and Oshtemo (coarse-loamy) mixed, mesic, Typic Hapluadalfs formed under glacial outwash (Crum and Collins, 1995). The crop rotation treatments at the Biodiversity Gradient Experiment included: monoculture corn (*Zea maize*, mC), corn with 1 red clover (*Trifolium pretense* L.) cover crop (C_{1cov}), corn-soy (*Glycine max*, CS), corn-soy-wheat (*Triticum aestivum*, CSW), CSW with red clover (CSW_{1cov}), CSW with red clover and cereal rye (*Secale cereal* L., CSW_{2cov}), and a spring fallow treatment that was just plowed every spring but contains 7-10 naturally-occurring plant species in the region (Table 1). This spring fallow treatment is considered the benchmark for plant diversity in the region, and under same tillage. Plantings of cover crop were dependent on the main crop in rotation (Smith and Gross, 2006). The experiment was in a randomized complete block design, which included four blocks or replicates of each treatment. All plots received the same tillage at 15 cm depth, and no fertilizer or pesticides were applied to these plots.

2.2. Soil sampling

We sampled soil from six crop diversity treatments, but to eliminate any immediate crop effect all the treatments were sampled in the corn phase and a spring fallow treatment (Table 1) on November 1, 2012. In each plot, we collected five soil cores (5 cm diameter, 10 cm depth) and then homogenized the cores in the field. 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 the remaining sample, soil chemical properties (total carbon, total nitrogen, ammonium, nitrate, pH, texture) were analyzed as originally reported elsewhere (McDaniel et al., 2014a; McDaniel et al., *In press*). Labile C was measured as permanganate oxidizable C (POXC). Overall biological activity and amount of potentially mineralizable carbon (PMC) and nitrogen (PMN) were analyzed using the aerobic incubation method (McDaniel et al., *In press*).

2.3. Bacterial community sequencing

We extracted DNA using the MoBio Power Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA). DNA concentration was adjusted to a standard concentration of 20 ng μl^{-1} and used as template. 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 (16S rRNA) (Caporaso et al., 2012). 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 Muscarella et al. (2014). Briefly, we assembled the paired-end 16S rRNA sequence reads using the Needleman algorithm (Needleman and Wunsch, 1970). 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 (Edgar et al., 2011). We aligned our sequence data set with the bacterial

185 SILVA-based bacterial reference database (Yilmaz et al., 2013). During data analysis,
186 operational taxonomic units (OTUs) were binned at 97% sequence identity and phylogenetic
187 classifications of bacterial sequences performed. Sequences were processed using the software
188 package *mothur* v.1.35.1 (Schloss et al., 2009; Kozich et al., 2013).
189
190 *2.4. Composition and abundance of disease suppression genes*
191
192 We classified disease suppressive taxa as the subset of soil microorganisms possessing
193 genes that are required for the production of antifungal compounds 2,4-diacetylphloroglucinol
194 (DAPG) and pyrrolnitrin (PRN) (Garbeva et al., 2004b; Haas and Defago, 2005). We targeted
195 *phlD* and *prnD*, which are known to code for a subset of DAPG producers and PRN producers,
196 respectively in environmental samples (according to methods in Latz et al. 2012). We assessed
197 the composition of disease suppressive microorganisms by targeting the *phlD* gene using
198 terminal restriction fragment length polymorphism (T-RFLP) (von Felten et al., 2011). For *phlD*
199 gene amplification, the forward primer B2BF (5′-ACCCACCGCAGCATCGTTTATGAGC-3′)
200 and reverse primer FAM-BPR4 (5′-CCGCCGGTATGGAAGATGAAAAAGTC-3′) yielded a
201 629 bp product. In each 25 µL PCR reaction, we combined 5% dimethylsulfoxide, 0.8 mg ml^{-1}
202 bovine serum albumin, 1× GoTaq Colorless Master Mix (Promega, Madison, WI), 0.2 µM of
203 each primer and 5 µL of template DNA. Reactions were cycled with an initial denaturation at 94
204 °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,
205 with a final extension carried out at 72 °C for 10 min (von Felten et al., 2011). The amplified
206 PCR products were purified with QIAquick PCR purification kit (Qiagen, Valencia, CA). After
207 purification, amplicons generated from each sample were digested in multiple restriction

208 enzymes overnight in 12 µL reaction mixtures containing 4 µL of PCR product, 1×enzyme buffer
209 (von Felten et al., 2011). After digestion, the enzymes were inactivated for 5 min at 80 °C, and
210 the digested products were purified according to the purification kit protocol (Qiagen, Valencia,
211 CA). For T-RFLP analysis, we combined 1.5 µL of the digested product with 9 µL of HiDi
212 formamide (Applied Biosystems, Foster City, CA) and 0.4 µL of internal size standard ABI
213 GeneScan LIZ 600 (Applied Biosystems, Foster City, CA). The samples were incubated for 3
214 min at 96 °C and then stored on ice prior to fragment analysis. We determined the length and
215 relative abundance of terminal restriction fragments (T-RFs) using an ABI 3130×1 Genetic
216 Analyzer (Applied Biosystems, Foster City, CA). Electrophoresis conditions were 60 °C and 15
217 kV with a run time of 41 min using POP-7 polymer. The resulting data was analyzed using
218 GeneMapper Software 4.0 (Applied Biosystems, Foster City, CA). The peak detection limit was
219 set to 50 fluorescence intensity units.

