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Surveying for biotic soil crust lichens of shrub steppe habitats in the Columbia Basin

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Abstract: Arid lands of eastern Oregon and Washington support a great diversity of biotic soil crust organisms that are often overlooked in biotic inventories. Land managers are increasingly interested in incorporating these species in their surveys so that they can protect unique habitats and prevent uncommon species from becoming threatened. Current monitoring guidelines for rangeland health sometimes take into account the percent cover by soil crusts; however, there is very little guidance for surveying for uncommon species. Furthermore, because soil crust species and habitats are unfamiliar to most botanists, finding these species presents special challenges. We outline suggestions for future surveys with these goals.

Key words: biotic soil crust, lichens, bryophytes, surveys, calcareous soils, sagebrush, *Texosporium*.

Introduction: The Columbia and Great Basins of central and eastern Oregon and Washington are home to beautiful and diverse biotic soil crusts. These communities are a mixture of interwoven fungi, algae, cyanobacteria, lichens, and bryophytes that grow in and on the soil in drylands. The biotic crust is not only important in ecosystem function, but also harbors some species that are uncommon or rare (Root et al. 2011). These biotic crusts are threatened by development, livestock grazing, changing fire regimes, and invasion by exotic grasses (Belnap & Lange 2001, Root & McCune 2011). Agricultural and developed sites are managed for non-native vegetation and have almost no soil biotic soil crust lichen communities. In more wild areas, invasion by exotic grasses and mechanical soil disturbance caused by livestock trampling and human activities can substantially diminish biotic crust abundance (Eldridge 1998, Belnap et al. 2001, Ponzetti & McCune 2001). Because more than 90% of the native shrub-steppe in Oregon and Washington has been disturbed from its native conditions by agriculture, grazing or other anthropogenic factors (Noss et al. 1995), there is reason to be concerned that some biotic crust species may be at risk.

While the biotic soil crust as a whole can be quite well-developed (**Fig. 1**), individuals within the crustal community can be quite subtle; the entire visible thallus of an individual may be less than 1 mm in size. They range in color from white to black, pink, blue, and yellow. Some species such as *Psora decipiens,* are distributed on nearly all continents whereas others, such as *Texosporium sancti-jacobi* and *Trapeliopsis bisorediata,* are endemic to western North America. Biotic crust lichen communities are patterned in relation to climate, vegetation, soil chemistry, texture (Root & McCune 2011). Differences in species composition affect the degree to which biotic crust communities stabilize soil (Eldridge 1998) and fix nitrogen (Beymer & Klopatek 1991). Biotic crust communities are not equally damaged by disturbances; in general, the more three-dimensional species seem most susceptible (Belnap et al. 2001) and lichens growing on sandier soils appear to be more easily disturbed (Root & McCune 2011).

Searching biotic soil crust communities for species of particular interest is not easy. While a manual for identification of lichen species in soil crusts of the Columbia Basin exists (McCune & Rosentreter 2007), botanists have little guidance on methods for searching for these species and their habitats. To address this need we present guidelines for surveys of biotic soil crust lichens in dryland ecosystems with a special emphasis on documenting rich, well-developed sites as well as new records of uncommon and rare species. Target organisms currently include lichens and bryophytes although many species of algae, cyanobacteria and non-lichenized fungi are also known to be associated with the biotic crust community. The specific objectives of such surveys may include: (1) locating new populations of uncommon species, (2) cataloguing species present and evaluating their relative abundance, (3) describing habitat associations of target species, or (4) monitoring potential threats on target communities. Other protocol suggestions (reviewed in Belnap et al. 2001) may be appropriate in cases where monitoring the presence and abundance of a crust is a higher priority than examining species-specific habitat associations.

Our focus is the intermountain Columbia Basin of Washington, Oregon, British Columbia, Montana, and Idaho, with special emphasis on the study area of Root & McCune (2011). This is a mosaic of arid and semi-arid steppe dominated by bunchgrasses, *Juniperus occidentalis,* and several species of sagebrush (*Artemisia* spp.) with elevations ranging from 170-1745 m. In this area we conducted both intuitive and stratified random sampling. While the habitats we discuss are specific to this area, we hope that surveying principles and some of habitat information will transfer to other ecoregions.

