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Quick Start Guide to Soil Methods for Ecologists

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Review

Quick start guide to soil methods for ecologists

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ABSTRACT

Increasingly biologists and ecologists are becoming aware of the vital importance of soil to processes observed aboveground and are incorporating soil analyses into their research. Because of the dynamic and heterogeneous nature of soil, proper incorporation of soil analysis into ecological studies requires knowledge and planning. Unfortunately, many ecologists may not be current (or trained at all) in soil science. We provide this review, based on our cumulative >60 years of work in soil science, to help familiarize researchers with essential information to appropriately incorporate soil analyses into ecological studies. Specifically, we provide a brief introduction into soils and then discuss issues related to soil sterilization, choosing a soil for a greenhouse project, sampling soils, and soil analyses.

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Introduction to soil

Soil is the dynamic layer of unconsolidated materials and biota covering the land surface that differs from the underlying geological "parent" material with respect to chemical, physical, biological, and morphological properties. The weathering of parent materials results in an organized blend of minerals, organic matter, water, biota, and air that supports higher plants and ecosystems. Soil is more than a sum of its parts; soil is a body of nature with distinctive characteristics and organization (Jenny, 1980). Pioneering soil scientists in the late 1800s recognized that soil is a product, as well as a component of the environment (Evtuhov, 2006). The rate of soil formation and soil type formed is dependent on parent material (rocks, alluvium, etc.), location on the landscape (topography), length of weathering (time), and action of climate and organisms.

Plant growth and performance depends on many soil properties (i.e., soil texture, pH, fertility, and organic matter content). Soil comprises a substantial portion of a plant's environment and as such, inclusion of soil analysis in ecological studies may provide valuable insight that cannot be otherwise gained. Soil is complex. Soils at any given site and at any given time are unique and often a factor that is influential to plant performance may not be obvious (e.g., a pH driven Fe limitation). The temporal and spatial heterogeneity in soil can pose challenges for researchers. The fundamental information provided here will provide some background for researchers to properly include soil in ecological studies. This paper discusses topics such as choice of soil for greenhouse studies and issues with soil sterilization. Further, we discuss issues that should be considered when sampling, processing, and analyzing soils. We also provide a general work-flow that can be easily adapted to most ecological studies and finally, we suggest some soil methods references that we have found useful. We hope that the information provided in this 'quick start' guide will be useful as a starting point for ecologists interested in including soils in their research.

Choosing a soil for greenhouse and field studies

Choice of soil can greatly affect the results of a greenhouse or field study by shifting the direction or magnitude of ecological processes. Soil choice is very important in ecological studies because soil factors often determine the outcome of plant performance, plant–plant interactions, and plant–soil interactions (Aerts, 1999; Perkins et al., 2011). Ecological questions often generally ask how plants perform in a natural environment-thus in natural soil. Attempting to research processes and patterns that occur on the landscape using artificial growing media or using altered or amended soil is inappropriate and will not produce valid results.

Among the choices for substrate for greenhouse studies are: artificial growing media (e.g., potting soil or peat), commercially available soil, and field collected soil. Artificial media will have levels of nutrients, organic matter, water retention, or texture significantly different from natural or field conditions. The use of artificial media may be appropriate for non-ecological greenhouse research such as producing seeds or removing maternal effects from field collected seed, but not for investigating ecological processes or mechanisms that occur in the field. We suggest, when possible, researchers should collect their own soil from field sites that are typical for their study question. If the amount of substrate needed is very large it might be necessary to obtain soil from a commercial purveyor. When this is the case, it is absolutely necessary to inquire about the origin of the soil and to perform soil analysis prior to use in order to ensure that the soil is as close to field conditions as possible. We also suggest that the field soil should not be amended in order to most closely match field conditions. For example, when sand is added to increase drainage, soil particle size distribution is changed, soil nutrients are changed, and the soil microbial community is changed. Similarly when soil is fertilized or amended (for example, with compost), of course soil nutrients are affected, but so is soil pH, microbial activity, and cation exchange capacity; further, the effects of amendment may differ with both type of compost or fertilizer and type of soil being amended (Duong et al., 2012).

