



Responses of ectomycorrhizal extraradical mycelium and associated bacteria to drought and warming

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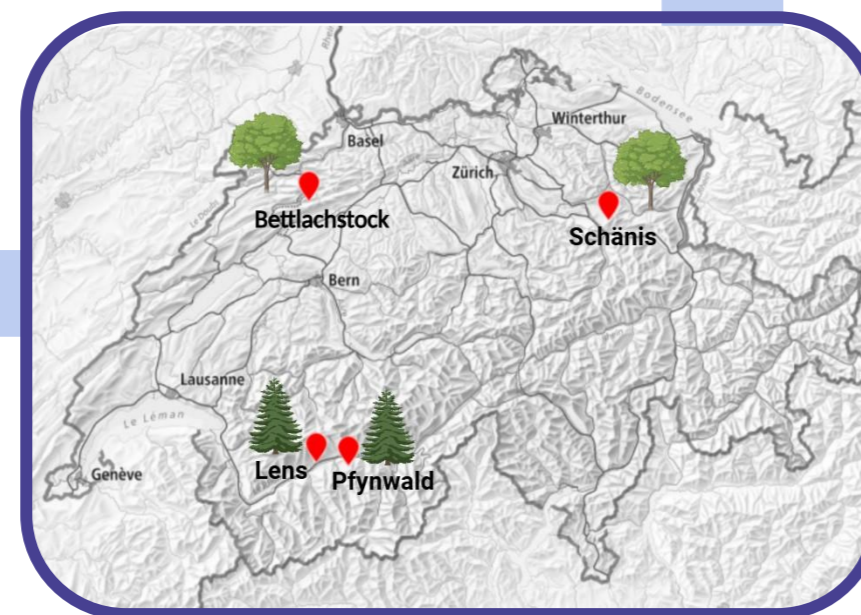
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Background

- A recent estimate suggests that global plant communities allocate 9.07 Gt CO₂e per year to **ectomycorrhizal fungi (EMF)**, of which >50% is directed toward production of **extraradical mycelium**, which represents ~30% of the belowground microbial biomass^a.
- Rich bacterial communities engage with the extraradical mycelium of EMF**, promoting mycorrhizal-plant symbiosis, enhancing mycorrhizal colonization, and buffer adverse condition^b.
- Forests help to mitigate global change by storing carbon in the soil. However, **forests themselves are threatened by global warming and frequent droughts**. The way in which forests respond is largely driven by soil microorganisms^c.



Experimental design

Field experiment to assess how **climate change and mycorrhizal network connectivity shape composition of soil microbial community**. The experiment was implemented across 2 *Fagus sylvatica* (beech) and 2 *Pinus sylvestris* (pine) forests. At each site, 3 replicate clusters were established for a total of 576 sampling units. The experiment ran for two years (2023-2025).



Tree seedlings were grown in modified tubes with mesh windows of varying sizes under climate stress.

Climate treatments: drought (1), warming (2), control (3), warming x drought (4)	Mycorrhizal manipulation treatments: • Full myco - allow fungal in/out growth, • Reduced myco - reduce it, • No barrier
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In-growth sandbags were buried in the soil in the two beech forests during the growing season and used to **"bait" the EMF and their associated bacteria**.



Methods

- Microbial biomass in the in-growth bags was measured by phospholipid fatty acid analysis (PLFA)
- 16S metabarcoding assessed soil microbial community composition
- Plant growth response was measured using basal stem diameter