Site Selection: Depending on the primary objectives and scale of the survey, locations may be selected using a stratified random or intuitive sampling design. Stratified random site selection offers the advantage of inference to the landscape because every potential site within a stratum has the same probability of being selected. It is an ideal tool when the primary goals of a survey require an unbiased sample. These goals include evaluating species' relative abundance, estimating number of populations in an area or detecting and monitoring the effects of threats to biotic soil crusts. Intuitive site selection allows targeting habitats that may be promising for uncommon species based on local knowledge or attributes that have not been mapped. Furthermore, it allows opportunistic sampling in convenient habitats or those that may be unusual based on previous herbarium specimens. However, it cannot be scaled up to make broader statistical inferences because the sites selected may not be representative. Intuitive surveys are most effective for locating new populations of uncommon species, describing habitat associations of uncommon species, and generating the most complete species list for a parcel. We suggest weighing the importance of the goals to determine an appropriate mixture of random and intuitively located plots.

We suggest stratification of random plots by habitat type or geography. The EPA ecoregions (Clarke & Bryce 1997) divide major habitats across the United States; these are especially useful for excluding forested habitats unlikely to support biotic crusts. Stratification by the finest level of these ecoregions ensures sampling in a wide range of habitats; since the ecoregions are spatially distinct, using them for stratification across a district also generates plots somewhat evenly spread across the study area. In our surveys, we accomplished this using the free program, Hawth's Tools (Beyer 2004), in ArcGIS (ESRI) to randomly select a predetermined number of points in each ecoregion (Clarke & Bryce 1997). Hawthe's Tools, which we used, will soon be replaced by the free Geospatial Modelling Environment, accessible through the same website. In the first round of sampling, we selected an equal number of plots in each ecoregion to force ourselves to visit the range of habitats in the district (Root & McCune 2011). Later, we sampled ecoregions proportional to the area that they covered in the district to allow us to further explore the most common habitats. When species of interest are known in advance, allocating a greater proportion of sample plots to ecoregions known to have many populations may be most efficient. Estimating the number of populations in a district requires estimating the number of populations in each stratum and combining them by weighting each estimate by the amount of land covered by each stratum (Lohr 1999, p. 99-103). When the target survey area is within just a few ecoregions, stratification by soil texture, plant association or geography may be appropriate. This approach could be implemented in the same way if a GIS layer mapping desired strata were available. Once randomly-located plots have been sampled in an area, the surveyors will have a more thorough understanding of habitats supporting uncommon species and be better equipped to intuitively locate additional plots in the most promising habitats for uncommon species.

Travelling by foot through the drylands of eastern Oregon and Washington is slow and many obstacles cannot be foreseen when pre-selecting sites. Restricting sample sites to those near roads may be necessary, but could be problematic if sites farther from roads provide very different habitats. Furthermore, many of the public parcels in eastern Oregon and Washington are completely surrounded by private land; these may also need to be excluded from sampling because of access restrictions. We recommend excluding habitats that are very unlikely to support biotic soil crusts, including: developed sites, those in current agricultural use and those with more than approximately 20% canopy cover by trees. Even though forested sites typically do

not support well-developed soil crust communities, those dominated by junipers (*Juniperus* spp.) can be an exception. Because conditions precluding soil crust development may not be foreseeable, we suggest pre-selecting a greater number of random plots than necessary so that if a site is found to be inappropriate, another site on the list can be substituted expeditiously.

Target Habitats for Intuitive Plots: Intuitive plots with the focus of capturing a complete species list should be spread across the dominant vegetation types and strategically placed to capture uncommon habitats known to support uncommon crust species. Furthermore, they should take full advantage of any prior information, such as herbarium specimens (Consortium of North American Lichen Herbaria. 2011), literature reports, and local botanists. Previous studies have found that different biotic crust communities are associated with grasslands, little sagebrush (*Artemisia arbuscula*), Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*), and juniper (*Juniperus occidentalis*) habitats (Root & McCune 2011). We suspect that other habitats with distinct vascular plant communities and soils could also provide habitat for different biotic crust communities. We describe below a few habitats that we have found to support rare or infrequent biotic soil crust species in central Oregon (these observations draw on the field experience of the authors, Root & McCune 2011 and Root et al. 2011).