220 We assessed the relative abundance of disease suppressive functional genes by targeting
221 *prnD* using quantitative PCR (qPCR) (Garbeva et al., 2004b). The partial *prnD* gene abundance
222 was quantified using a SYBR green assay with primers prnD-F (5′-
223 TGCACTTCGCGTTCGAGAC-3′) and prnD-R (5′-GTTGCGCGTCGTAGAAGTTCT-3′)
224 (Garbeva et al., 2004b). The 25 µL PCR reaction contained 1× GoTaq Colorless Master Mix
225 (Promega, Madison, WI), 0.4 µM of each primer, and 5 µL of template DNA. Cycling conditions
226 were as following: initial cycle 95 °C for 10 min, and 30 cycles of 95 °C for 15 s and 60 °C for 1
227 min. For the qPCR standard curve, *prnD* gene was amplified from soil genomic DNA. PCR
228 fragments were cloned to pGEM-T Easy Vector System according to the manufacturer's manual
229 (Promega, Madison, WI). Plasmids were extracted using the QIAprep Spin Miniprep kit (Qiagen,
230 Valencia, CA), and cloned fragments were verified by PCR and agarose gel electrophoresis.

231 Dilutions of plasmid DNA containing *prnD* gene were used to generate standard curves in
232 quantities ranging from 5.0×10^2 to 5.0×10^7 copies. We quantified the *prnD* gene in 25 µL
233 reaction volumes containing about 20 ng DNA template, 1×TaqMan Environmental Master Mix
234 2.0 (Applied Biosystems, Valencia, CA), 1× SYBR green I, and 0.4 µM of each primer.
235 Fragments were amplified with an initial denaturation step at 95 °C for 10 min, followed by 40
236 cycles of 95°C for 15 s, 60 °C for 1 min. For each sample, PCR reactions were run in triplicate.
237 We obtained standard curves based on serial dilutions of mixed PCR product amplified from soil
238 samples. Reactions were analyzed on a BIO-RAD CFX-96Real-Time System (Bio-Rad,
239 Hercules, California, USA).
240
241 *2.5. Statistical analyses*
242
243 We tested for differences in total bacterial diversity (based on Shannon Diversity index
244 H′) and *prnD* gene abundance in response to crop diversity treatment using analysis of variance
245 (ANOVA). We checked that data met assumptions of analyses, and we treated crop diversity
246 treatment as a fixed factor and block as a random effect. We used Tukey's Honestly Significant
247 Difference (HSD) tests to identify between-group differences in bacterial diversity and *prnD*
248 gene abundance.
249 To visualize patterns of microbial community composition, we used Principal
250 Coordinates Analysis (PCoA) of the microbial community composition based on the Bray-Curtis
251 dissimilarity coefficient for each possible pair of samples using the R statistical package (R Core
252 Development Team 2015). To test for differences in total bacterial communities and a subset of
253 previously identified biocontrol bacterial taxa (i.e., *Psuedomonas* spp. and *Streptomyces* spp.)

among crop diversity treatments, we used non-parametric permutational multivariate analysis of variance (PERMANOVA) implemented with the *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 *adonis* function carries out sequential tests (similar to Type I sums of squares) (Oksanen et al., 2010), the effect of the last soil factor or soil biological activity factor of the model was included in the final PERMANOVA model summary (Peralta et al., 2012). We also performed a similarity percentage analysis (SIMPER) using the *simper* function (R Statistics Package R version 3.0.2) (Clarke, 1993; Warton et al., 2012) to identify the bacterial OTUs responsible for community differences between monoculture corn and other crop diversity treatments and is based on the contribution of individual taxa to the average Bray-Curtis dissimilarity. We also performed multiple linear regression (gene abundance ~ 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 *prnD* using the *lm* function in the R Statistics Package R version 3.0.2 (R Core Development Team 2015).

3. Results

3.1. Bacterial community composition along a crop diversity gradient

276 A total of 12,539,359 sequence reads were generated, and we analyzed 47,261 OTUs for
277 bacterial community "patterns". A summary of soil attributes is presented in Table S1 and
278 elsewhere (McDaniel and Grandy, 2016). The crop diversity treatment significantly influenced
279 bacterial community composition (R^2 = 0.37, p < 0.001; Table S2a, Fig. 1). Bacterial
280 communities from the fallow plots and the most diverse crop rotations (CSW, CSW_{1cov},
281 CSW_{2cov}) were more similar to each other than the lower crop diversity treatments (C_{1cov}, CS)
282 (Fig. 1). The monoculture corn (mC) treatment was more distinct in bacterial community
283 composition than all other crop diversity treatments (Fig. 1). Bacterial diversity, as measured
284 using Shannon Diversity Index (H′), was surprisingly greater under lower crop diversity systems
285 than higher crop diversity systems, but highest in fallow treatments the most diverse non-
286 cropping system (crop rotation: $F_{6,20}$ = 10.16, p <0.0001; block: $F_{1,20}$ = 0.20, p = 0.6600; Fig. 2).
287 Among, the corn cropping systems, mC had the highest Shannon Diversity Index and decreased
288 by up to as much as 4 % in the most diverse rotation of corn-soybean-wheat with two cover
289 crops (CSW_{2cov}).

290 Bacterial community composition was best explained by soil texture (R^2 = 0.066, p<0.05,
291 Table 3a). However, bacterial community composition was marginally affected by soil moisture
292 (R^2 = 0.048, p < 0.10, Table 2). Labile C had an effect on bacterial community composition (R^2
293 = 0.074, p < 0.05), but potentially mineralizable C did not. Potentially mineralizable nitrogen
294 (PMN), however, which is produced in the same aerobic incubation as PMC (a biologically-
295 available N pool), explained significant variation in bacterial community composition (R^2 =
296 0.063, p<0.05, Table 3).

