Ecosphere

Crop rotational diversity increases disease suppressive capacity of soil microbiomes

Appendix S1

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Table S1. Soil properties measured along the KBS crop 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 (16S rRNA gene amplicon sequencing) 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	0.493	0.082	2.08	0.372	<0.001
Residuals	21	0.832	0.040		0.628	
Total	27	1.32			1	

Methods

We targeted *phlD* and *prnD*, which are known to code for a subset of DAPG producers and PRN producers, respectively in environmental samples (according to methods in Latz et al. 2012). We assessed the composition of disease suppressive microorganisms by targeting the *phlD* gene using terminal restriction fragment length polymorphism (T-RFLP) (von Felten et al. 2011). For *phlD* gene amplification, the forward primer B2BF (5′-ACCCACCGCAGCATCGTTTATGAGC-3′) and reverse primer FAM-BPR4 (5′-CCGCCGGTATGGAAGATGAAAAAGTC-3′) yielded 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 an 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 (von Felten et al. 2011). The amplified PCR products were purified using the 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 (von Felten et al. 2011). 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 were analyzed using the GeneMapper Software 4.0 (Applied Biosystems, Foster City, CA). The peak detection limit was set to 50 fluorescence intensity units.

Figure S1.

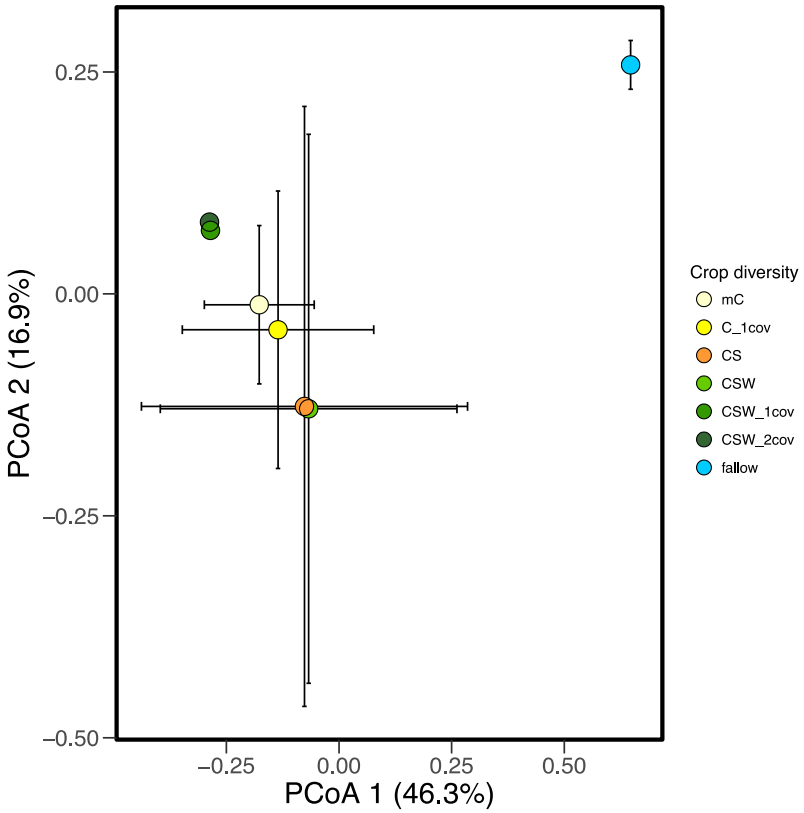

Figure S1. Ordination from Principal Coordinates Analysis of disease suppressive community based on T-RFLP of *phlD* gene (DAPG producers) T-RF relative abundance along crop diversity gradient.

Figure S2.

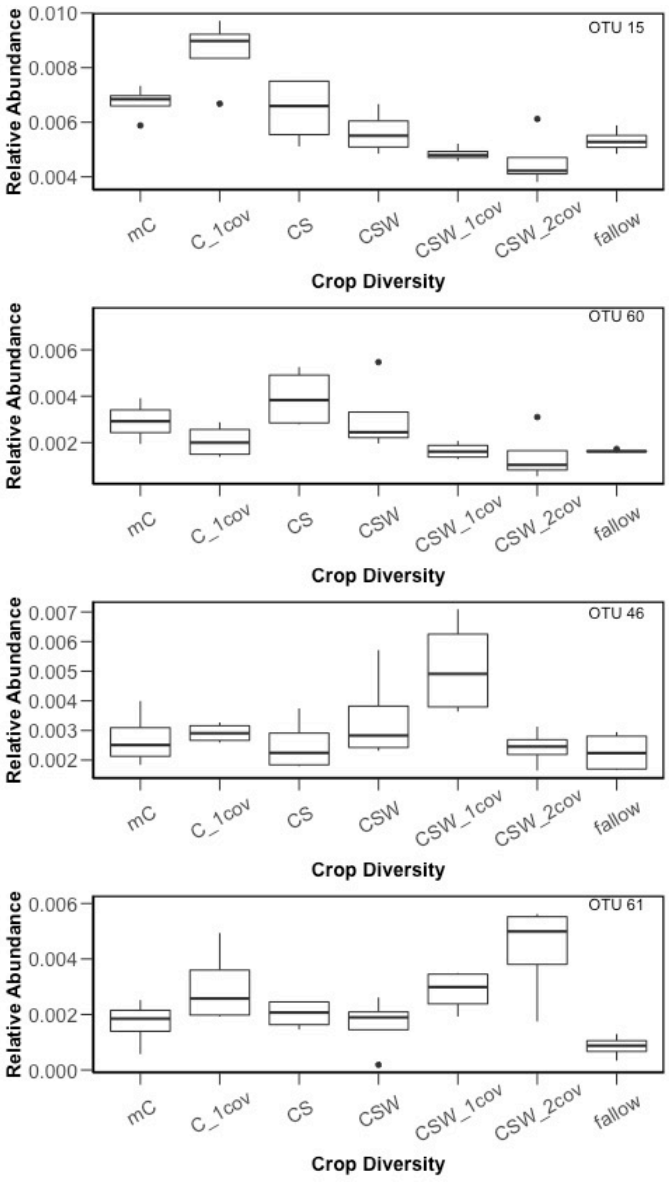

Figure S2. Boxplots of indicator bacteria relative abundance along crop diversity gradient based on indicator species analysis for a subset of taxa displayed in Table 4. [OTU15 = unclassified Betaproteobacteria represented C_{1cov} treatment; OTU60 = Acidobacteria Gp4 represented CS treatment; OTU46 = Acidobacteria Gp4 represented CSW_{1cov} treatment; OTU61 = unclassified Sphingomonadaceae represented CSW_{2cov}]