Metabarcoding of the rhizosphere microbiome of perennial ryegrass in response to *Epichloë festucae* var. *lolii* infection

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Abstract

Epichloë endophytes inhabit the intercellular spaces of cool-season pasture grasses, and can confer upon their hosts agriculturally desirable benefits such as heightened resistance to biotic and abiotic stresses. The mechanisms underlying many of these benefits are not well understood. Previously observed *Epichloë*-associated impacts towards the rhizosphere microbiome of their hosts could be a contributing factor, however the overall extent to which specific taxa in the rhizosphere microbiome of perennial ryegrass are affected by *Epichloë festucae* var. *lolii* infection remains to be elucidated. To assess this, two independent experiments were carried out in which clonal perennial ryegrass (NuiD) plants inoculated or uninoculated with *E. festucae* var. *lolii* (Lp19) originating from sterile tissue culture were grown in soil collected from a natural ryegrass pasture. After approximately two months of growth under controlled conditions in a growth cabinet, their prokaryotic and fungal rhizosphere microbiomes were compared using high-throughput metabarcoding.

For prokaryotes, endophyte infection had no significant impact on species richness or evenness of the rhizosphere microbiome of their hosts in either experiment. A very minor but significant shift in overall community composition was shown in the first experiment but not the second. At the level of phyla, aside from a minor 1.1% increase in the relative abundances of Bacteroidetes in the rhizosphere of infected compared with uninfected plants in the first experiment but not the second, there were no other significantly differentially abundant prokaryotic phyla due to endophyte infection. At the genus level rhizospheres of infected and uninfected plants showed a high degree of similarity in both experiments, with little variability between replicates within treatments. At the level of operational taxonomic units (OTUs), in the first experiment there was only one significantly differentially abundant OTU in the rhizosphere depending on endophyte infection, and nine in the second. However, all of which had relatively low abundances (<0.3%), and none were consistently significantly differentially abundant in both experiments.

For fungi, there were no significant impacts of endophyte infection on species richness or evenness of the rhizosphere in either experiment, nor were there any significant

endophyte-associated shifts detected in overall rhizosphere community composition. Taxonomic analyses found that in both experiments endophyte infected plants had decreased abundances of a single abundant OTU compared with uninfected plants, which was found to be significant across both experiments (P= 0.026). The OTU sequence mapped with moderate (76-90%) homology to a number of reference sequences assigned as belonging to the class Sordariomycetes. Given previously observed endophyte-associated effects on arbuscular mycorrhizal (AM) fungi, reads assigned as belonging to AM were filtered and analysed separately. This showed that there were no significant effects of endophyte infection towards AM diversity nor overall community composition in both experiments, although there was an endophyte-associated increase in the abundance of the AM family Acaulosporaceae in the first experiment but not the second.

Thus, aside from an endophyte-associated antagonism towards an abundant OTU in the rhizosphere likely of the class Sordariomycetes, *E. festucae* var. *lolii* had an otherwise minor impact on the prokaryotic and fungal rhizosphere microbiome of their perennial ryegrass hosts. The minor magnitude of endophyte-associated effects was further emphasized by analyses consistently showing that both prokaryotic and fungal rhizosphere community composition differed to a greater extent between plants of each experiment irrespective of endophyte infection than between plants of differing endophyte status within each experiment- at least in this cultivar-endophyte strain interaction under the conditions of this study.

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Table of contents

Abbreviations

µL	Microliter(s)
16S	Prokaryotic small subunit ribosomal RNA gene
3D	Three-dimensional
AM	Arbuscular mycorrhiza
BLAST	Basic local alignment search tool
bp	Base-pairs
BS	Bulk soil
Bt	*Bacillus thuringiensis*
D	Simpson's diversity index
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
E	Simpson's evenness index
E+	Endophyte-infected
E-	Endophyte-uninfected
E1	Experiment one
E2	Experiment two
EMP	Earth Microbiome Project
FC	Field capacity
FDR	False discovery rate
g	Gram(s)
h	Hour(s)
H_2O	Water
HCl	Hydrochloric Acid
ISR	Induced systemic resistance
ITS	Internal transcribed spacer region of the eukaryotic ribosomal RNA gene cluster
mg	Milligram
MHB	Mycorrhiza helper bacteria
MS	Murashige and Skoog medium

ng	Nanogram
OTU	Operational taxonomic unit
P	Phosphorus
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PERMANOVA	Permutational multivariate analysis of variance
PF	Plant-free
ppm	Parts per million
QIIME	Quantitative Insights into Microbial Ecology
qPCR	Quantitative PCR
R	Rhizosphere
RO	Reverse osmosis
rpm	Revolutions per minute
rRNA	Ribosomal RNA
spp.	Species
TBE	Tris-borate-EDTA
UNITE	User-Friendly Nordic ITS Ectomycorrhiza Database
VOCs	Volatile organic compounds
x g	G-force

List of figures

Appendix figures

List of tables

Appendix tables