Land-use and vegetation history can influence soil characteristics and should be thoughtfully considered before the choice of field soil is made. Land-use history, such as past disturbance (i.e., agriculture production or mining), can greatly impact soil properties and plant performance with effects lasting for decades (Evans and Belnap, 1999; Kulmatiski et al., 2006). The legacy of agriculture on soil properties can last for over a century (Morris et al., 2011). Other historical impacts to soils that may need to be included in soil selection are the presence of metals or pollutants from human activities such as mining (Li and Thornton, 1993). Vegetation history is a critical component to consider when selecting a soil for studies. Plants can have species-specific effects on soil nutrient availability, soil microbial communities and thus, performance of plants in subsequent generations (Bever, 1994; Bais et al., 2006; Perkins and Nowak, 2013). Thus, we recommend being mindful of land-use history and vegetation to make a thoughtful choice of soil collection areas.

After land use impacts and site vegetation history have been considered and a collection site chosen, technical issues of soil collection must be addressed. One must realize that soils in the field have developed over a long time but soil structure and horizontal and vertical distribution of carbon and nutrients in some cases can change in a few generations (Rau et al., 2011a). Good experimental design requires that all soil within one soil collection (or treatment) be homogenous, which requires your field collected soil to be thoroughly mixed. Mixing will disrupt both soil horizons (discussed below) and soil structure. This limitation should be acknowledged by the researcher or more complex soil collection protocols employed. More complex protocols include digging a soil pit to observe the vertical distribution of soil horizons. Then each soil horizon can be collected separately and used to re-create layered soil in the greenhouse pots or mesocosms. Alternatively, small soil monoliths (intact soil cores) can be collected from the field with the vertical layers of soil intact and used in the greenhouse. The more complex the soil collection protocol, the more time and labor intensive. We recommend that for small pot studies or short duration experiments, the simple method is acceptable; however for large pot studies or longer term experiments, the more complex soil collection protocols are preferred.

Soil sterilization

Soil sterilization is often attempted to examine the relationship between plant species and the soil microbial community, to separate the effects of the soil microbial community and soil nutrients, or to selectively investigate the contribution of one fraction of the soil microbial community on plant performance. Several methods are available for soil sterilization including irradiation, dry or moist heating, and application of biocides such as methyl bromide and mercuric chloride (Wolf and Skipper, 1994). Although all of these methods can sterilize soil (destroy both active and resting structures of microorganisms), they all also alter soil physical properties (Sinegani and Hosseinpur, 2010). The efficacy of irradiation for soil sterilization is dose dependent (e.g., fungi are more susceptible to radiation than bacteria, McNamara et al., 2003). Irradiation also dramatically increases mineral N, P, S, and Mn availability (Wolf and Skipper, 1994; McNamara et al., 2003), and considerably alters pH

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(McNamara et al., 2003). Moist heat (autoclaving) and dry heat can effectively sterilize soil, but also increases pH, decreases organic matter, and alters availability of heavy metals (Wolf and Skipper, 1994; Egli et al., 2006), especially Mn (Wolf and Skipper, 1994). Application of chemical biocides such as methyl bromide and mercuric chloride can sterilize soil and may be useful when a researcher wants a residual effect of sterilization (Wolf and Skipper, 1994). However, these biocides can increase pH, remain detectable in soil, and have ecological and human health impacts (Wolf and Skipper, 1994).

The limitations and implications of each sterilization method must be considered if an ecologist wishes to use a sterilization method in a study. After any sterilization attempt, sterility must be assessed. Before and after sterilization, soil nutrients must be measured and accounted for in the experimental design. Realize that after sterilization soil is not the same as the soil was before sterilization, just without an active microbial component! Soil nutrient and pH conditions have also changed. Without measuring and accounting for this change, comparing sterilized soil to unsterilized soil is inappropriate.

Sampling soils

Sampling soil can pose a challenge because of spatial and temporal heterogeneity in soil properties. Spatially, soils vary horizontally (across the land) and vertically (with depth). Horizontally, soil is influenced by biological processes such as the distribution of plants and perturbations of animals, and physical processes (such as erosion) that shift the distribution of soil particles and nutrients. Vertically, soil development is impacted by biological processes such as root distribution (with the accompanying organic acid and chelator secretion), and physical processes such as leaching of minerals through soil from shallower to deeper depths over time. Temporally, soil nutrients can be impacted within a season as labile nutrients are taken up by the plants during active growth and returned to the soil with litter and as plants senesce. On longer temporal scales, soil formation from the physical and biologically mediated breakdown of rock is an ongoing process but occurs on millennial time scales. Thus heterogeneity must be taken into account when soils are sampled in order to understand the soil properties that plants are actually exposed to during different parts of the plant life cycle. In this section we discuss issues that should be considered when designing a soil sampling protocol.

