Soil microbial community response along afforestation dynamics differs between two mountain areas in northern and central Italy

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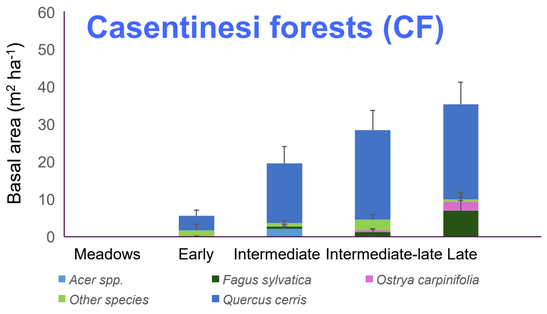
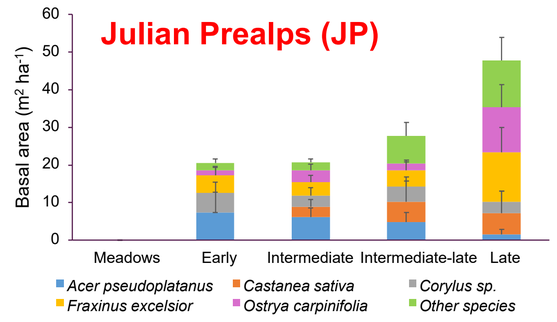
Abstract

This study investigates the response of soil microbial community to spontaneous afforestation - a natural rewilding process that has been ongoing for decades across both national and European levels following land abandonment. The main objectives are to: i) characterize changes in topsoil physico-chemical properties and the associated microbial community response along the successional gradient, ii) assess causal relationships between soil variables and microbial diversity and composition. The research was conducted in two Italian sites: the Foreste Casentinesi National Park (CF) and Julian Prealps (JP). In both areas, five successional stages were identified based on historical orthophotos (1954-2020) and replicated across four chronosequences: meadow-pasture (M-P), early (E), early-mid (E-M), mid-late (M-L), and late (L) stages of afforestation. Topsoil samples (0–10 cm depth) were analysed for pH, bulk density (BD), organic carbon (OC) and total nitrogen (N). Soil microbial communities were characterized through environmental DNA extracted from fine soil fractions, followed by DNA metabarcoding using ITS and 16S rRNA gene markers for fungi and bacteria, respectively. The results reveal that, along the afforestation gradient, topsoil becomes increasingly acidic and less compact (lower BD), with a concurrent rise in organic matter content. However, these trends are modulated by site-specific variability. Notably, overall microbial diversity was lower at CF compared to JP, a pattern likely linked to more homogeneous environmental conditions and reduced above-ground diversity. Bacteria and fungi exhibited distinct responses to forest regrowth following meadow abandonment. Fungal communities - mainly composed of *Ascomycota* and *Basidiomycota* – peaked in diversity at intermediate successional stages. In contrast, bacterial communities, dominated by *Proteobacteria* and *Verrucomicrobiota*, showed greater site specificity, especially at lower taxonomic rank. Overall, the findings highlight the ecological relevance of nature-based solutions such as rewilding, not only in fostering microbial diversity but also in contributing to climate neutrality and biodiversity conservation at larger scales.



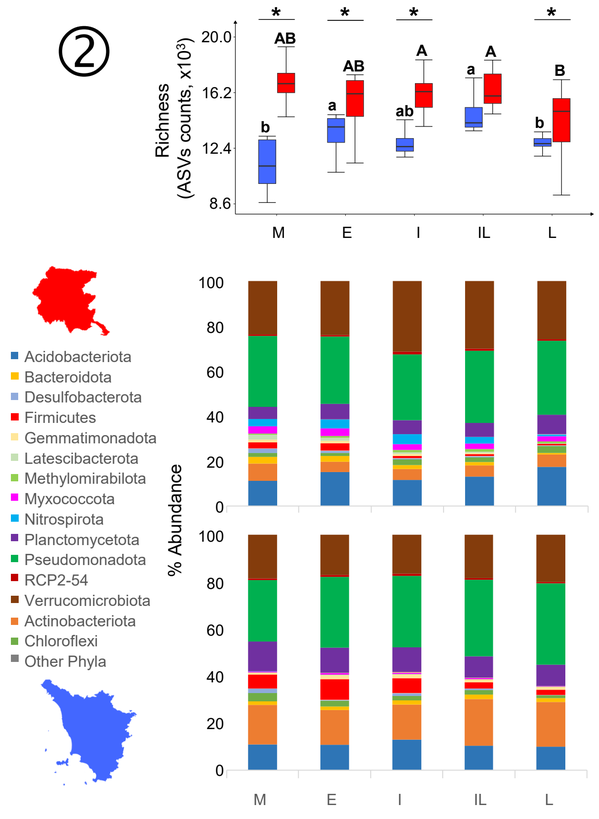


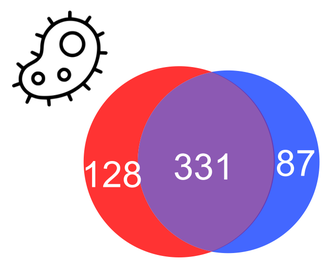
RESULTS

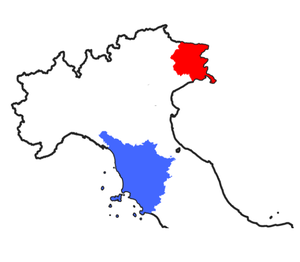
① Location of the two study areas (C) where forest density (basal area of trees with DBH > 5 cm) increased with successional stage (L and R).

