Forest floor sample collection protocols

Supplies

30 × 30-cm wooden quadrats

2-3 kitchen knives per quadrat

Paper bags – a mix of sizes – smaller for Oi and larger for Oa

Sharpie marker

Latex gloves to keep hands clean and samples uncontaminated

Large backpack or mesh laundry bag to carry out samples

Folding saw to cut large wood

Hand clippers for cutting small wood

Overview

Separation of forest floor can be very subjective. Forest floor mass and nutrient pools will be determined by collecting three samples per plot from a 0.09-m² (30 × 30-cm) quadrat. A kitchen knife is used to slice through the forest floor by tracing the interior of the quadrat. We work to standardize separation of O horizons as follows:

Oi horizon is the surface layer and represents the most recent litterfall (i=input). Note that litter may reside in this horizon for 1 to 3 years. Collection of this layer tends to be the ‘light, fluffy, unconsolidated’ material on the surface. This material is readily identifiable to species. It shows no sign of physical or biological decomposition such as, degraded cuticle layer, blocky decomposed edges, or presence of fungal hyphae. Note that holes made by insects while the leaf is in the overstory usually appear rounded and may show signs of ‘healing’ around the hole opposed to physical/microbial decomposition which usually appears as a blocky breakdown or overall thinning of the leaves. This layer is usually present in all forest types.

Oe horizon is the mid-layer and represents material with initial to mid-stage decomposition. This litter shows signs of physical decomposition and/or microbial colonization. This layer usually appears to be more dense or packed together, compared to the Oi-horizon, fungal hyphae are often visible. This is a transitional layer and as such can be difficult to identify. The transition from Oi to Oe is a combination of visual (fungi presence) and physical (surface and edge degradation of leaves). This layer is present in most forests, in cove forests with a high tulip poplar population this may be the bottom layer.

Oa horizon is the bottom forest floor layer and represents organic material which is no longer recognizable as to original material source (leaf or flower or bud). In this layer the organic material is in particles, and may have roots present (please collect as few roots as possible). Identifying the end point for collection of this layer can be difficult in sites with very dark soils for example, high elevation sites. In soils with clear color differences between Oa and A horizon, collect as much Oa as possible while minimizing sample contamination with mineral soil. In dark surface soils, one must sometimes use changes in texture to identify the transition from Oa to A. A horizon mineral soil will include sand particles and therefore have a slightly gritty feel as compared to the Oa which is slick in these systems. In many southern Appalachians forests it will also have signs of mica present. The Oa horizon may be absent in cove forests with high tulip poplar population (see above) and will be absent in pure pine stands (mor forest floor type).

Wood should be collected separately. Wood includes acorns, hickory nuts, and any other type of seed, as well as twigs <10-cm diameter (but see protocols for Rhodo Removal). Wood of all
decay classes should be included – if you can tell that it is wood, even if it is fragile and breaks apart easily (i.e., decay class 5), it should be included in the wood sample. If the forest floor plot occurs under large dead branches which have fallen to the ground, these dead branches are included if any part of the branch is touching the ground.

Samples will be placed in paper bags, oven dried to a constant weight at 60°C, and weighed. The samples will be composited by plot and layer, ground to <1-mm, and analyzed for total C, N, P, Ca, Mg, K, and Al concentrations. Total C and N will be determined by combustion on an Elementar Flash EA 1112 series (Thermo Scientific, Waltham, MA). Total Ca, Mg, K, and P will be determined by dry-ashing a subsample at 480°C, digesting it in HNO3 acid, and analyzing it on an inductively coupled plasma spectrophotometer (ICP, Brown et al. 2009).

Project Protocols

**Rhododendron Removal:** Forest floor samples will be collected in winter (Dec-Feb) before treatments and every winter thereafter. Forest floor mass and nutrient pools will be determined by collecting three samples per plot from a 0.09-m$^2$ quadrat of forest floor and separating into 3 categories: Oi, Oe + Oa horizons, and wood (≤7.5-cm diameter).

**Terrestrial Gradient:** Forest floor samples will be collected in winter (Dec-Feb). Collections will occur every 5 years. Oi, Oe, Oa, and wood (≤10-cm diameter) will be collected separately. Forest floor plot locations will be randomly generated based on the x,y coordinates of the 10-m gridded stakes. Locations that have already been sampled (since 2013) are listed below to prevent these locations from being re-sampled:

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**Bolstad Soil Moisture Plots:** Forest floor samples will be collected in winter. Collections will occur every 10 years. Oi, Oe, Oa, and wood (≤ 10-cm diameter) will be collected separately. Forest floor subplot locations will be located 14-m (slope corrected) from plot center at 3 random compass bearings. For 2015/2016 collections, the bearings were 15°, 162°, and 282°.