

1 **Supplemental Information**

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3 **Narrow Grass Hedges Reduce Tylosin and Associated Antimicrobial Resistance Genes in**

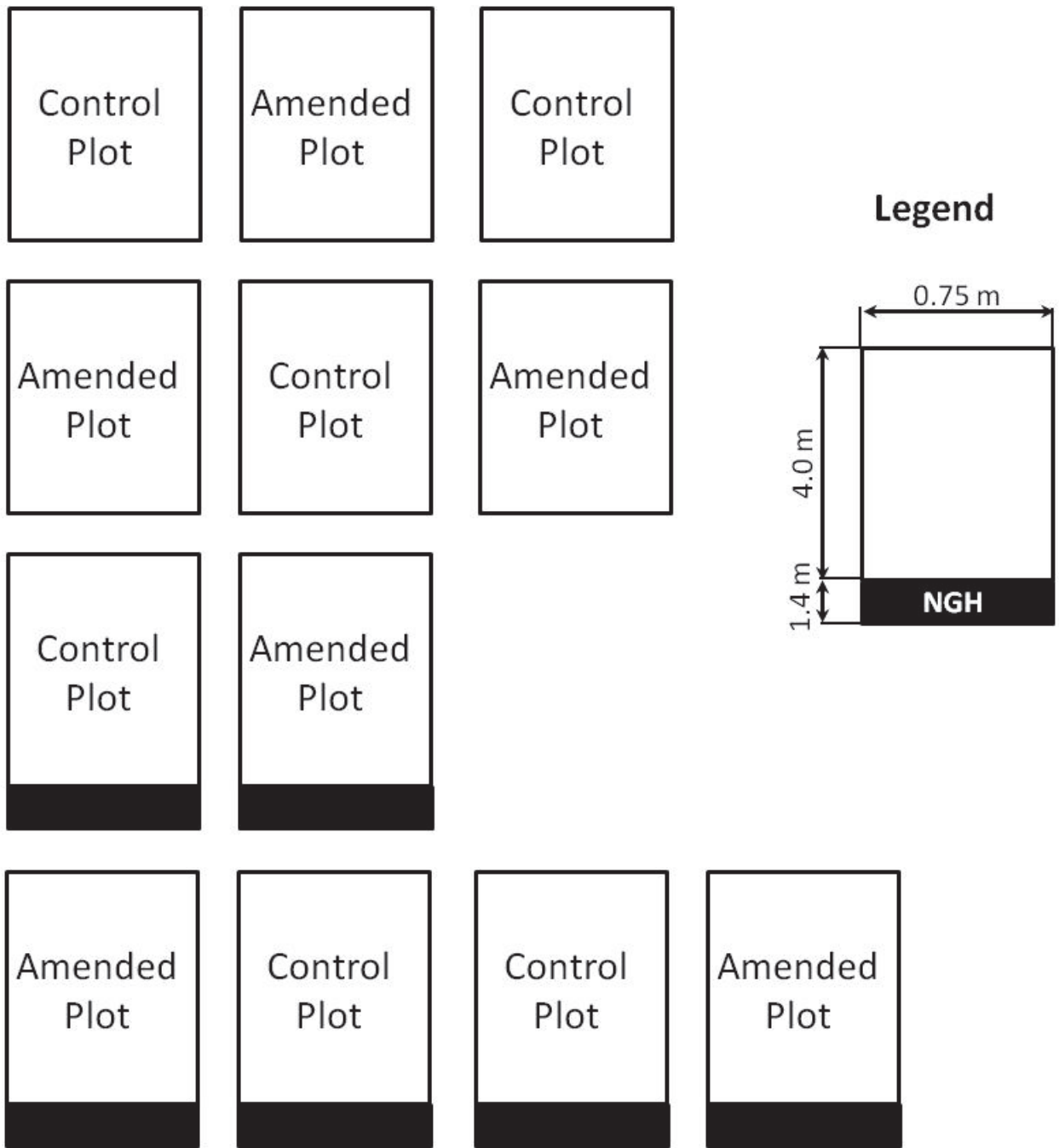
4 **Agricultural Runoff**

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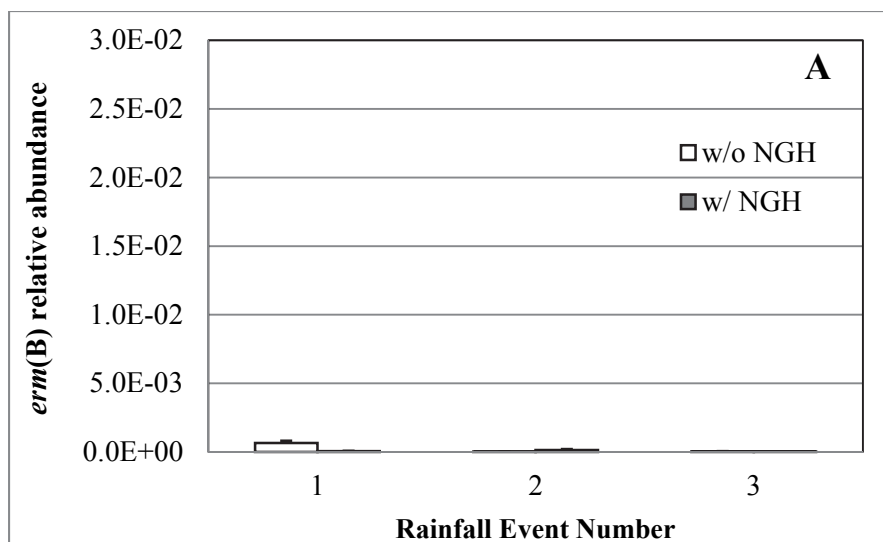
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18 Pages – 8
19 Figures – 3
20 Tables – 3



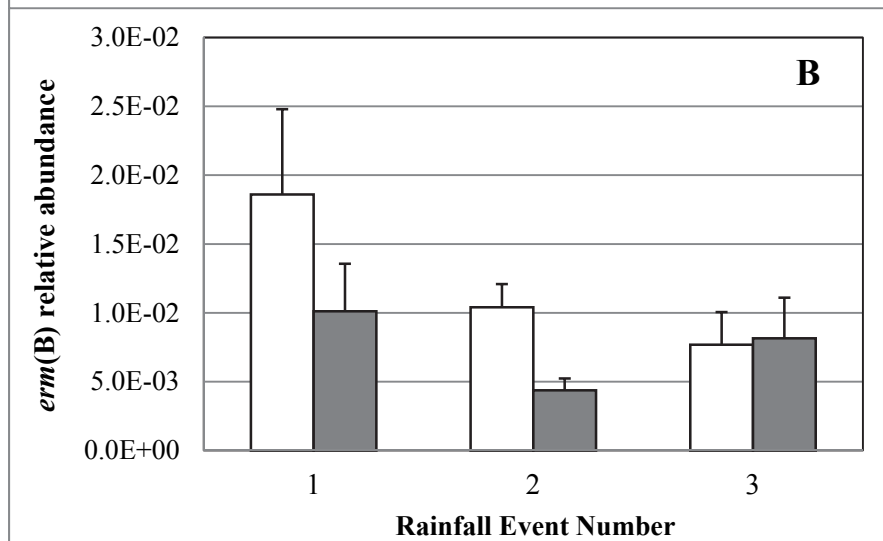