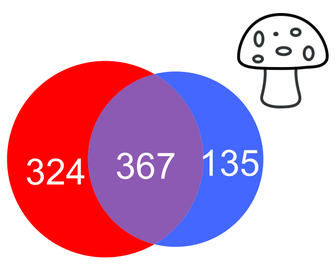
② α-diversity of soil fungal and bacteria communities (assessed by eDNA 16S and ITS metabarcoding) a the two areas: overall genus richness (Venn diagrams, C), genetic richness (boxplots, L and R) and composition at phylum level (barplots, L and R) across stages.

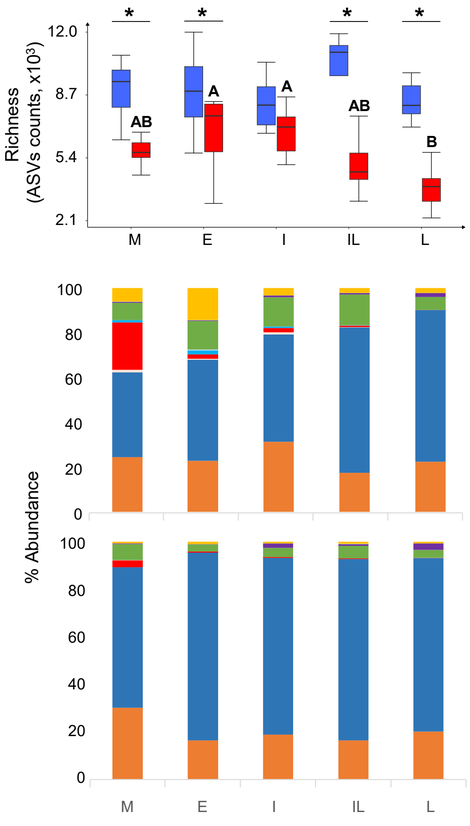
③ β-diversity of microbial communities across stages (PCoAs, C) and relationships with soil chemical-physical properties (RDA tables, L and R).