297 The bacterial taxa primarily responsible for treatment differences between mC and the
298 other crop diversity treatments are *Sphingomonadales* spp. and *Acidobacteria* subgroup Gp6

299 (Table S3). When we compared a subset of taxa representing broad biocontrol bacterial
300 community (composed of *Streptomyces* spp. and *Pseudomonas* spp.), there was no significant
301 pattern in community composition across the crop diversity treatment (PERMANOVA; crop
302 rotation: $R^2 = 0.321$, $p = 0.132$; Table S4).
303
304 *3.2. Disease suppression functional potential in soils along a crop diversity*
305
306 Crop diversity affected the composition and abundance of disease-suppression soil
307 microorganisms. We observed a significant shift in the composition of disease-suppression
308 microorganisms (represented by *phlD* gene T-RFLP) along the crop diversity gradient
309 (PERMANOVA; crop rotation: $R^2 = 0.52$, $p = 0.037$; Fig. 3, Table S2b). The *phlD* community
310 composition in the fallow treatment was different from other cropping systems (Fig. 3). The
311 relative abundance of dominant T-RF 280 bp fragment group, previously identified as an
312 important disease suppressive bacterial population (von Felten et al., 2011), accounted for about
313 70% of the disease suppressive community under fallow conditions. In addition, the disease
314 suppressive functional group T-RF 582 bp was a dominant group, representing about 31-97%
315 relative abundance across all crop diversity treatments. In addition, *prnD* gene abundances in
316 cropping systems were higher than under fallow conditions (crop rotation: $F_{6,20} = 7.51$, $p =$
317 0.0003; Fig. 4). In cropping systems, the *prnD* gene in CSW_{2cov} treatment was the most
318 abundant, and the gene abundance was significantly higher than in CSW and fallow treatments
319 (Fig. 4). Our diversity benchmark, the fallow treatment (i.e., lowest crop diversity), showed the
320 lowest *prnD* gene abundances (Fig. 4). Based on multiple linear regression analysis, plant and
321 soil factors significantly influenced *prnD* abundance (Adjusted R^2=0.40, F=4.571, p=0.005).

322 Crop species number (p=0.003), soil carbon (p=0.002), and soil moisture (p=0.0005)
323 significantly influenced *prnD* gene abundance (Table 4).
324
325 **4. Discussion**
326
327 We found that crop rotation history changed bacterial diversity and disease suppression
328 potential in agricultural soils in the current study. Contrary to our prediction, bacterial diversity
329 decreased with increasing cropping diversity (Fig. 2). In contrast, disease suppressive potential
330 of the soil microbial community increased with crop diversity, with the lowest suppressive
331 potential in the no crop fallow treatments (Fig. 4). A possible explanation for this pattern in
332 belowground biodiversity is the contribution of cover crop species to the rotation and the
333 interaction with weedy plant species associated with these plots and. We observed that without
334 crop plants (as reflected in the no crop fallow treatment), disease suppressive potential was
335 significantly diminished compared to crop treatments, possibly due to reduced selection for soil
336 microorganisms with disease suppression traits. The composition of the soil microbial
337 community may be more important than diversity to soil suppressive function. Thus, crop
338 rotation has the potential to impact diseases suppressive function, providing evidence for
339 sustainable biocontrol of soil-borne pathogens.
340
341 *4.1. Crop diversity decreases belowground (bacterial) diversity*
342
343 Crop rotation history decreased bacterial diversity and increased disease suppression
344 potential in this 12-year crop diversity study. Contrary to our prediction, however, crop diversity

345 decreased soil bacterial diversity (Fig. 2). A recent meta-analysis showed that the crop rotation
346 effect increased soil bacterial diversity (i.e., Shannon's diversity index H′) most notably in the
347 first 5 years of treatment, but crop rotations occurring in greater than 5 years were more variable
348 in diversity (Venter et al., 2016). Although, a few of studies included in the meta-analysis were
349 based on high throughput sequencing approaches (i.e., pyrosequencing) also found decreases in
350 bacterial diversity with increasing crop diversity (Alvey et al., 2001; Yin et al., 2010; Reardon et
351 al., 2014; Venter et al., 2016).

352 The pattern of reduced bacterial diversity (based on 16S rRNA gene sequencing) was
353 lower in soils with higher cropping diversity did not align with our initial predictions. There are a
354 number of potential reasons for this pattern. One explanation for this pattern in belowground
355 biodiversity is the presence of weedy plant species. Increasing crop diversity within a rotation is
356 used as a strategy for reducing reliance on synthetic herbicides and been correlated with reduced
357 weed diversity. Early observations at this long-term crop diversity study revealed decreasing
358 weed abundance with increasing crop diversity during the 2002-2004 seasons (Smith and Gross,
359 2007). Specifically, the monoculture treatments (including mC) had an average of 13 weed
360 species per m^{-2}, but the most diverse cropping systems (CSW_{2cov}) had only 5 or 6 in 2003. Thus,
361 while our crop diversity is lower in monocultures, they actually may have greater total plant
362 diversity compared to more diverse rotations when weeds are included. Last, instead of diversity,
363 the Shannon Index might also be looked at as an indicator of a shift in microbial carbon usage.
364 More specifically, a study by McDaniel and Grandy (McDaniel and Grandy, 2016), using the
365 very same soils we used in this study, found that catabolic evenness (a diversity measure of the
366 catabolism of a suite of 31 carbon compounds) also decreased with increasing crop diversity.

This indicates that this trend is not just structural, but also functional, and may indicate specialization.

