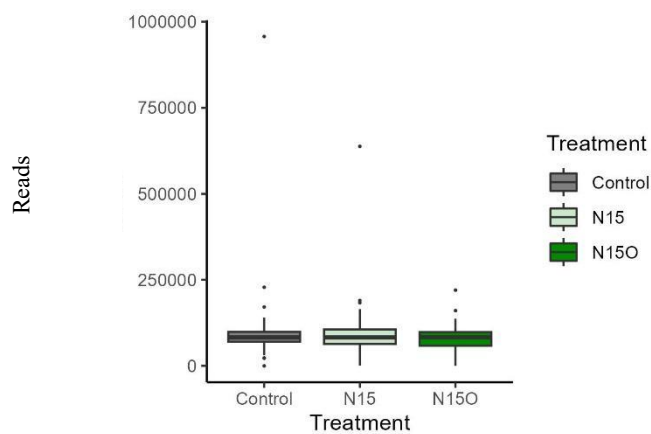


Supplementary material

Material S1. Effect of Historical Nitrogen Application

The leaf samples were categorized based on their historical nitrogen application, representing trees that received three yearly doses of nitrogen fertilizers (0, 15, and 150 kg/ha [65]). Our analysis revealed no significant effect on the number of Amplicon Sequence Variants (ASVs) reads (Fig. S1).

Fig. S1 Boxplot illustrating the response of aspen leaf-associated ASV reads to historical fertilization [65]



ANOVA Summary:

Effect	DFn	DFd	F	p
Treatment	2	334	0.534	0.587

term		conf.low	conf.high	p.adj	
Control	N15	540.9433	-19051.58	0.99	n.s.
Control	N150	-8007.5568	-28095.74	0.61	n.s.
N15	N150	-8548.5001	-30974.89	0.64	n.s.

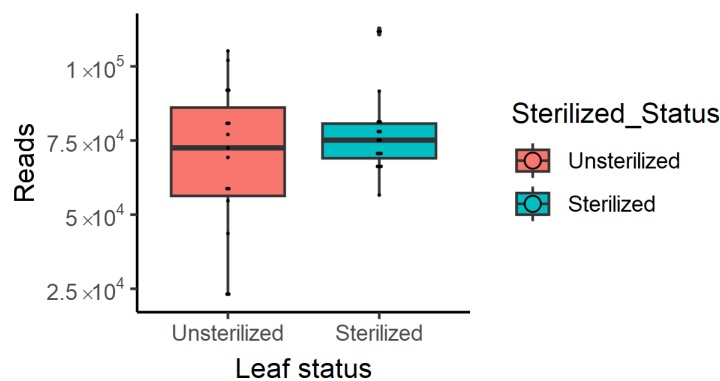
Material S2. Sterilized vs. Unsterilized Samples

To assess the impact of sterilization on sequencing reads equivalent to the number of Amplicon Sequence Variants (ASVs), we collected an additional set of samples during the initial sampling event, comprising 31 samples. These samples were divided into two groups: 16 samples were subjected to sterilization, while 15 samples were left unsterilized.

For sterilization, leaves were initially submerged in 70% ethanol for 5 seconds, followed by immersion in 2% sodium hypochlorite for 4 minutes. Subsequently, the leaves were rinsed and washed twice with distilled water for 10 seconds, and excess water on the leaves was removed using tissue paper. The sterilized and non-sterilized leaves were then stored at -80°C for subsequent analysis.

Upon analysis, no significant differences were observed in the number of ASV reads between sterilized and unsterilized samples (Fig. S2).

Fig. S2. Boxplot depicting the impact of leaf sterilization on the number of fungal reads in a sample.



Summary; $t = -1.4357$, $df = 14$, $p\text{-value} = 0.17$. The true mean difference was not equal to 0 (95-% confidence interval: -23447.38 - 4643.78). The sample estimate of mean difference: -9401.8