Horizontal variation

In most soils, systematic spatial variation of soil properties exists even under apparently homogenous vegetation such as pastures and turfgrass. Where vegetation is more heterogeneous, soil properties will have even more substantial spatial variation, (i.e., higher nutrient accumulation under intact vegetation compared to unvegetated interspace i.e., 'islands of fertility'). Thus, the issue of sampling or collecting soils in the horizontal direction is complicated (Tiedemann and Klemmedson, 1973; Schlesinger et al., 1996; Halvorson et al., 1997). Therefore in order to avoid experimental errors, a thoughtful and precise protocol to take unbiased samples is important.

Three methods of sampling to account for horizontal variation include: simple random sampling, stratified random sampling, and systematic sampling. A random sampling design involves taking soil samples at predetermined random points within the entire study area. Stratified random sampling design entails dividing the study area into 'strata' or areas where soil has a common characteristic (i.e., under a shrub or in an interspace) and taking samples from random locations within each strata. Proper replication is important in stratified random sampling because often substantial variation can occur within strata as well as among strata. Systematic sampling requires a regular grid be established and samples taken from predetermined points regardless of strata (Peterson and Calvin, 1986). One soil methodological study that compared sampling designs recommends a systematic sampling regime except where variation is periodic (e.g., row crops) and where there is an obvious fertility gradient, in these situations stratified random sampling is preferred (Peterson and Calvin, 1986). We recommend that researchers thoughtfully consider their own questions regarding horizontal variation in soil characteristics and choose a sampling scheme judiciously.

Finally, one should very carefully consider whether to combine replicate samples into a 'composite sample' or not. If a plot is the unit of study, and, for example, vegetation biomass and nutrient content will be expressed on a plot basis, it is acceptable to use a composite soil sample. Using a composite soil sample can also reduce cost and analytical time (Boone et al., 1999). One should be aware, however, that any potential for evaluating soil property variation within a plot or finding any 'nutrient hotspots' (Schimel and Bennett, 2004; Johnson et al., 2011) will be lost by bulking samples.

Vertical variation

A fundamental feature of soils is horizonation (stratification into physically and chemically different layers or 'horizons'). Unfortunately, exact boundaries between soil horizons are often indistinct. Consequently, the determination of horizons and boundaries are often arbitrary and will vary with investigator and field conditions (such as moisture, Federer, 1982). For example, the top and usually most distinct soil horizon is the organic (O) horizon that consists of organic matter can be very difficult to precisely separate from the underlying 'A' horizon (mineral soils high in organic matter). Further, horizon depths are often quite variable on the landscape, especially in disturbed areas. A constant depth of sampling might not include the entire 'A' horizon in samples where the horizon is thick and might include some amount of the underlying horizon in sample points where the horizon is thin.

Two sampling options exist for accounting for soil horizons, sampling by horizon or sampling by depth. Sampling by horizon is only possible if pits are dug at each sampling location to determine the exact depth of each horizon; or if the soil is friable (easily crumbled or broken up) and free of stones, an open-faced punch auger can be used to determine horizons while coring. In the event that pits are dug, the possibilities for re-sampling in the future near the same location are diminished. Thus, we generally recommend sampling by depth, noting the issues that doing so presents. In practice, a common soil science procedure is to dig a pit in the general area of the sampling to determine the nominal depths of the major horizons and use that to establish sampling depths. When physically sampling soil, it is imperative to sample soil proportionately, that is, to take samples from a constant horizontal area, as in a core, so as not to bias the samples with more or less of one depth or another. Thus, if soil samples are to be taken by shovel or trowel, it is important to keep the sampling hole proportional and avoid the natural tendency to narrow the hole area with depth creating a biased sample (Fig. 1). We recommend taking soil samples with either a corer or using the unbiased trowel protocol (Fig. 1).

