## Supplementary Information

Article title: A rapid approach to profiling diverse fungal communities using Oxford's MinION™ nanopore sequencer

**Supplementary Method 1:** Optimized protocol for fungal DNA extraction from hardwood root-tips (or other plant material) using OPS Diagnostics Synergy™ 2.0 Plant DNA Extraction Kit. Any text in blue was adjusted or standardized from the original protocol. Any text in red was an added step not included in the standard protocol. Centrifuge speeds and times can be found in the protocol supplied with the kit. For tough samples, we choose to use the zirconium oxides satellites, but the steel balls have also worked efficiently.

1. Weigh out 25-30 milligrams (mg) of dry plant tissue and place in Synergy™ tube\*.
- Fine roots/root-tips were lyophilized after field collection, and extractions were completed within one week.
2. Place cap on tubes, place on bead beater and perform a 30-60 sec dry grind.
- Including this dry grind step results in better homogenization of tough tissues.
3. Remove from bead beater, and add 750 microliters (µl) of homogenization buffer\*+ to each tube.
4. Place cap on tubes, place on bead beater and perform a 1 min wet grind.
5. Incubate ‘milkshake’ at 37°C for 15 min.
6. Transfer tubes to a centrifuge and extract supernatant.
7. Add 7-10 µl RNase A solution\*+ and incubate at 37°C for 15 min.
8. Add 7/10 volume isopropanol and incubate at -20°C for 10 min.
9. Transfer solution to spin column\* and centrifuge.
10. Wash column with 70% ethanol and centrifuge.
11. Repeat step 10.
12. Elute from spin column by adding molecular grade water or TE, then centrifuge.
- For this step, we chose to do a two-step elution with 60ml TE each time. Then the solution was further purified by a Polyethylene Glycol (PEG) precipitation. Based on personal observations, PCR reactions had higher success with an extra purification step.

\* Included in kit.

+ Included in kit, but more than the standard amount is used. Extra solution can be requested from OPS Diagnostics. We use more RNase A solution, and purchase an extra bottle of the CTAB extraction buffer.