4.2. Crop diversity enhances plant pathogen suppression

The diversity of plant pathogen suppressive (PPS) microbial community increased with crop diversity treatment (Fig. 4). In addition, we found that the increased crop diversity, via rotation, increased the abundance and composition of a specific plant pathogen suppression gene. Together, these results suggest that cropping diversity may increase the disease suppressive functional potential of agricultural soils. These findings are consistent with previous studies suggesting that plant diversity can enhance protection against soil-borne pathogens by fostering antagonistic soil bacterial communities (Latz et al., 2012; van der Putten et al., 2016). One potential explanation for this effect is changes in plant root exudation, which may lead to enrichment of plant growth promoting rhizobacteria (PGPRs) (Badri et al., 2009; Chaparro et al., 2012). In previous studies, interactions among the total microbial community and soil-borne pathogens in the plant rhizosphere have influenced both plant growth and productivity (Bakker et al., 2010; Penton et al., 2014).

The addition of cover crops to rotations, in particular, strongly increased disease suppressive potential. This along with evidence from previous studies shows that crop rotations may prevent many forms of crop disease caused by *Fusarium* spp., *Phytophthora*, and *Rhizoctonia* spp. (Raaijmakers et al., 2009; van der Putten et al., 2016). Soil microbial diversity has been implicated as important for soil disease suppression; sterilized soils lose suppressive capacity, and adding soil microorganisms to sterilized soil facilitates disease suppression functional capacity (Garbeva et al., 2006; Brussaard et al., 2007; Postma et al., 2008). Biocontrol

391 bacteria can also provide disease suppression against plant pathogens by way of the following
392 mechanisms: competition for iron, antibiosis, lytic enzymes, induction of system resistance of
393 host plants (Doornbos et al., 2012). Plants can also recruit specific biocontrol microorganisms in
394 some cases. A previous study suggests that beneficial pseudomonads are recruited depending on
395 the most dominant soil-borne pathogen infecting crop species. Specifically, *Pseudomonas* spp.
396 capable of DAPG production are more effective at controlling *G. faminis var. tritici,* while
397 *Pseudomonas* spp. capable of producing phenazines were enhanced in fields where phenazine-
398 sensitive *Rhizoctonia* plant pathogen were most dominant (Berendsen et al., 2012; Mavrodi et
399 al., 2012). In the present study, we analyzed a subset of previously reported biocontrol bacterial
400 taxa (e.g., *Pseudomonas* spp. and *Streptomyces* spp.) across the crop diversity gradient; however,
401 we did not detect distinct changes in putative biocontrol community composition (Table S4).
402 The gene abundance results suggest that incorporation of cover crop in rotations increases
403 *prnD* gene abundance, a gene associated disease suppressive microbial taxa capable of producing
404 antifungal compound pyrrolnitrin (PRN) (Garbeva et al., 2004b; Haas and Defago, 2005).
405 Specifically, cover crop species may have important effects on the *prnD* gene abundance and
406 disease suppressive functional potential in soils. The *prnD* gene abundance in cropping systems
407 is higher than in fallow treatment. In addition, there are no reports linking the functional group
408 T-RF 280 to a specific genotype of laboratory strains (von Felten et al., 2011). In our study, this
409 bacterial group is capable of producing 2,4-diacetylphloroglucinol (DAPG) and affects DAPG
410 community composition (Fig. 3). The T-RF 582 bp was also reported to be a major DAPG-
411 producing functional *Pseudomonas kilonensis* in a previous study (von Felten et al., 2011). The
412 abundance of DAPG and PRN producers increasing with plant diversity has been previously
413 observed (Latz et al., 2012). Compared to agricultural soils, the PRN producers were more

414 frequently detected in grassland or grassland-derived plots (Garbeva et al., 2004a; Garbeva et al.,
415 2004b). The *prnD* gene abundance increased in the presence of grasses, but the legume species
416 tended to decrease the DAPG and PRN producer abundance (Latz et al., 2012). Without crop
417 plants (as reflected in the fallow treatment), we observed that disease suppressive potential
418 significantly declined. Disease suppression traits such as antifungal production may not be
419 needed and are not maintained in the community. When agricultural management is absent, there
420 is reduced selection for soil microorganisms with disease suppression traits. Together, our
421 findings combined with previous studies suggest that the land-use regime, plant diversity, and
422 plant species are involved in structuring disease suppressive microbial communities.
423
424 *4.3 Crop-to-bacterial feedback mechanisms and links to the "rotation effect"*
425
426 Disease suppression may have a major role in what is colloquially referred to as "the
427 rotation effect." The increases in yields seen by farmers over the millennia (Karlen et al., 1994)
428 may be due in large part to disease suppression. Crop rotations may also provide other important
429 benefits like enhanced nutrient provisioning to plants, improvement of soil physical properties,
430 increases in soil C, and increases in soil microbial and faunal activity that also could be
431 responsible for the increased yields responsible for the rotation effect (Ball et al., 2005; van der
432 Putten et al., 2016). Our study provided evidence that crop rotations alter soil bacterial
433 community composition, but the mechanisms through which this occurs can include chemical,
434 physical, and biological changes to the soil environment. Crops can influence soil properties in a
435 variety of ways, including chemically and physically. Chemically, plants provide carbon to the
436 soil environment through root exudation of recently assimilated photosynthate, composed of

437 soluble, low molecular weight organic compounds (Neumann and Romheld, 2007). As a
438 consequence, the increased C flow from root exudates can stimulate soil microbial activity.
439 Changes in plant inputs through variation in either root exudation rates or chemical composition
440 are likely a major factor to how crops and crop rotations, can alter belowground microbial
441 communities. Our study focused on soil bacterial community composition. It has been identified
442 that crop rotation also influences soil fungal and faunal communities, which are also important
443 members of the soil food web (McLaughlin and Mineau, 1995). Changes in root exudates have
444 been observed to shift microbial community composition and stimulate a diverse microbial
445 community (Hooper et al., 2000; Stephan et al., 2000; Paterson et al., 2009; Dijkstra et al., 2010).
446 Physically, crop diversity (especially rotations) can enhance soil properties like improving plant
447 water availability by lowering bulk density, increasing soil pore space, and increasing soil
448 aggregate formation (Tilman et al., 2002; McDaniel et al., 2014b; Tiemann et al., 2015), which
449 could have indirect influence over the soil bacterial community as well. Biologically, soil
450 microorganisms can provide disease suppression against plant pathogens through competition for
451 nutrients, antibiosis, and induction of system resistance of host plants (Doornbos et al., 2012).