Temporal variation

Many soil conditions, especially biologically sensitive properties (e.g., soil ammonium and nitrate, NH_4^+ and NO_3^-), fluctuate over

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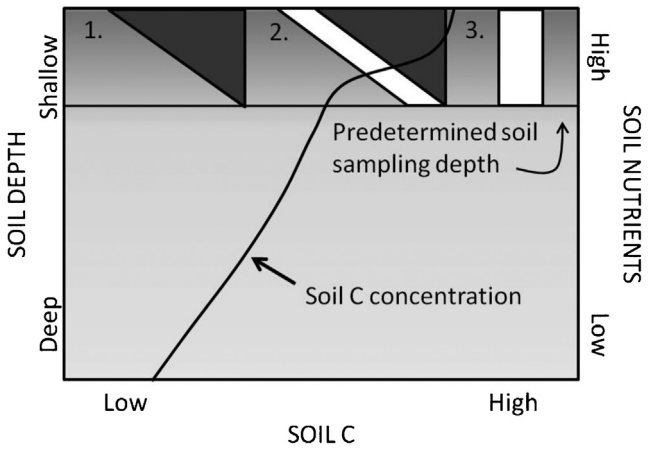

Fig. 1. Schematic representation of soil nutrient concentration with depth and sampling methods. Sampling proportionally by depth is important due to vertical variation in soil nutrients. (1) Biased sampling using a trowel in which proportionally more soil volume is taken from shallower depths (should be avoided). (2) Unbiased sampling with a trowel in which the first scoop (dark) is discarded and the second layer (white) is collected. (3) Unbiased proportional sampling with a core (recommended protocol).

time and any soil sample represents a snapshot that is only representative of the soil conditions at the time of sampling. Temporal variation is usually not a problem for soil properties that change very slowly such as soil texture or total nutrient concentrations. For highly dynamic properties, such as soil NH_4^+ and NO_3^-, temporal variation over the short term (months or even days) is important. For example, soil nutrient availability may be higher during times of the year when plants are dormant (due to a lack of nutrient uptake by plants). Sampling during the season in question or during the growing season is logical and acceptable. However, remember that conditions at other times of the year may influence processes observed during the growing season; e.g., high levels of NO_3^- in the soil before spring growth can influence seed germination (Baskin and Baskin, 1998). Depending on the research question, either a single or multiple soil sampling dates may be needed.

Advances in polymer chemistry have made it possible to use ion exchange resins to measure integrated soil nutrient availability over time (Skogley et al., 1996; Johnson et al., 2005). Resins may come as loose beads, or bulk sheets, or they may be enclosed in nylon, pvc, or plastics. Resins are typically left in the soil for a period spanning days to weeks and rely on diffusion to exchange soil solution ions with ions bound to the resin. Simply, as the soil solution passes over the resins, the nutrients in the soil solution are sorbed to the surface of the resins and subsequently extracted in the lab for analysis. The measurement of nutrients from resins generally do not generate absolute values for soil nutrient content, but may be used as an index to determine treatment or season effects on soil nutrient availability (Johnson et al., 2005). The amount of nutrients adsorbed on resins is correlated with the amount of soil water to which the resins were exposed. Therefore, it is essential that a measure of soil water be available to calibrate the nutrients measured from resins.

Estimating soil mass

Obtaining representative soil nutrient concentrations is adequate for some purposes (e.g., generating site fertility indexes), but for more detailed examination of soil nutrients on the landscape (i.e., estimating total soil nutrient content or nutrient pool sizes), soil mass must be taken into account. All methods of estimating soil mass have sampling problems and errors, and can be more trying than taking samples for chemical analysis. Three fundamental things are needed in order to obtain estimates of soil mass: bulk density of the soil (described below), percent coarse fragments (rocks, stones, and boulders greater than 2 mm diameter), and depth of the soil sample.

Bulk density is the dry mass of intact soil (<2.0 mm particle size) in a given volume, and is generally expressed as grams per cubic centimeter ($g\,cm^{-3}$). It is not necessarily measuring the mass of the soil that is a challenge, but accurately measuring the volume of the soil that can be an issue. As soils are sampled, their structure is changed and air pore space is altered- soils can become more or less aerated and 'fluffy'. So often it is best to determine the volume of soil by the size of the pit where the soil came from. Bulk density can be measured using various methods (clod, core, excavation, quantitative pits, and radiation) which are fully reviewed by Blake and Hartge (1986). Many of these methods have been developed largely for agricultural soils which are low in large coarse fragments. Non-agriculture soils often contain substantial amounts of large coarse fragments. When large stones or rocks are abundant accurate assessment of the volume of soil is difficult if stones only partially protrude into the sampling pit. Accordingly, Johnson et al. (2007) developed a method which estimates volume from rock density, root density, and bulk density of the <1 cm fraction (which is sieved and weighted in the field). This method assumes that the bulk density samples taken by coring from the soil pit (between large rocks) represents the bulk density of the <1 cm fraction field sieved and weighed.

