Using niche occupancy from microbiome data to reveal potential fungal pathogens of established perennial ryegrass roots

Nigel L. BELL[1*], Bernadette M. PROUT[1], Faith MTANDAVARI[1], David E. HUME[2],
Sandra YOUNG[3], Emily M. GERARD[3], Lee T. AALDERS[1] and Shengjing SHI[3]

[1]*AgResearch Group – Bioeconomy Science Institute, Ruakura Research Centre, Hamilton, New Zealand*

[2]*AgResearch Group – Bioeconomy Science Institute, Grasslands Research Centre, Palmerston North, New Zealand*

[3]*AgResearch Group – Bioeconomy Science Institute, Tuhiraki, Lincoln, New Zealand*

*Corresponding author: nigel.bell@agresearch.co.nz

Perennial ryegrass is the most widely sown grass in Aotearoa New Zealand (AoNZ) grazed pastures. Poor persistence of perennial ryegrass pastures in the northern part of the country is an on-going issue for farmers. Both above- and below-ground fungal plant diseases are likely to be part of the persistence issue, and a better understanding of the range of pathogens present across the country will help in the design of new approaches to overcome their effects. Microbiome data gives a culture-independent measure of potential pathogen, and beneficial, microbiota presence and relative abundance, including in different niches. This may be a useful tool to improve our knowledge of potentially pathogenic and beneficial microbiota for further study.

Forty plots of three-year-old perennial ryegrass plants were sampled from each of four dairying regions across AoNZ. Samples were taken in autumn and separated into soil, rhizosphere soil, roots, shoots and phyllosphere niches, giving a total of 800 niche samples. Fungal communities were determined by extracting DNA and analysing using ITS MiSeq amplicon sequencing. Percentage of reads of individual amplicon sequence variants (ASVs) amongst the five niches was used to identify any that were preferentially present and abundant in particular niches, as an indication of potential pathogen status.

For roots there were ca. 100 fungal ASVs across all sites which were almost exclusively associated with this niche (>80% of reads). This high niche occupancy rate was particularly striking for 10 closely related ASVs whose sequences matched *Slopeiomyces* or *Gaeumannomyces* spp., with many of these species known as grass pathogens. For shoots, only ca. 20 ASVs across all sites were as closely associated with this niche. This included species such as *Neoascochyta* sp., known for causing disease in grasses along with *Epichloë* sp., known in AoNZ for their mutualistic interaction with fescues and ryegrasses. These patterns of occurrence may change temporally but do show that potential pathogens and beneficials could be identified in microbiome data by their niche occurrence pattern. Clearly, isolation and plant growth testing of the most prevalent of these fungi is now needed to understand their impact on ryegrass growth and therefore what part they may be playing in poor plant persistence.

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