452 Cover crops are the most salient feature of these crop rotations affecting the soil bacterial
453 community in general. This is not surprising, since cover crops have been shown to influence
454 several soil properties, which likely have indirect effects on the soil bacterial community
455 composition. Soil properties like total C, total N, pH, and bulk density and porosity have all been
456 shown to increase with cover crops (Bullock, 1992; Liebman and Dyck, 1993; Tilman et al.,
457 2002; McDaniel et al., 2014a; McDaniel et al., 2014b; Tiemann et al., 2015). In our study, higher
458 crop diversity but lower soil bacterial diversity supported higher disease-suppression functional
459 potential in soils. We posit that rotation has a filtering effect on soil microorganisms, whereby

460 crop diversity selects for antagonistic microorganisms with disease suppressive potential. Our
461 study further supports the hypothesis that plant diversity can support biocontrol functional
462 potential by enhancing antagonistic properties of resident soil microorganisms against soil-borne
463 pathogens. The soil microbial community composition may be more important than soil
464 microbial diversity to soil ecological function. Increasing evidence supports that crop species and
465 soil type have been shown to shape the soil microbial composition and function (McDaniel et al.,
466 2014b; Tiemann et al., 2015; Venter et al., 2016); thus, land management can have a large effect
467 on soil microbial processes and microbial community composition (Jangid et al., 2008; Lauber et
468 al., 2008; Orr et al., 2015).
469
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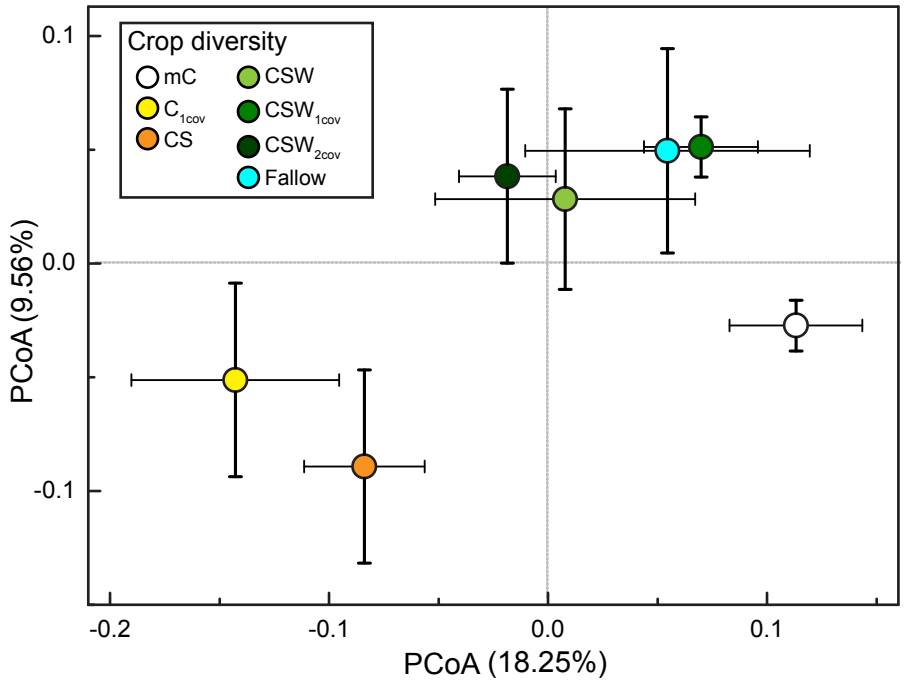
660 FIGURES

661 Figure 1



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663 Figure 1.Ordination from Principal Coordinates Analysis depicting soil bacterial communities
664 along a cropping diversity gradient. Symbols are colored according to cropping diversity
665 treatment (mC=monoculture corn; C_{1cov} =corn/1 cover crop; CS=corn/soy;
666 CSW=corn/soy/wheat; CSW_{1cov} =corn/soy/wheat/1 cover crop; CSW_{2cov}=corn/soy/wheat/2 cover
667 crops; fallow=spring fallow, tilled annually).

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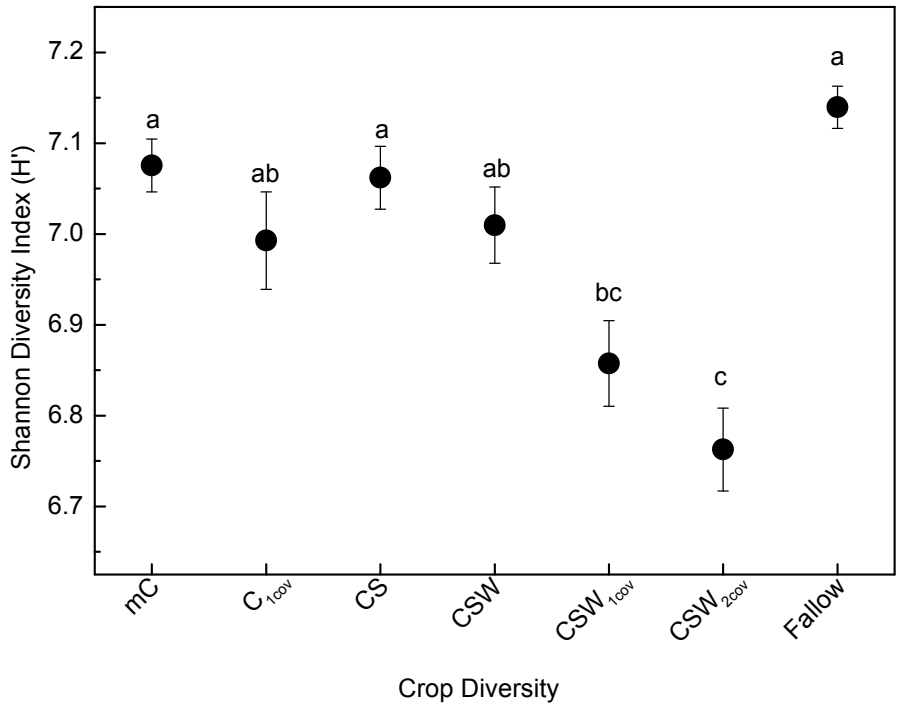
674 Figure 2



