Protocol for Harvesting Roots, Rhizosphere, and Bulk Soil from Field samples

1. Dig up roots of 2 plants and place in a large pan/tray/bucket (take the plants from two different areas of the plot).
2. Remove excess soil manually by brushing/shaking roots or by using a trowel (wear gloves).
3. Collect some of this bulk soil sample in a labeled quart size Ziploc bag and place on ice. (about 1/3 of a bag).
4. Excise roots from the 2 plants: collect a variety of roots from each plant, approximately 6-8 roots per plant. From soy we collected all of the lateral roots and approximately a 4-inch section of the tap root tip. Place the roots in, a labeled 50 ml tube containing 35 mls of phosphate buffer, cutting roots as needed, to fit into tube.
5. Vigorously shake tube for 60 sec to wash off rhizosphere soil from the roots
6. Remove roots, and briefly place on a paper towel.
7. Place roots into labeled empty 50ml tube.
8. Place both tubes (roots, rhizosphere) on ice.
9. When finished you should have three samples for each plot, = bulk soil, rhizosphere and roots.
10. The roots are then brought into the lab and surface sterilized with household bleach containing 0.01% Tween for 30 seconds, then 70% ethanol for 30 seconds followed by three washes with deionized sterile water. We then blot the roots dry and cut up the roots and store them at -80^{o}C. The next step with the roots is to grind them with liquid nitrogen to homogenize before doing the DNA extraction. For the rhizosphere we filter with a course filter to remove root particles then spin down, resuspend in a smaller volume and store in eppendorf tubes until we do the DNA extraction. The soil we just store in a ziplock bag in the cold room. Aim for 250 grams as that is what one lab needs for their chemical analysis.

Phosphate Buffer: 6.33 gr/L NaH_2P0_4, 8.5 gr/L Na_2HP0_4 anhydrous, 200ul/L Silwet L-77