More recently we have begun utilizing a diamond tipped rotary core device coupled to a gasoline power head in order to obtain estimates of bulk density, soil mass, coarse fragment content, and root biomass (Rau et al., 2011b). The rotary core device cuts cleanly through large rock fragments and coarse roots eliminating bias from including or excluding large coarse fragments which protrude partially into quantitative pits. Core bits with an internal diameter of 5–9.5 cm are large enough to proportionally sample soil, roots, and rocks without creating significant surface disturbance, making the core device ideal for repeated monitoring or small plot studies. Comparisons of the rotary core to quantitative pits showed that there was no consistent trend for the core to over or underestimate soil or rock mass compared to quantitative pits (Rau et al., 2011b). We found that the rotary core is appropriate for quantifying soil physical parameters such as bulk density, soil mass and coarse fragment content, as well as C and N content. The possibility exists of an increase of soil nutrients due to grinding rocks, so exchangeable cation and perhaps also extractable *P* values should be regarded with caution (Levine et al., in press). Rotary core samples should have no issues for organic C or N contents. We recommend that the rotary core be considered as a soil sampling method in ecological studies that require landscape scale assessments and repeated measures of soil parameters to any appreciable depth.

Soil sample processing and analysis

Thousands of published methods are available to quantify soil attributes, and the preferred methods depend, of course, on the questions asked (see Table 2 for recommendations of good soil references). We will describe some of the protocols that have proven useful in understanding plant–soil relationships, determining the nutrient content of soil, and are sensitive in a wide range of conditions (Table 1). We have had robust results with the protocols described below; however other protocols may certainly be appropriate and rigorous. The first step in good soil analysis is an appropriate sampling regime based on a sound statistical design, as discussed above. The second step is to determine, for a particular

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Table 1
Quick start guide to soil analyses. See text for more details. Time sensitive properties are susceptible to rapid change after soil samples are collected and should be processed as quickly as possible.

Soil property	Analysis method	Time sensitive?	Reference
Soil mass	Quantitative pit or Rotary core	No	Johnson et al. (2007), Rau et al. (2011b)
Texture	Many methods available	No	Gee and Bauder (1986)
pH	Standard pH meter	Moderate	Thomas (1996)
Organic matter	Several methods available	Moderate	Nelson and Sommers (1996)
Soil solution nutrients	Immiscible displacement	Yes	Mubarak and Olsen (1976)
Mineral N	KCl extraction	Yes	Keeney and Nelson (1987)
N mineralization potential	30 day incubation/KCl extraction	Moderate	Hart et al. (1994)
P	Depends on soil pH	Yes	Olsen et al. (1954) (for alkaline soil), Bray and Kurtz (1945) (for acid soil)
K and Micronutrients	DTPA chelate-extraction	Moderate	Lindsay and Norvell (1977)

nutrient, what pools are to be quantified. The third step is proper sample handling and timely processing. In soil, different pools of nutrients exist ranging from readily available, water extractable, labile pools (i.e., in the soil solution), to less readily available recalcitrant pools (i.e., sorbed to soil particles or in organic matter). Water-extractable pools are the most readily available to plants, but for certain nutrients, this pool can supply less than 5% of a plant's total needs (Blank, 2008). Nutrients in the soil-solution are replenished over time from the more recalcitrant pools. It is important to note that there is no perfect method to accurately predict plant-available nutrients. Plants have many species-specific strategies to obtain limiting soil nutrients and no one method can account for those strategies. The protocols described here address a range of nutrient pools, from labile pools to more recalcitrant pools.

The work flow for a very general soil sample collection and processing protocol is diagramed in Fig. 2. Generally, soil samples are collected, composited or not (see discussion above), transported to a laboratory, sieved to remove coarse fragment, roots, and biota, and analyzed (some methods described below). It is essential to realize that soil characteristics are subject to start changing from the moment the soil sample is collected and some characteristics change much more rapidly and are more time sensitive than others (Table 1), thus proper timing in soil analysis is imperative. To assure that minimal changes occur once soil samples are collected, we recommend processing samples for properties that are vulnerable to rapid change the same day soil samples are collected. Some researchers add extracting agents in the field just after soil is collected and the coarse fragment (everything above 2 mm) sieved out (which is a protocol that we recommend if the resources are available, Van Miegroet, 1995). We recommend not collecting more soil samples that will be analyzed for properties that are susceptible to rapid change than can be processed the same day. However, other soil properties are much less dynamic and processing need not be immediate. Many researchers will store and air-dry these samples to processes later. A necessary step in analyzing soil samples is to remove a subsample of the soil to determine the water content of the sample. Generally, a 5–10 mg sample is weighed before and after drying in a 105 °C oven until constant weight is reached (Jarrell et al., 1999). This allows the calculation of the exact amount of soil used for analysis.