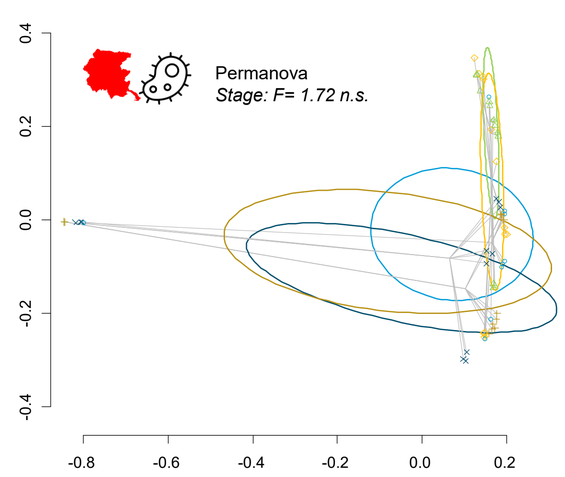


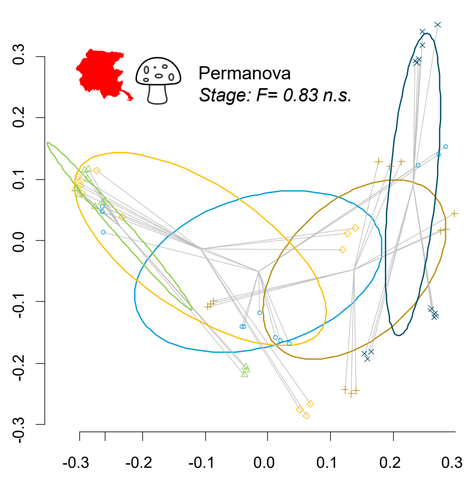


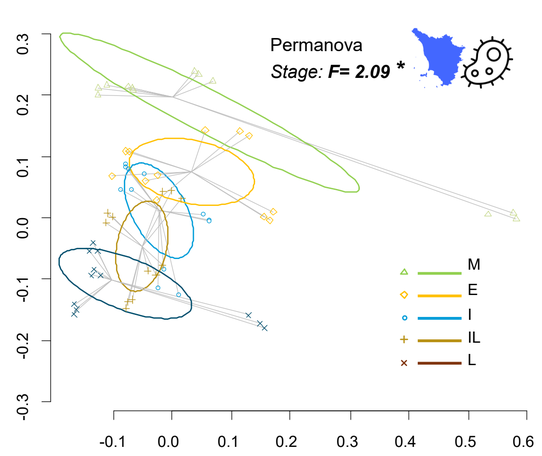


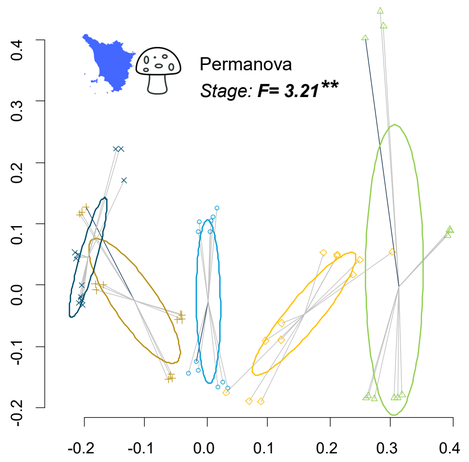












RDA RESULTS

soil predictor	P	P
BD	0.2	0.08
OC	**0.001**	0.06
C_{stock}	0.1	0.3
N	**0.001**	0.3
C:N	**0.01**	0.3
W	0.1	0.7
pH	**0.01**	**0.001**

RDA RESULTS

soil predictor	P	P
BD	0.9	0.5
OC	0.5	0.6
C_{stock}	**0.01**	0.6
N	**0.001**	0.7
C:N	**0.01**	0.2
W	0.9	**0.001**
pH	**0.001**	0.1

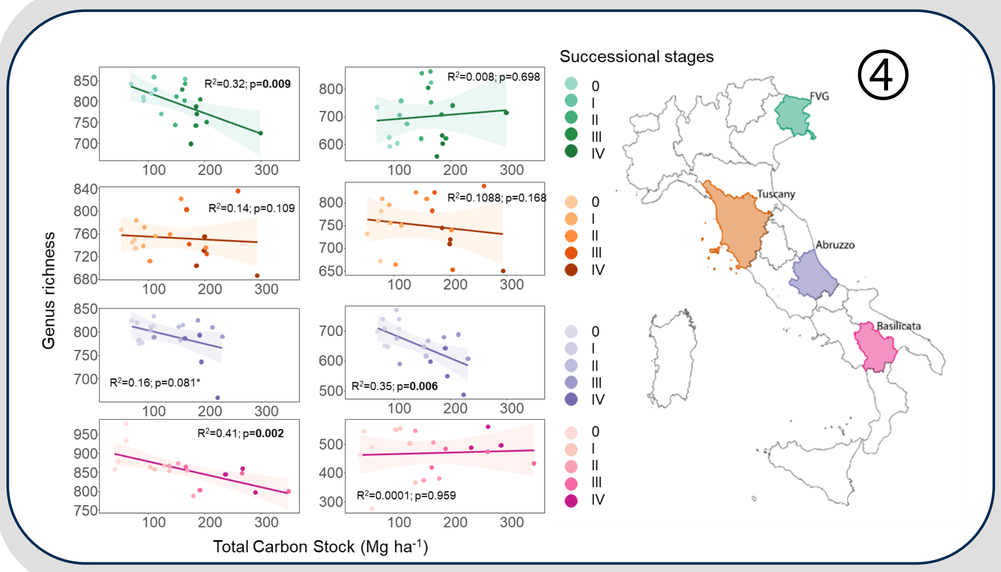
Conclusions:

Spontaneous reforestation on abandoned crop and/or grass lands consistently alters topsoil by increasing acidity and soil organic matter (SOM) while decreasing soil density. These changes are largely influenced by the density and diversity of trees, through litterfall and decomposition.

Microbial diversity peaks at intermediate successional stages. However, bacteria and fungi respond differently:

- **Fungi:** As SOM increases in later stages, Basidiomycota become dominant, replacing other fungal phyla.
- **Bacteria:** Major bacterial phyla show no consistent response to soil changes, likely due to diverse ecological strategies within these groups.

Microbial community composition was strongly influenced by geography. The northern site, with greater soil variation, showed less distinct successional patterns than the more uniform central Italian site, where community shifts, especially for fungi, were clearly related to forest successional dynamics.



Perspectives:

Building on our findings, we are going to extend the scope of the research to improve the understanding of microbial dynamics along forest successions across broader geographic scales.

- at National-scale: We have moved southward along a latitudinal gradient across Italy, from Friuli in the north to the island of Pantelleria. Promising preliminary results (④) show above-ground ecological factors affect soil microbial diversity.
- at European-Scale: We are concluding our sampling contribution within the Horizon EU WildCard project, expected to provide multi-taxa and multi-scale data from all European Ecoregions, further widening and deepening our knowledge of the forest rewilding process.

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