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676 Figure 2. Total bacterial diversity (mean ± SEM based on Shannon Diversity Index H′) in
677 response to long-term crop diversity treatment. Different letters above points reflect significant
678 differences in gene abundance along crop diversity gradient at $p < 0.05$ (Tukey's HSD *post-hoc*
679 analysis).

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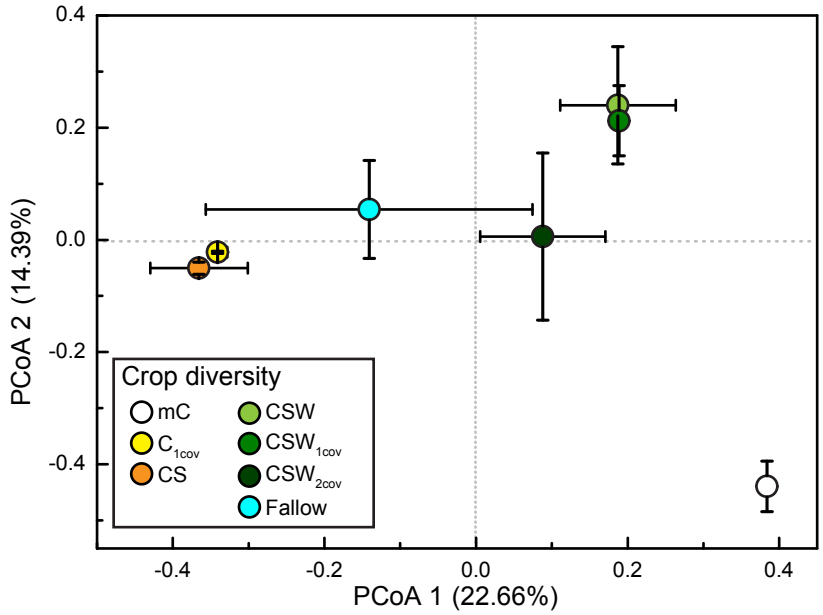
688 Figure 3



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

691 based on T-RFLP of *phlD* gene (DAPG producers) T-RF relative abundance along crop diversity

692 gradient.

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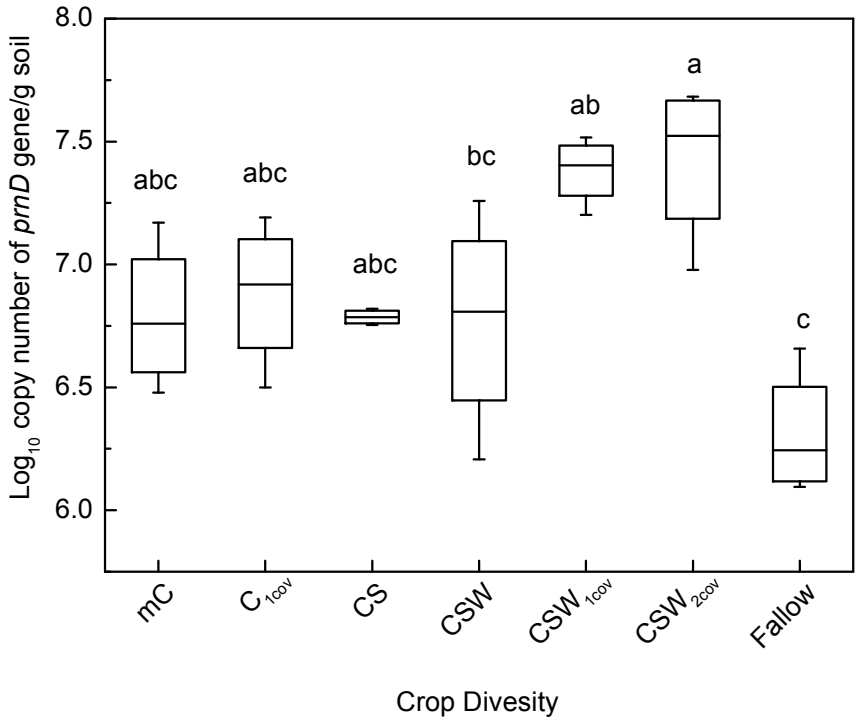
703 Figure 4



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

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TABLES

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.

Crop diversity treatment description	Number of crop species
(1) Continuous monoculture (mC)	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

732 Table 2. Summary of the contribution of (A) soil factors (original data from McDaniel et al.
733 2014) and (B) soil biological activity (original data from McDaniel et al. XXX) on bacterial
734 community variation at the KBS Biodiversity Gradient Experimental Plots based on
735 permutational MANOVA (PERMANOVA). Soil factor effects were considered to significantly
736 contribute to community variation at $P < 0.05$.