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Supplemental Fig. S1. Schematic showing plot layout. The plots are not drawn to scale. Each row of plots were tested in the same week.

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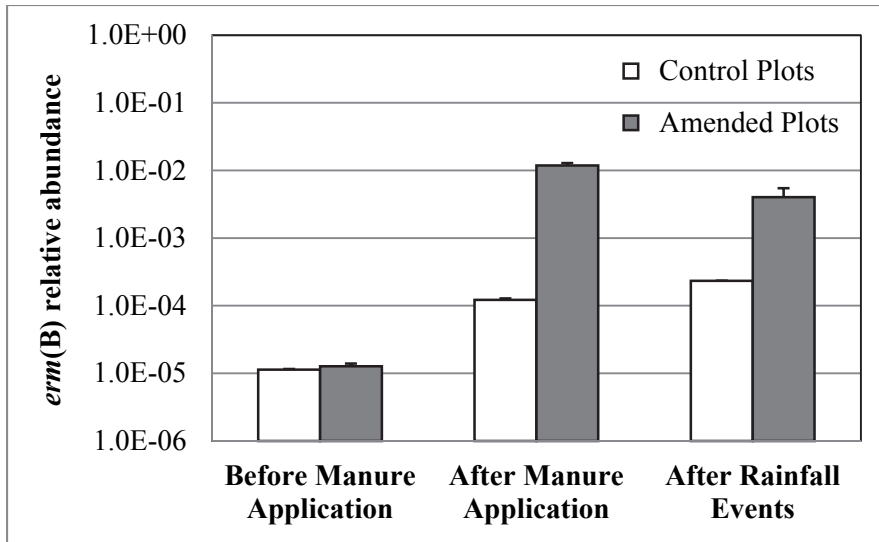


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Supplemental Fig. S2. The relative abundance of *erm(B)* in runoff during the three rainfall events from control (A) and amended (B) plots. Error bars represent standard errors from triplicate field experiments.