Table 2
A brief list of soil methods reference books that we find most useful. Note this list is not a comprehensive list of all soil reference books and other books are probably just as useful as these.

Title	Publisher	Year
Methods of Soil Analysis (especially Parts 1, 2 & 4)	Soil Science Society of America	1982, 1994, 2002, respectively
Soil Sampling and Methods of Analysis	Canadian Society of Soil Science	1993
Soil Survey Manual[a]	Natural Resources Conservation Service, United States Department of Agriculture	1993

[a] Available online.

Fundamental soil properties

Soil texture, pH, and organic matter are fundamental soil properties that affect the entire soil system. Therefore, they should be quantified as part of a rigorous soil protocol. Soil texture is the proportion of the various sizes of soil particles (sand, silt, or clay) and is quantified by particle size analysis. Soil texture is one of the most basic soil properties and influences plant growth, water infiltration, nutrient holding capacity. Soils comprised mostly of sand are generally more droughty and lower in plant nutrients and organic matter than soils than contain more clay. Clayey soils hold more water and nutrients than sandy soils, but drainage is often slow resulting in poor aeration, excessive runoff, and increased resistance to physical penetration by plant roots. Texture can be roughly evaluated by the 'texture-by-feel method' and quantitatively evaluated with a hydrometer, pipette method, or tubidometer method (Gee and Bauder, 1986). Commercially available automated instruments are available to quantify particle size distribution using light scattering technology (Segal et al., 2009). These instruments have utility in reproducibility, simplicity, small samples size, and a wide range of particle sizes quantified, but are expensive. All of these quantitative methods are fairly reliable (Kettler et al., 2001), thus we recommend researchers utilize whichever method for which they have resources.

Soil pH affects nutrient availability, elemental toxicities, biological activity, and subsequently, plant growth. For example, at high pH, Fe may be present in the soil but bound to soil particles and therefore unavailable for plant uptake; conversely, at low pH, Fe is displaced from soil particles and becomes available for plant uptake; further at extremely low pH, Fe availability can increase to the point of toxicity (Brady and Weil, 2008). Therefore, plants growing in three soils with the same Fe content can either have Fe deficiency if the soil pH is high, acceptable Fe levels if the soil pH is neutral, or too much Fe (Fe toxicity) if the soil pH is extremely low. Most productive arable soils have intermediate pH values (5.5–7; Brady and Weil, 2008) but wild land soils have more extreme variation. Soil pH is routinely quantified using a glass electrode (Thomas, 1996). Soil pH measurement is done by suspending soil in an aqueous electrolyte solution and then measuring the pH of the solution. Thus, pH measurement is sensitive to salt types and concentration in the electrolyte solution and in the soil. Often the

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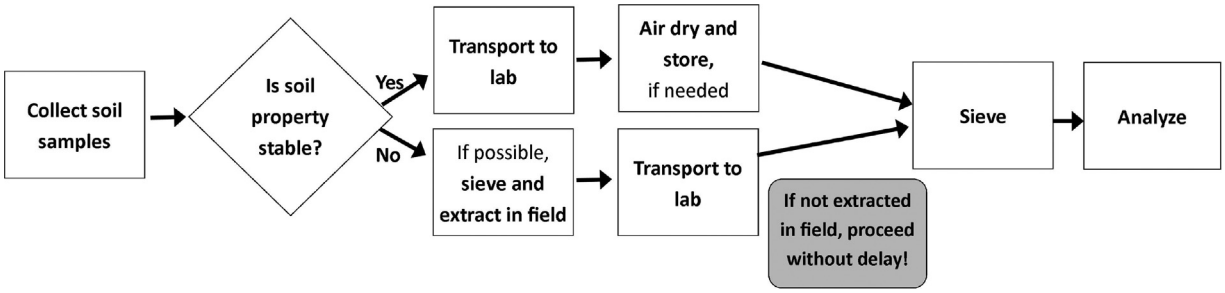

Fig. 2. Diagram of a very general work flow for collection and processing of soil samples. Each step should be thoughtfully addressed. For example, 'transport to lab' may involve keeping soil samples on ice in a cooler or in bags air-drying in the back of a truck. This work-flow can be adapted to most every study.

electrolyte solution used is 0.01 M $CaCl_2$, however, we have found that 0.05 M $CaCl_2$ provides more reliability in arid wildland soils that have variable salt content. We recommend that researchers follow the instructions from their pH meter manufacturer, examine the reliability of pH readings in solutions with varying electrolyte concentrations to develop their protocol, and then, always process all the soil samples in a study using the same protocol.