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738 (a) Soil Factors

Effect	df	SS	MS	F	R^2	p-value
Sand	1	0.088	0.088	2.243	0.066	0.014
Silt	1	0.088	0.088	2.239	0.066	0.020
Clay	1	0.087	0.087	2.207	0.065	0.024
pH	1	0.057	0.057	1.444	0.043	0.143
Nitrate	1	0.023	0.023	0.593	0.018	0.893
Ammonium	1	0.019	0.019	0.496	0.015	0.966
Nitrogen	1	0.043	0.043	1.086	0.032	0.326
Carbon	1	0.036	0.036	0.921	0.027	0.491
Moisture	1	0.064	0.064	1.622	0.048	0.078
Residuals	18	0.707	0.039		0.534	
Total	27	1.325			1	

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744 (b) Soil Biological Activity

Effect	df	SS	MS	*F*	R^2	*p*-value
PMN	1	0.083	0.083	1.821	0.063	0.049
PMC	1	0.062	0.062	1.358	0.047	0.146
POXC	1	0.097	0.097	2.125	0.074	0.028
Residuals	24	1.100	0.046		0.830	
Total	27	1.325			1	

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760 Table 3. Summary of multiple linear regression to test the influence of disease suppressive
761 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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Table S1 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 (*McDaniel et al. 2014).

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

*McDaniel MD, Grandy AS, Tiemann LK, Weintraub MN. 2014. Crop rotation complexity regulates the decomposition of high and low quality residues. Soil Biol Biochem 78:243-254.

Table S2 Effects of crop rotation on total bacterial community composition based on 16S rRNA amplicon sequencing (a) and disease suppressive community composition based on *phlD* gene T-RFLP (b) 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 gene

	df	SS	MS	*F*	R^2	*p*-value
Rotation	6	0.493	0.082	2.08	0.372	<0.001
Residuals	21	0.832	0.040		0.628	
Total	27	1.32			1	

(b) *phlD* gene T-RFLP

	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	

Table S3 Summary of similarity percentages (SIMPER) of the top 10 bacterial taxa responsible for differences in bacterial community composition between monoculture corn and each of the crop diversity/fallow treatments.

CS-C

OTU ID	average	sd	ratio	ava	avb	cusum	taxonomy
Otu000001	0.0031	0.0019	1.6782	0.0291	0.0267	0.0117	Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified
Otu000002	0.0028	0.0016	1.7098	0.0361	0.0305	0.0221	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000004	0.0024	0.0010	2.4510	0.0190	0.0183	0.0312	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000003	0.0023	0.0019	1.2195	0.0192	0.0174	0.0398	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000005	0.0016	0.0011	1.4928	0.0126	0.0126	0.0457	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000028	0.0012	0.0004	3.4057	0.0051	0.0051	0.0504	Bacteria;unclassified;unclassified;unclassified;unclassified;unclassified
Otu000064	0.0012	0.0013	0.9627	0.0017	0.0030	0.0550	Bacteria;Firmicutes;Bacilli;Bacillales;unclassified;unclassified
Otu000017	0.0011	0.0010	1.1353	0.0052	0.0061	0.0593	Bacteria;Verrucomicrobia;Spartobacteria;Spartobacteria_order_incertae_sedis;Spartobacteria_family_incertae_sedis;Spartobacteria_genera_incertae_sedis
Otu000007	0.0011	0.0009	1.2244	0.0063	0.0081	0.0635	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae;unclassified
Otu000006	0.0011	0.0008	1.4008	0.0067	0.0076	0.0675	Bacteria;Verrucomicrobia;Spartobacteria;Spartobacteria_order_incertae_sedis;Spartobacteria_family_incertae_sedis;Spartobacteria_genera_incertae_sedis

C-C1cov

OTU ID	average	sd	ratio	ava	avb	cusum	taxonomy
Otu000001	0.0051	0.0033	1.5290	0.0267	0.0358	0.0178	Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified
Otu000002	0.0027	0.0015	1.7418	0.0305	0.0292	0.0271	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000004	0.0025	0.0013	1.8750	0.0183	0.0178	0.0358	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000008	0.0020	0.0013	1.4620	0.0032	0.0071	0.0427	Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Micrococcaceae;unclassified
Otu000005	0.0016	0.0010	1.5955	0.0126	0.0104	0.0484	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000009	0.0015	0.0011	1.3491	0.0054	0.0080	0.0535	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae;unclassified
Otu000003	0.0014	0.0010	1.3998	0.0174	0.0169	0.0585	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000011	0.0014	0.0008	1.7199	0.0061	0.0089	0.0634	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae(97);unclassified(93)
Otu000028	0.0013	0.0009	1.4952	0.0051	0.0036	0.0681	Bacteria;unclassified;unclassified;unclassified;unclassified;unclassified;
Otu000049	0.0013	0.0012	1.0088	0.0027	0.0047	0.0725	Bacteria;Acidobacteria;Acidobacteria_Gp7;Acidobacteria_Gp7_order_incertae_sedis;Acidobacteria_Gp7_family_incertae_sedis;Gp7

CSW -C

OTU ID	average	sd	ratio	ava	avb	cusum	taxonomy
Otu000001	0.0037	0.0025	1.4701	0.0256	0.0267	0.0127	Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified
Otu000003	0.0034	0.0026	1.3177	0.0238	0.0174	0.0242	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000004	0.0031	0.0024	1.3097	0.0194	0.0183	0.0349	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000005	0.0025	0.0016	1.5315	0.0119	0.0126	0.0433	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000002	0.0024	0.0018	1.3352	0.0302	0.0305	0.0513	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000006	0.0019	0.0016	1.2433	0.0111	0.0076	0.0579	Bacteria;Verrucomicrobia;Spartobacteria;Spartobacteria_order_incertae_sedis;Spartobacteria_family_incertae_sedis;Spartobacteria_genera_incertae_sedis
Otu000064	0.0018	0.0020	0.9285	0.0037	0.0030	0.0642	Bacteria;Firmicutes;Bacilli;Bacillales;unclassified;unclassified
Otu000025	0.0013	0.0015	0.8854	0.0057	0.0040	0.0687	Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;Flavisolibacter
Otu000016	0.0013	0.0008	1.6594	0.0069	0.0069	0.0732	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000028	0.0013	0.0008	1.6126	0.0042	0.0051	0.0777	Bacteria;unclassified;unclassified;unclassified;unclassified;unclassified