Microbial biomass analysis in sand bags

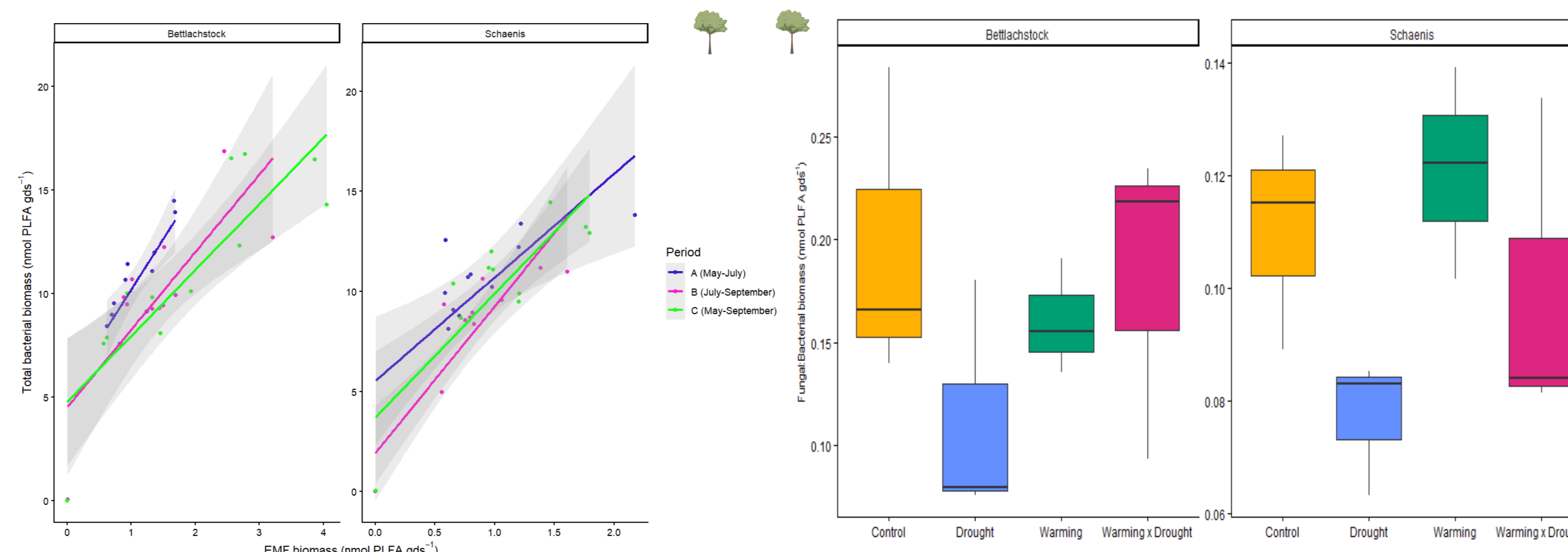


Fig. 1: Linear regression of total bacterial and EMF biomass (nmol PLFA gds⁻¹) across sampling periods at Bettlachstock (Bett) and Schanis (Sch) (R² = 0.56–0.79, p < 0.01 for all periods).

Fig. 2: Fungi-to-bacteria biomass ratio across climate treatments at Bett and Sch (LME, Type II Anova). At Sch, drought significantly reduced the ratio (p = 0.0016). No significant effects were detected at Bett.

- Across treatments and sampling windows, bacterial biomass was positively correlated with EMF biomass in the in-growth bags (Fig. 1)
- After the entire growing season, **drought reduced the fungal-to-bacterial biomass ratio in the in-growth bags** (Fig. 2)
- Across treatments and experimental sites (beech forests), **plant growth and fungal-to-bacterial biomass were positively correlated** (Fig. 3)

