

# Bacterial & Fungal Communities Associated with Alfalfa in Soils with Stand Establishment Failure

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Declining alfalfa acreage in the United States in recent decades is due, in part, to challenges associated with productivity and management. Successful alfalfa establishment is critical to a healthy and productive stand. However, numerous plant pathogenic oomycetes, fungi, and bacteria can negatively impact the health and productivity of a stand. In addition to plant pathogens, a vast diversity of beneficial microbes play important roles in plant nutrient acquisition, growth and development, and responses to biotic and abiotic stressors. However, there is little known about the composition or diversity of alfalfa-associated microbiomes or their relationships with known or suspected plant pathogens. The goal of this work is to characterize the microbiomes of alfalfa rhizospheres and roots, identify potentially overlooked pathogens, and prioritize pathogen-associated microbes. Alfalfa plants were sampled from multiple locations with histories of stand establishment problems in 2020 and 2021 after the first cutting. DNA was extracted from rhizosphere soil and alfalfa roots and bacterial and fungal marker genes (16S-V34 and ITS1 regions of rRNA, respectively) were amplified and sequenced (Illumina MiSeq, 2x300bp) to characterize microbial communities of alfalfa. Location and root compartment were strong drivers of alfalfa-associated microbial communities. However, despite variation in microbial composition among locations, a core group of microbial taxa found in the rhizosphere and root endosphere were identified. These taxa included both potentially beneficial (eg. *Ensifer*, *Streptomyces*, Glomeraceae) and pathogenic (eg. *Fusarium*, *Plectosphaerella*) taxa. Further, random forest regression identified candidate taxa negatively and positively associated with the abundances of common oomycete pathogens quantified via qPCR. For example, the most abundant bacteria explained ~45% of variation in the abundances of *Aphanomyces euteiches* in the rhizosphere, with Rhizobiaceae, *Streptomyces*, and *Pseudomonas* being positively associated with *A. euteiches* populations. Finally, analysis of co-occurrence networks incorporating pathogen abundances identified additional pathogen-associated taxa and network modules. Together, these results shed light on microbiome members that consistently associate with alfalfa in locations with stand-establishment challenges and suggest key pathogen-associated taxa that may play roles in disease development and plant health.