Methodology for the test on leaf preservation methods for microbial and fungi endophytic and epiphytic communities’ analyses

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Different leaf preservation methods were tested to assess their effect on bacterial and fungal epiphytic communities, and to assess if they allow to distinguish the variability of communities between sampling sites. Precisely, we evaluated the impact of leaf freezing or drying on epiphytic microbial communities and compared these treatments to the immediate processing of samples upon sample collection. The sampling campaign took place on October 4th, 2022. Leaves of *Populus grandifolia* were collected from 3 individuals (20 leaves per individual) per site, at 3 sites (i.e., Base plein air de Ste-Foy, 46.790370, -71.326906; Parc des Chutes de la Chaudière, 46.71794013482146, -71.2826022498374; St-Étienne de Lauzon, 46.662724778407444, -71.29950419910787). *P. grandifolia* was selected for this assay because its leaves stay green until later in the Fall, as opposed to *P. tremuloides* which had already changed color at the time of sampling. The 20 leaves from each individual tree were pooled in a bag and then distributed in the different treatments (5 leaves/treatment): 1) Fresh leaves; 2) Frozen samples (-20°C for 3 weeks); 3) Dried for 1 week and frozen for 3 weeks; 4) Dried for 3 weeks and frozen for 3 weeks. The drying treatment consisted of placing the leaves in a Ziploc bag containing 2 silica gel pouches and putting them in a growth chamber at 21°C and 45% humidity in the dark.

For all treatments, epiphytic communities were extracted by adding 50ml PBST buffer to the Ziploc bags containing the leaf samples. Bags were briefly shaken to cover the leaves with PBST, and then the leaves and buffer were recovered in a 50ml tube and vortexed for 3 minutes at maximum speed. The leaves were removed from the tube and the buffer was centrifuged at 4000 g and 4°C for 20 minutes. The supernatant was removed but keeping 2 ml in which the pellet was resuspended and transferred into a 2ml tube. A centrifugation of 1 minute at 15000 g was performed and the supernatant discarded. The pellet was resuspended in 800 µl of CD1 solution (QIAGEN DNeasy Powersoil Pro kit) and transferred into a PowerBead tube provided with the kit. DNA extraction was then performed using the QIAcube following the manufacturer’s instructions.