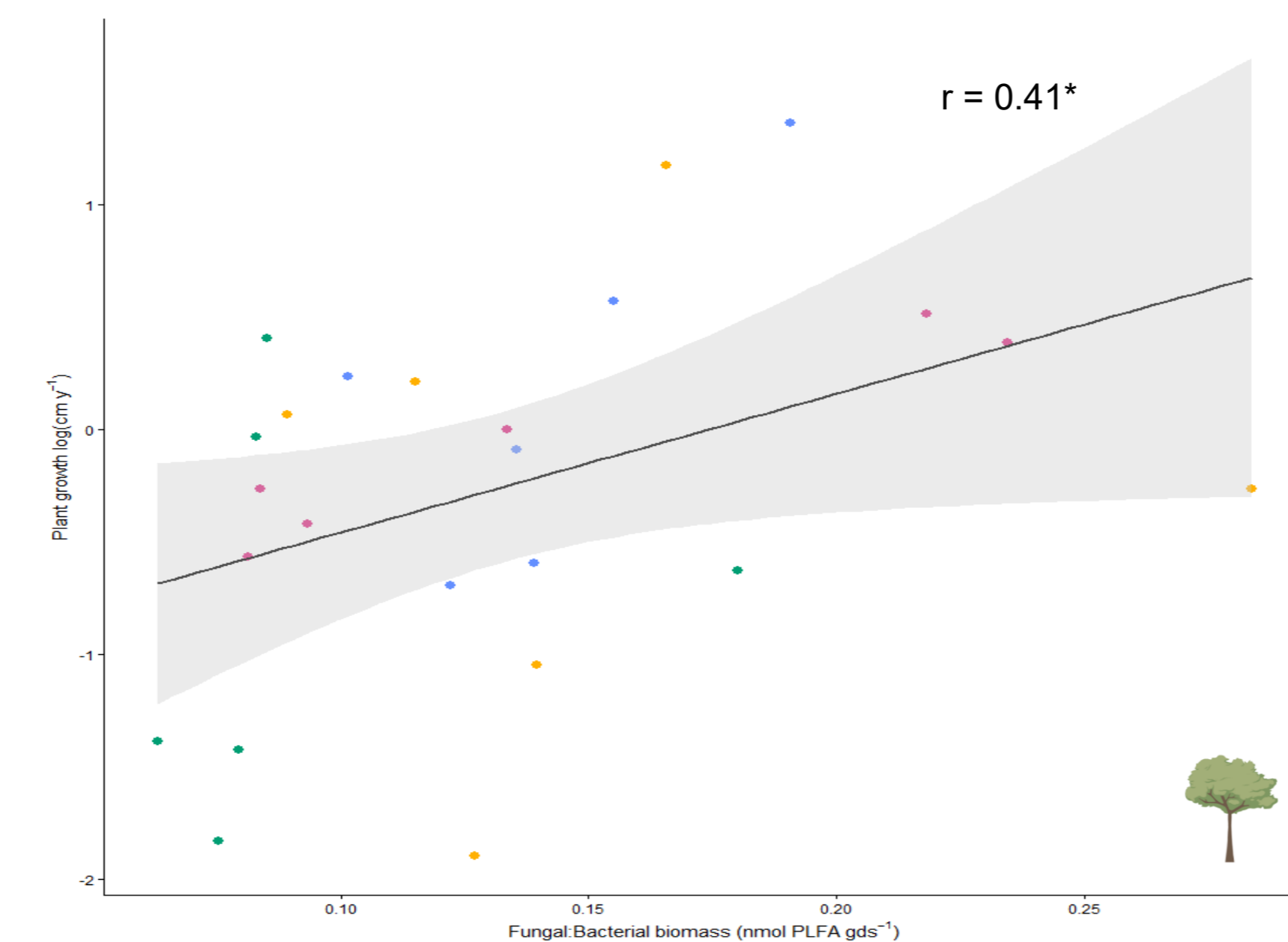


Fig. 3: Linear regression of fungi-to-bacteria biomass (nmol PLFA gds⁻¹) and plant growth log (cm y⁻¹) in Bett and Sch.

Conclusions

- Tight coupling between extraradical mycelium and bacterial communities under climate stress
- Bacteria associated with the extraradical mycelium of EMF are comparatively less sensitive to drought than the fungi

Soil bacterial community composition analysis

- Strong warming/drought effect** on the bacterial community composition (Fig. 4)
- In the **pine forests** there is a **significant mycorrhizal connectivity effect** (Fig. 4)
- The main climatic driver of bacterial community composition varies even within the same forest type (Fig. 5)

