EGU24-21677, updated on 20 May 2024
https://doi.org/10.5194/egusphere-egu24-21677
EGU General Assembly 2024

Exploring alpine seedling dynamics: microsite preferences and physiological performance in the French treeline ecotone

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Seedling establishment is a major bottleneck in plant community dynamics and is particularly critical for tree advance in the treeline ecotone. However, the characteristics and availability of safe sites for tree regeneration in alpine ecosystems remain unclear, while the criteria for safe sites may differ between tree species. Tree seedlings in the treeline ecotone are exposed to multiple environmental stressors that may differ from those affecting adult trees. Understanding the response of seedlings to different combinations of abiotic and biotic constraints is essential for predicting future treeline shifts. We therefore aimed to: 1) evaluate differences in microsite preferences of the conifers *Larix decidua, Pinus uncinata*, and *P. cembra* at treeline sites with two different types of bedrock chemistry, and 2) study the response of these species plus two further treeline-forming tree species, *Picea abies* and *Sorbus aucuparia*, to microclimatic manipulation. We evaluated microsite preferences at four sites in the upper treeline ecotone in the French Alps, two with calcareous and two with siliceous bedrock, and compared, at each site, the microsite characteristics of 50 tree species individuals with 50 random microsites, describing the substrate, ground cover, macro- and microtopography, and nearest shelter of each microsite. In a field experiment, also in the French Alps, seedlings were planted in 40 plots arranged in five blocks with the following treatments: Day warming, Day warming + watering, Night warming, Night warming + shade, Shade, Control, Watering, and Vegetation cover. We evaluated survival, growth, and biochemistry (chlorophyll fluorescence and nonstructural carbohydrates) of two seedling cohorts (planted in two consecutive years). We found that microsites were similar, and mostly sheltered, in both bedrock types, and the occupied microsites were a good representation of the available microsites in the respective areas, suggesting that safe-site availability does not limit the establishment of these species in the treeline ecotone. In the experiment, the two seedling cohorts responded differently to the treatments, but in general the vegetation treatment had the strongest effect on seedling performance in all the species studied. Our results imply that, contrary to our expectations, seed availability, rather than safe site availability, is a primary constraint for tree establishment in these alpine-treeline ecotones. Furthermore, in our experiment, the presence of vegetation affected seedling performance more than shading or warming, but given the

differences between cohorts, this result must be carefully considered.