

The interplay between forest succession and soil properties shapes microbial community dynamics after land abandonment

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This study investigates the response of soil microbial communities to spontaneous afforestation, a natural rewilding process increasingly occurring across European landscapes after land abandonment. The research was conducted at two Italian sites: the Foreste Casentinesi National Park (CF) and Julian Prealps (JP). In both areas, five successional stages (I-V) of afforestation were identified based on historical orthophotos (1954-2020) and replicated across four chronosequences. Topsoil samples (0–10 cm) were analyzed for soil properties, including pH, bulk density, organic carbon, total nitrogen, and water content. Soil microbial communities were characterized through environmental DNA extracted from fine soil fractions, followed by DNA metabarcoding using ITS and 16S rRNA gene markers to target fungi and bacteria, respectively. We hypothesized that: i) topsoil properties change along the afforestation dynamics, thereby controlling the pattern of soil microbiota α -diversity; ii) microbial β -diversity differs between the two study areas as a result of the interplay between afforestation dynamics and soil properties.

Results showed that topsoil became increasingly acidic and less compact (i.e., lower bulk density), accompanied by a rise in organic matter content. However, these trends were modulated by site-specific variability. Microbial α -diversity generally peaked at intermediate successional stages and declined in late forests. Bacterial communities were dominated by *Verrucomicrobiota* and *Pseudomonadota*, while fungal communities were dominated by Ascomycota and Basidiomycota, the latter increasing as afforestation progressed. β -diversity differed markedly between the two areas. In CF, but not in JP, a significant successional trajectory was evident, particularly for fungi. Bacterial community composition in CF was most associated with soil pH, and fungal composition with soil water content, while in JP multiple soil properties explained community variation.

In conclusion, successional effects on soil microbiota were strongest in the more homogeneous CF environment, while site-specific soil filtering predominated in JP.