Calcareous soils. Calcareous soils (**Figs. 2, 3, 4**) often support biotic crust lichens that are uncommon in the largely basaltic Columbia Basin, but are often broadly distributed in the northern hemisphere and common in calcareous regions. Perhaps the paucity of other vegetation and unusual chemistry precludes other competition. In central Oregon, we have found calcareous soils to be richly covered in *Phaeorhiza sareptana, Psora cerebriformis, P. decipiens* and *Toninia sedifolia* with smaller components of *Aspicilia rogeri, Circinaria (Aspicilia) hispida, Heppia lutosa* and other unusual species. Several of the species that are rare in the Great Basin may be found in Oregon or Washington by more closely examining our calcareous habitats. Even in areas with noncalcareous soils and bedrock, a buried hard, whitish, caliche layer that fizzes on contact with hydrochloric acid may be present. This caliche contains calcium carbonate and other minerals (**Fig. 5**). When the caliche is turned up by soil disturbance (especially small mammals and badgers), local pockets of calciphiles develop on this upturned caliche, especially after abandonment by the disturbance agent. Lichens on exposed calcium carbonate deposits can also be found where the caliche layer intersects steep faces of rock outcrops and in association with seepage deposits over and through bedrock.

Along Rivers. Along the John Day, Deschutes, and Columbia Rivers, we observed uncommon biotic crust lichens on canyon slopes and rims (**Fig. 6**). In these habitats, which may be influenced by the humidity from the river, we found populations of *Acarospora schleicheri, Fuscopannaria cyanolepra, Texosporium sancti-jacobi* (**Fig. 7**), *Ochrolechia turneri, Rhizocarpon diploschistidina* and *Trapeliopsis bisorediata*. Often, these sites supported rich biotic crust communities even when they were heavily invaded by cheatgrass (*Bromus tectorum*). Canyon rimrock often has thin soil, sparse vegetation, and a stony surface, all of which appear to reduce grazing impacts but foster biotic crusts. They are relatively dry and windy, favoring certain species (*e.g. Texosporium*), while diminishing species favored on cooler, moister sites (*e.g. Fuscopannaria cyanolepra* and *Ochrolechia upsaliensis*). Similarly, canyon slopes can be relatively inaccessible to cattle. Canyon slopes provide a mixture of relatively moist and dry habitats, depending on aspect.

Bunchgrasses. Well-developed native bunchgrass habitats (**Figs. 1, 8**) with *Poa sandbergii* and *Pseudoroegnaria spicata,* especially on steep hillslopes, often had well-developed biotic crust communities. Particularly on north-facing aspects, these sites supported *Fuscopannaria cyanolepra* (**Fig. 9**) and *Tetramelas (Buellia) terricolus* on soil organic matter. They also often had abundant *Acarospora schleicheri* (**Fig. 10**), *Diploschistes muscorum, Ochrolechia upsaliensis* and *Placynthiella* spp.

Artemisia rigida on thin gravelly soils. *Artemisia rigida* is a small deciduous *Artemisia* that occurs on poorly drained, flat, stony sites, usually with sparse vascular plants. These barren, gravelly sites often have shallow standing water in winter, but are very dry in summer. Here the biotic soil crusts experience limited competition from vascular plants. Biotic crusts are typically abundant where there is sufficient soil, as well as in rock crevices. They support populations of vagrant *Xanthoparmelia* as well as *Acarospora schleicheri* and *Diploschistes muscorum*. Poorly drained flats can support vagrant *Dermatocarpon* species. Rock crevices often support a lichen community transitional between soil-dwelling and rock-dwelling species, including numerous infrequent crustose and squamulose species (*e.g. Buellia badia, Cladonia* spp., *Lecanora phaedropthalma, Toninia ruginosa*).