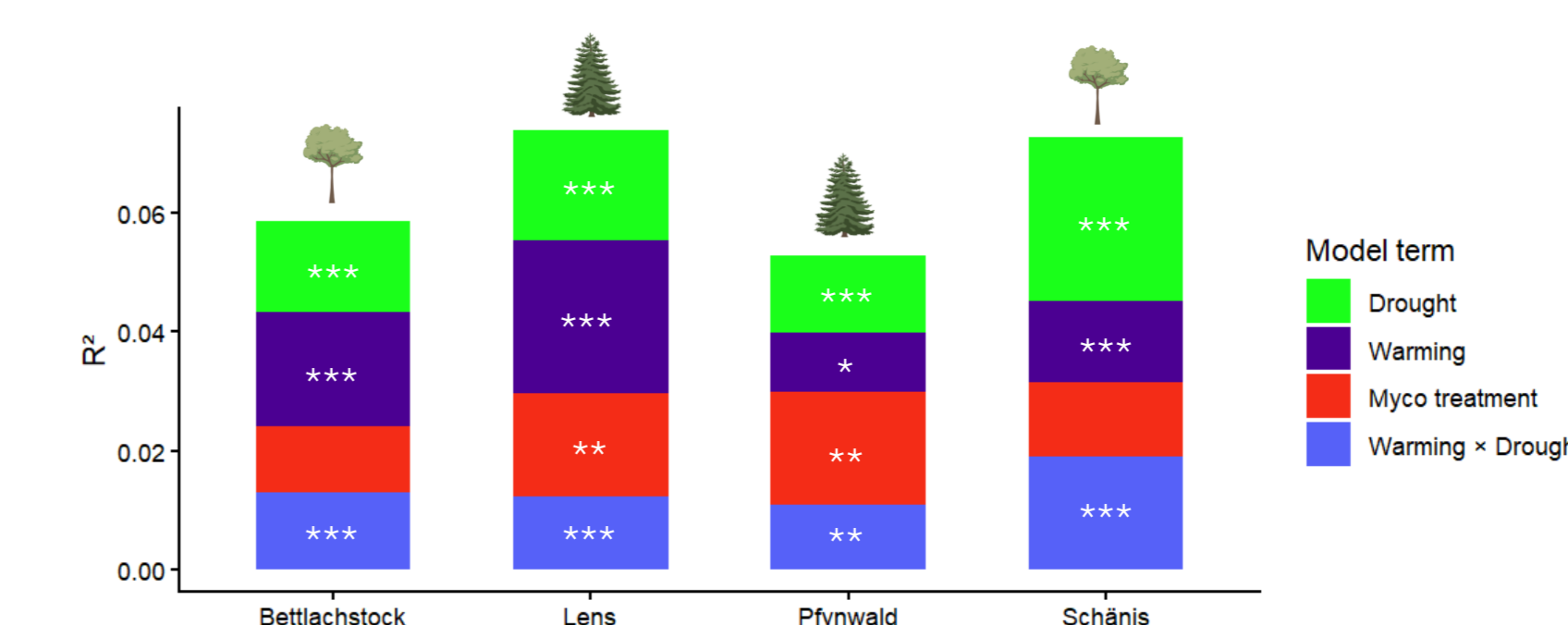


Fig. 4: Stacked bar chart showing the proportion of variance (R²) and p-value (**, ***) in bacterial community composition explained by warming, drought and mycorrhizal manipulation treatment as estimated by PERMANOVA, for each experimental site.

Conclusions

- Local factors modulate bacterial community responses to climate change
- Mycorrhizal connectivity emerges as a key driver of bacterial community assembly in pine forests