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31 Supplemental Fig. S3. The relative abundance of *erm(B)* in soil before manure application, after
 32 manure application, and after three rainfall events in control and amended plots. Error bars
 33 represent standard errors from triplicate field experiments.

Supplemental Table S1. Relevant information of the qPCR reactions used in this study.

Target Gene	Primer	Sequence (5'-3')	Annealing Temp (°C)	Linear Range (copies/20µL)	R ²	Efficiency (%)	Reference
<i>erm(B)</i>	ermB-F	GGTTGCTCTTGCACACTCAAG	65	10 ¹ -10 ⁹	0.996	94.4	(Koike et al., 2010)
	ermB-R	CAGTTGACCGATAATTCGATTG					
16S rRNA	1369 F	CGGTGAATACGTTTCYCGG	56	10 ³ -10 ⁹	0.979	82.4	(Suzuki et al., 2000)
	1492 R	GGWTACCCTGTTACGACTT					

Supplemental Table S2. The absolute abundance of *erm*(B) and the 16S rRNA gene (average \pm standard error) in runoff from control and amended plots with and without NGHs.

Rainfall Event	Control Plots		Amended Plots	
	w/o NGH (copies/mL)	w/ NGH (copies/mL)	w/o NGH (copies/mL)	w/ NGH (copies/mL)
<i>erm</i> (B)				
1	$(1.33 \pm 0.19) \times 10^3$	$(2.02 \pm 0.53) \times 10^2$	$(4.85 \pm 2.44) \times 10^6$	$(7.61 \pm 2.35) \times 10^4$
2	< MDL	$(1.65 \pm 0.84) \times 10^2$	$(1.12 \pm 0.32) \times 10^5$	$(3.14 \pm 1.92) \times 10^4$
3	< MDL	< MDL	$(9.30 \pm 3.14) \times 10^4$	$(4.59 \pm 1.73) \times 10^3$
16S rRNA gene				
1	$(2.31 \pm 0.31) \times 10^6$	$(5.63 \pm 2.22) \times 10^6$	$(1.32 \pm 0.66) \times 10^8$	$(9.48 \pm 4.07) \times 10^6$
2	$(8.97 \pm 1.89) \times 10^6$	$(3.78 \pm 1.36) \times 10^6$	$(9.37 \pm 2.29) \times 10^6$	$(7.28 \pm 2.79) \times 10^6$
3	$(5.12 \pm 0.55) \times 10^6$	$(4.63 \pm 0.99) \times 10^5$	$(1.21 \pm 0.06) \times 10^7$	$(1.57 \pm 0.30) \times 10^6$

Supplemental Table S3. The absolute abundance of *erm*(B) and the 16S rRNA gene (average \pm standard error) in top soils of the amended plots before manure application, after manure application and after three rainfall events. Standard errors were calculated based on triplicate field experiments.

Gene	Before Manure Application (copies/g soil dw)	After Manure Application (copies/g soil dw)	After 3 Rainfall events (copies/g soil dw)
<i>erm</i> (B)	< MDL	$(3.91 \pm 1.43) \times 10^7$	$(8.18 \pm 2.20) \times 10^6$
16S rRNA	$(2.40 \pm 0.34) \times 10^9$	$(2.88 \pm 0.71) \times 10^9$	$(2.12 \pm 0.77) \times 10^9$

REFERENCES

- Koike S., Aminov R.I., Yannarell A.C., Gans H.D., Krapac I.G., Chee-Sanford J.C., Mackie R.I. (2010) Molecular Ecology Of Macrolide-Lincosamide-Streptogramin B Methylases in Waste Lagoons and Subsurface Waters Associated with Swine Production. *Microbial Ecology* 59:487-498. DOI: DOI 10.1007/s00248-009-9610-0.
- Suzuki M.T., Taylor L.T., DeLong E.F. (2000) Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. *Applied and Environmental Microbiology* 66:4605-4614. DOI: Doi 10.1128/Aem.66.11.4605-4614.2000.