Survey Methodologies: *Timing*. Biotic soil crusts are present year round; however, surveys are easiest to accomplish in fall and spring when the soil is damp; this makes the colors more vibrant and the observer less likely to overlook species. When surveys are necessary in summer, we recommend carrying a plant-misting spray-bottle to moisten the survey area prior to observation. Late spring is a good time for sampling if the ephemeral bryophytes portion of the crust is sought. These include the thalloid liverworts *Athalamia*, *Mannia*, and *Riccia*. Many of those species are short-lived annuals, persisting as resting spores through summer and fall.

Protocol. Once sites have been selected, plot size also depends on study objectives. Large plots are most effective for discovering the greatest number of species present and large-scale habitat associations. Smaller plots allow for more precise estimates of abundance and are better for tracking subtle changes in communities over time or examining small-scale factors.

If surveying aims to thoroughly characterize the biotic crust community by maximizing species observed, we recommend a protocol modified from the Forest Health Monitoring Protocol (FHM, McCune et al. 1997), but adapted to terrestrial species rather than epiphytes. Circular plots 34.7-m in radius (approximately 1 acre or 0.38 ha) are searched for terricolous lichens or bryophytes for two hours or until no new species had been found for a half hour. This method has been applied to other treeless ecosystems, for example, arctic tundra (Holt et al. 2009, DeBolt 2008, 2010, Root & McCune 2011) and is comparable to other lichen plots throughout the country. To see the biotic crusts, the surveyor must crawl or spot-check sites (**Fig. 11**) with a handlens or magnifying eyeglasses. A drawback to this approach is that different surveyors are likely to be more attuned to different taxa and habitats; were the same plot visited by multiple surveyors, they would likely result in somewhat different species lists. The same problem has been more thoroughly studied using the FHM method in forests and has been found to be sufficiently consistent to allow large-scale comparisons (McCune et al. 1997); however, future research should determine whether this is also true for soil crusts.

In one-acre plots, we suggest allocating the time within a plot to more promising microhabitats including places with finer soils, under shrubs, among basalt outcrops, and near caliche exposure. Rock outcrops can often have well-

developed crust communities in the interspaces even in otherwise poorly-developed areas (**Fig. 12**). At grazed sites with sandy soils, biotic crust communities are often developed only under shrubs (**Fig. 13**). Poorly drained flats without gravel (**Fig. 14**) and unstable slopes (**Fig. 15**) typically do not support well-developed soil crusts. To focus exclusively on biotic crust communities, we recommend including organic matter integrated in the soil matrix and organic matter but excluding lichens and bryophytes growing on pebbles, rock or fallen twigs; inclusion of these substrates would introduce new saxicolous and epiphytic species.

Microplots placed at random locations within one-acre plots could provide representative estimates of abundance and evaluation of smaller-scale factors. These smaller plots may also be more consistent than one-acre plots if monitored over time by different personnel. Microplots centered on target species populations within one-acre plots could facilitate description of their micro-habitat associations. Several small-scale approaches have been employed for measuring biotic soil crusts including line and point intercept (Belnap et al. 2001), 20 x 50 cm Daubenmire (1959) micro-plots along a transect (Ponzetti & McCune 2001), and larger 4 x 0.5-m mesoplots (Ponzetti et al. 2007). We feel that the best size depends on the scale of the factor being investigated and on the target precision of abundance estimates; smaller plots are quite variable and time-consuming but can provide more precise estimates of abundance that allow for detection of subtle trends in relative abundances (McCune & Lesica 1992) .
To characterize a site and describe biotic crust communities, permanent plots are not necessary. If monitoring trends over time in permanent plots is an objective, it is important not to alter the community in the plot. It is very difficult to sample one-acre plots without some trampling; furthermore, biotic crust species must be collected for identification and vouchering. Micro-plots may be better for long-term monitoring because the observer can sit outside the plot and collect voucher specimens from similar individuals outside the plot; however, it is possible that the specimens of nearby soil crusts that appear similar to those in the plot are, in fact, different species.

Site Documentation. At the site, record the GPS location of collections and the plot center and mark plots on a map. Characterizing the soil texture and pH as well as the vascular plant community may be useful in refining habitat associations in the future (DeBolt 2010). Soil texture and chemistry can be extremely variable across the plot; if the aim is to characterize a large plot, we suggest collecting several subsamples and mixing them for analysis (Root & McCune 2011). However, smaller-scale soil samples may be useful in describing microhabitat associations of uncommon species. Texture can be assessed by feel; however, this qualitative variable can be difficult to use in analysis and we found that our wet-sieved soil texture estimates were more useful.