Soil organic matter is a reservoir for nutrients and water and is necessary for biological activity in soil. Organic matter contributes to good soil structure which promotes drainage and root penetration (Sollins et al., 1999). High organic matter soils generally have greater plant productivity than low organic matter soils. Soil organic matter can be split into a number of pools each with their own protocols, issues, and usefulness. Determination of these pools is complex enough that it is outside the scope of a 'quick start' guide, however determination of total soil organic matter and total soil C is often sufficient. Total soil organic matter can be determined via high-temperature combustion or loss-on-ignition methods. An older method, the Walkley–Black procedure is no longer recommended due to toxic waste products and unreliable results (Sollins et al., 1999). High-temperature combustion converts soil carbon to carbon dioxide that is measured using either thermal conductivity or an infrared gas analyzer. Advantages of high temperature oxidation include automation, consistency, low detection limits, and very complete oxidation of soil carbon. Important considerations in sample preparation include grinding to a fine powder to assure sample homogeneity and pre-treatment of samples to remove inorganic carbon sources such as calcite and dolomite (Nelson and Sommers, 1996). Organic matter content can be calculated from organic carbon values by simply multiply organic carbon by 1.72 (an empirically derived based on the finding the soil organic matter contains approximately 58% carbon, Nelson and Sommers, 1996). A second option is quantifying organic matter using loss on ignition, wherein the difference in weight of a soil sample before and after ignition at 400 °F for 16 h is often considered the organic matter content of a soil (Nelson and Sommers; 1996; Sollins et al., 1999).

Nutrients in the soil-solution

The soil-solution is the aqueous phase (water) surrounding soil particles. The nutrient pool in the soil solution represents the most readily available source of nutrients to plant roots. Typical solutes quantified in the soil-solution include anions: chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), sulfate (SO_4^{-2}), ortho-P, many organic acids and the cations: calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium (Na^+), and (K^+). The concentration of solutes in aqueous extracts is very reactive, thus is useful for examining effects of soil microenvironments, plant species, microbial processes, and disturbance regimes such as wildfire. For example, the soil-solution near roots differs considerably from that away from roots because root exudation can increase soil-solution levels of many nutrients including N, P, and micronutrients (Marschner, 1995). Quantification of anions and cations in the soil-solution can have great utility. Nutrients in the soil-solution are sensitive to environmental conditions, thus we recommend extraction from freshly collected soil. The soil solution extraction protocol that we recommend is a modification (Mubarak and Olsen, 1976) of the saturated paste method (United States Salinity Laboratory, 1953) called immiscible displacement.

Nitrogen

Mineral N

For many terrestrial ecosystems, the availability of N is the major driver for plant growth (LeBauer and Treseder, 2008) and mineral N is a form of nitrogen that is readily available for plant uptake. Mineral N originates from mineralization of soil organic matter; e.g., NH_4^+—N, NO_2^-N, NO_3^-N, and N-containing monomers which include amino acids (Schimel and Bennett, 2004). Mineralization is the conversion of an element from an organic form to an inorganic form as a result of microbial decomposition (Brady and Weil, 2008). There is still uncertainty regarding the relative species-specific balance of uptake of these N-forms; NO_3^-—N and NH_4^+—N dominate, but N-containing monomer uptake may be more widespread and more important than previously thought (Kielland, 1994). Levels of mineral N in soil are a balance between mineralization of organic matter and microbial plus plant uptake. Ecosystems with strong coupling of mineralization with uptake generally, at any one time, have low mineral N availability. Disturbance in its many forms can decouple mineralization with uptake resulting in much higher N availability. As one would expect, measurement of mineral N is a highly used soil analytical procedure. Given the linkage of mineral N to microbial processes, we recommend rapid processing of samples once collected.

