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FRANKIA ASSEMBLAGES ASSOCIATED WITH *ALNUS RUBRA* AND *ALNUS VIRIDIS* ARE STRONGLY INFLUENCED BY HOST SPECIES IDENTITY

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This study examined the effect of host species identity on the structure of *Alnus*-associated *Frankia* bacterial assemblages in the Pacific Northwest, United States, using two approaches. First, *Frankia* in nodules were sampled from six stands of *Alnus rubra* or *Alnus viridis*. Second, a bioassay was conducted where *A. rubra* and *A. viridis* seedlings were grown in different soils collected from these two hosts. *Frankia* genotypes were characterized with *nifH* sequences and bacterial assemblages were compared using taxon- and divergence-based analyses. Strong host associations were evident in the field; the dominant *Frankia* genotypes showed significant associations with either *A. rubra* or *A. viridis*, and there were host-associated groupings at the assemblage level as well. In the bioassay, host associations among *Frankia* genotypes were evident but less pronounced, reflecting an interaction between host species and other factors. Although nodule abundance varied among bioassay treatments, seedling dry mass was not strongly correlated with either nodule quantity or soil chemistry. Collectively, our observation and experimental results indicate that host identity is a major factor influencing the genotype composition and abundance of *Alnus*-associated *Frankia* assemblages.

Keywords: *Frankia*, *Alnus*, host association, *nif*H, edaphic factors, symbiosis, microbial ecology.

Online enhancements: supplemental figures.

Introduction

Frankia is a genus of nitrogen-fixing actinomycete soil bacteria that associate with ~200 plant species across eight families (Benson and Silvester 1993; Wall 2000; Chaia et al. 2010). This symbiosis occurs in a variety of habitats on all continents except Antarctica (Benson and Dawson 2007) and plays an important role in ecosystem nutrient cycling as well as succession (Chapin et al. 1994; Compton and Cole 1998; Vogel and Gower 1998). The structure (i.e., composition and diversity) of *Frankia* assemblages has been shown to be influenced by a range of biotic and abiotic factors (Dawson 2008). For example, many studies have found that biogeographic factors (e.g., climate, elevation) significantly affect the structure of *Frankia* assemblages at multiple spatial scales (Benson and Dawson 2007 and references therein). Others have demonstrated that edaphic factors (e.g., soil type, pH) can also strongly influence the composition and diversity of *Frankia* assemblages (Griffiths and McCormick 1984; Sheppard et al. 1988; Crannell et al. 1994; Nickel et al. 1999).

A biotic factor that has received considerable attention is host species identity. Although many *Frankia* strains can nodulate more than one host species in inoculation trials (Baker 1987; Huss-Danell 1991; Chaia et al. 2006), multiple studies have identified host species as a major determinant of *Frankia* assemblage structure in natural settings. Distinct *Frankia* assemblages have been observed among allopatric hosts across different species (Rouvier et al. 1996; Clawson et al. 1999; Navarro et al. 1999) and genera (Rouvier et al. 1996; Simonet et al. 1999). More strikingly, studies have also found that distinct *Frankia* assemblages associate with different sympatric host species both within (Anderson et al. 2009; Pokharel et al. 2010) and across (Huguet et al. 2001) genera. While some studies have found no evidence for a correlation between *Frankia* genotypes and host plant species (Vanden Huevel et al. 2004), there appears to be general support for the importance of host species in influencing both the composition and diversity of *Frankia* assemblages (Oakley et al. 2004; Mirza et al. 2009).

Despite considerable prior research regarding host identity effects, very few molecular-based investigations of *Frankia* assemblages have experimentally assessed the effect of host plant identity on assemblage structure independent of confounding environmental factors. Oakley et al. (2004) found that the structure of local *Frankia* assemblages associated with eight *Ceanothus* species in seven biogeographic regions in California were more strongly influenced by host species than by other factors, such as geographic location and environmental conditions. In the complementary greenhouse experiment, *Frankia* assemblages on *Ceanothus cordulatus* seedlings matched those of *C. cordulatus* individuals in the field, indicating that host plant species, independent of environmental factors, was a primary determinant of *Frankia* assemblage composition. In contrast, Simonet et al. (1999) found that *Casuarina* individuals grown in the greenhouse had different patterns of *Frankia* composition than those sampled in the field, suggesting that environmental factors play a significant role in regulating *Frankia* assemblage structure. Given these contrasting results, additional studies using both observational versus experimental comparisons on other

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