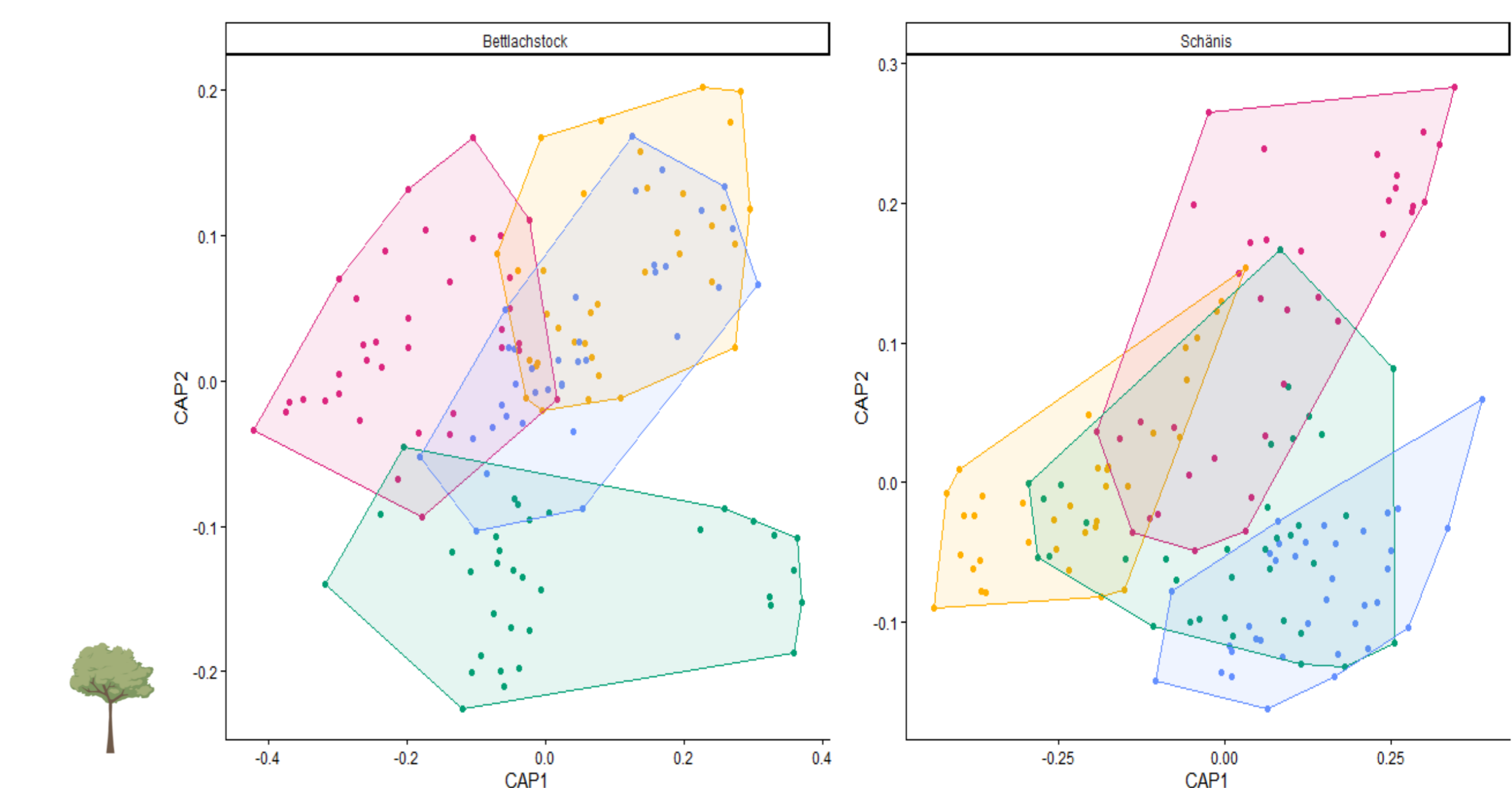


Fig. 5: db-RDA of bacterial community composition at Bett and Sch. Polygons represent climate change groups. At both sites, warming (Bett: F = 3.24, p < 0.001; Sch: F = 2.01, p < 0.001) and drought (Bett: F = 2.65, p < 0.001; Sch: F = 4.16, p < 0.001) and their interaction (Bett: F = 2.15, p < 0.001; Sch: F = 3.03, p < 0.001) significantly drove bacterial community composition.

Take-home message

Warming and drought reshape soil microbial communities in a site-specific manner, with drought emerging as the primary driver of EMF and their associated bacteria decline, threatening the below-ground networks that support forest ecosystem functioning.