The most common method to quantify mineral N availability is the 2.0 M KCl extraction (Keeney and Nelson, 1987). This concentration of KCl extracts the principal forms of mineral N in soil, NH_4^+—N, NO_2^-—N and NO_3^-—N. These forms are then quantified using an auto-analyzer to separately analyze NH_4^+—N and NO_2^-—N + NO_3^-—N. Most instruments have the ability to separately analyze NO_2^-—N and NO_3^-—N. Remember that this procedure is a one-time snapshot of mineral N availability. Mineral N can vary widely both spatially and temporally, thus a one-time measurement can have limited utility (Farley and Fitter, 1999). In summary, quantification of mineral N, in a proper temporal and spatial sampling scheme, is a robust predictive measurement for plant growth and a host of biologically mitigated soil processes.

N mineralization potentials

If one were to budget tissue content of N of a mature plant with pre-growth soil mineral N, tissue N content would greatly exceed soil mineral N content (Johnson et al., 2007; Perkins et al., 2011).

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In other words plants are often observed to take up more N that was available in the soil before plant growth, thus soil N must be constantly replenished via mineralization of soil organic matter. While measuring soil N at one point in time provides a snapshot of soil fertility, measuring N mineralization provides an indication of how that soil fertility is recharged over time. Soil researchers continually seek to perfect methods that quantify N mineralization potential. Still, the potential quantity of N that can be mineralized over a period of time is an important soil property and highly predictive of an ecosystem's plant productivity (McGuire et al., 1992). We have experimented with several methods (field and laboratory) to quantify soil N mineralization potentials and routinely use a laboratory 30-day, moist, aerobic incubation in the dark procedure (Hart et al., 1994) followed by KCl extraction (Keeney and Nelson, 1987). We find that this procedure is convenient, reproducible, and produces reliable data.

Phosphorus and Potassium

Phosphorus

Phosphorus is often considered the second most limiting nutrient for plant production after N (Lajtha et al., 1999) and in more developed landscapes is the limiting factor to plant growth (Wardle et al., 2004). The plant-available pool of P contains a relatively small proportion of total P in the soil system. Like N, plants require replenishment of the plant-available P pool and have evolved mechanisms to foster movement of organic-bound P and P that is sorbed to mineral surfaces (Marschner, 1995) into the plant-available pool. Many protocols have been developed to quantify potentially available soil P pools and each works best for specific soil types. We routinely use two protocols: the Olsen et al. (1954) procedure is best adapted to alkaline soils and the Bray and Kurtz (1945) procedure is appropriate for acid soils. An important exception to the latter is in andic soils (derived from parent material that has a significant portion of volcanic material), where we find that the Bray method is ineffective because of the high ortho-P adsorption properties of these soils (at times indicating zero extractble P); in this situation the bicarbonate P method has proven superior (Johnson et al., 1997; Susfalk, 2000).

Potassium

Like N and P, K is a macronutrient (required in large amounts) for optimum plant performance. However, K seems to receive less attention in the ecological literature than N or P (Tripler et al., 2006). Potassium levels in soil have been observed to influence primary production (Tripler et al., 2006), competitive relationships between plants (Tilman et al., 1999), priority effects and plant–soil feedbacks (Perkins and Nowak, 2013), and have strong seasonal fluctuations (Tripler et al., 2006). Therefore, perhaps the ecological significance of K deserves more attention. Soil K is determined using the same method as micronutrients (the DTPA chelate-extraction method described below).

Micronutrient availability

The correct balance of soil micronutrient availability is critically important for optimal plant performance (Marschner, 1995). Micronutrients are essential nutrients that plants require in small amounts; thus 'micro' refers to amount needed not essentiality (Brady and Weil, 2008). Micronutrients include B, Cl, Cu, Fe, Mn, and Zn. Quantifying availability of micronutrients in soil is problematic because they are present in such minute quantities. We have experimented with the DTPA chelate-extraction method, which was developed to identify micronutrient deficiencies in calcareous soils (Lindsay and Norvell, 1977). DTPA-extraction is not perfect and data generated must be judiciously examined in relation to site-specific soil characteristic and vegetation (O'Connor, 1988). However, the method is quick, reproducible, and readily indexes micronutrient availability among soil types.

Conclusion

The increasing desire for ecologists to incorporate soils in their research, i.e., acknowledge the aboveground impacts of belowground conditions, is very positive and has potential to increase understanding of many ecological process and patterns. If a researcher does not have at least a basic understanding of soil, the potential for making fundamental errors (and thus generating incorrect and unreliable information) is high. This paper provides basic information on choosing a soil for a field or greenhouse study, soil sterilization, soil sampling, and analysis. By providing this information, we hope that ecologists will be better prepared to address some of the challenging issues with incorporation of soil into ecological studies.

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