CSW1cov-
C

OTU ID	average	sd	ratio	ava	avb	cusum	taxonomy
Otu000001	0.0080	0.0029	2.7324	0.0427	0.0267	0.0233	Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified
Otu000002	0.0053	0.0017	3.2004	0.0198	0.0305	0.0388	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000008	0.0037	0.0026	1.4427	0.0107	0.0032	0.0497	Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Micrococcaceae;unclassified
Otu000004	0.0029	0.0020	1.4488	0.0143	0.0183	0.0582	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000025	0.0021	0.0018	1.1963	0.0074	0.0040	0.0644	Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;Flavisolibacter
Otu000006	0.0018	0.0014	1.2519	0.0108	0.0076	0.0697	Bacteria;Verrucomicrobia;Spartobacteria;Spartobacteria_order_incertae_sedis;Spartobacteria_family_incertae_sedis;Spartobacteria_genera_incertae_sedis
Otu000003	0.0018	0.0012	1.4489	0.0151	0.0174	0.0749	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000005	0.0016	0.0009	1.7331	0.0115	0.0126	0.0796	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000064	0.0014	0.0009	1.5663	0.0038	0.0030	0.0838	Bacteria;Firmicutes;Bacilli;Bacillales;unclassified;unclassified
Otu000028	0.0014	0.0010	1.3996	0.0032	0.0051	0.0879	Bacteria;unclassified;unclassified;unclassified;unclassified;unclassified

CSW2cov-
C

OTU ID	average	sd	ratio	ava	avb	cumsum	Taxonomy
Otu000001	0.0135	0.0041	3.3287	0.0538	0.0267	0.0354	Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified
Otu000002	0.0059	0.0022	2.7222	0.0188	0.0305	0.0507	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000004	0.0036	0.0025	1.4686	0.0118	0.0183	0.0602	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000008	0.0036	0.0020	1.7843	0.0104	0.0032	0.0697	Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Micrococcaceae;unclassified
Otu000003	0.0035	0.0022	1.6243	0.0168	0.0174	0.0789	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000006	0.0033	0.0032	1.0351	0.0130	0.0076	0.0876	Bacteria;Verrucomicrobia;Spartobacteria;Spartobacteria_order_incertae_sedis;Spartobacteria_family_incertae_sedis;Spartobacteria_genera_incertae_sedis
Otu000035	0.0026	0.0024	1.0971	0.0072	0.0031	0.0944	Bacteria;Acidobacteria;Acidobacteria_Gp1;Acidobacteria_Gp1_order_incertae_sedis;Acidobacteria_Gp1_family_incertae_sedis;Gp1
Otu000021	0.0022	0.0015	1.4605	0.0086	0.0042	0.1001	Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;unclassified;unclassified
Otu000009	0.0020	0.0010	2.0627	0.0094	0.0054	0.1054	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae;unclassified
Otu000027	0.0020	0.0014	1.4213	0.0073	0.0039	0.1106	Bacteria;Proteobacteria;Alphaproteobacteria;unclassified;unclassified;unclassified

fallow-C

OTU ID	average	sd	ratio	ava	avb	cusum	taxonomy
Otu000001	0.0029	0.0018	1.6218	0.0237	0.0267	0.0101	Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified
Otu000004	0.0024	0.0015	1.5848	0.0197	0.0183	0.0185	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000002	0.0022	0.0009	2.3624	0.0261	0.0305	0.0262	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000005	0.0015	0.0009	1.5931	0.0125	0.0126	0.0314	Bacteria;Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;Acidobacteria_Gp6_family_incertae_sedis;Gp6
Otu000009	0.0015	0.0007	2.1148	0.0083	0.0054	0.0365	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Oxalobacteraceae;unclassified
Otu000028	0.0013	0.0009	1.4890	0.0034	0.0051	0.0411	Bacteria;unclassified;unclassified;unclassified;unclassified;unclassified
Otu000003	0.0013	0.0008	1.5674	0.0171	0.0174	0.0456	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000016	0.0013	0.0007	1.7643	0.0046	0.0069	0.0500	Bacteria;Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;Acidobacteria_Gp4_family_incertae_sedis;Gp4
Otu000020	0.0013	0.0009	1.3536	0.0069	0.0044	0.0544	Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;unclassified
Otu000006	0.0011	0.0008	1.4200	0.0076	0.0076	0.0584	Bacteria;Verrucomicrobia;Spartobacteria;Spartobacteria_order_incertae_sedis;Spartobacteria_family_incertae_sedis;Spartobacteria_genera_incertae_sedis

Table S4. Effects of crop rotation on biocontrol bacterial community (composed of *Streptomyces spp.* and *Pseudomonas spp.*) at the KBS Biodiversity Gradient Experimental Plots based on permutational MANOVA (PERMANOVA) results. Rotation effect was considered to significantly contribute to community variation at $P < 0.05$.

	df	SS	MS	*F*	R^2	*p*-value
Rotation	6	0.768	0.128	1.654	0.321	0.132
Residuals	21	1.626	0.077		0.679	
Total	27	2.394			1	