Specimen Collection and Processing. Most biotic crust species are sufficiently difficult to identify that collecting a piece will be necessary. For larger samples we suggest collecting into labeled #1 or #2 brown paper bags or small plastic Petri dishes. Smaller samples can be padded with tissue or collected in small coin envelopes that fit nicely inside bags and provide additional protection from fragmentation and crushing. Samples should be stored safely in boxes on returning to one's vehicle and air-dried as soon as possible; if collected in moist weather they can grow moldy within a few days. We have found that processing specimens requires approximately the twice amount of time as fieldwork. Each specimen from each site should be examined under a dissecting microscope; often several species will be present in the same sample and very small pieces of interesting but obscure species may be discovered in this phase. Species should be separated for study with other

related taxa so that consistent taxonomy can be applied in challenging groups. Typically, there will be many poorly-developed thalli that cannot be identified to genus or species because they lack the necessary structures. Collection of several individuals increases the chances that one will be identifiable. Once dried and separated, lichen specimens can be glued to an archival card or a small box or Petri dish and placed in a packet for storage (**Fig. 16**).

Care in the curation process is essential as specimens can easily become unidentifiable piles of soil upon handling (Rosentreter et al. 1988). We recommend blowing lichens free of surface dust, slicing off excess soil using a razor and setting them, soil down, in a shallow dish filled with a mixture of glue and water, being careful to allow the glue to permeate soil but not the lichen thallus. Elmer's glue works well and will permeate the substrate; once dry, this will harden the soil. Once the substrate is stabilized, glue the specimen onto a card or in an archival box and surround it with full-strength glue. Small pieces of foam glued around specimens on cards can protect them from damage when filed in herbarium cases. More thorough instructions can be found in McCune & Rosentreter (2001) and Rosentreter et al. (1988) and ideas for curation in general can be found by accessing the Northwest Lichenologists webpage (http://home.comcast.net/~nwlichens/Curation.htm).

Surveyor Qualifications. Individual thalli are quite inconspicuous and require an eye for detail; new surveyors should spend time in the field learning to see these species with those who have had practice. Further practice in the lab will be necessary to distinguish taxa that differ primarily in microscopic characteristics. To be successful, surveyors need: (1) access to the primary literature regarding the latest taxonomy of lichens and bryophytes, (2) familiarity with dissection and compound microscopes for identification of bryophytes and crustose lichens, (3) familiarity with routine chemical tests and possibly thin layer chromatography (TLC) to identify lichen compounds, (4) access to an herbarium with reference specimens, and (5) a working relationship with experts in bryology and lichenology who can collaborate to examine particularly challenging groups. In addition to knowledge of the biotic soil lichen flora, surveyors should be proficient at interpreting vascular plant communities and soils to identify target habitats. Aspiring soil crust surveyors can hone their skills by participating in field trips and workshops, many of which are announced or sponsored by the Northwest Lichenologists (information about these can be found at http://home.comcast.net/~nwlichens/events.htm) or the International Association of Bryologists (IAB blog - Atom).

Resources for Identification. Several resources are available to identify biotic soil crust lichens, including: McCune & Rosentreter (2007), Nash et al. (2002, 2004, 2008) and Rosentreter et al. (2007). Bryophyte resources include: Norris and Shevock (2004), Lawton(1971), Flowers(1973), McIntosh (1986), and the Bryophyte Flora of North America (Flora of North America Editorial Committee 2007; other volumes in progress).

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Fig. 1: A well-developed soil crust on a bunchgrass site at 250 m in Gilliam County, Oregon. Note yellow patches of *Acarospora schleicheri*, gray-green patches of *Aspicilia reptans,* and white patches of *Diploschistes muscorum*. All photos were taken by Heather Root unless otherwise noted.

Fig. 2: Calcareous soil patch with orange field vest on it has little vascular plant cover and several uncommon biotic soil crust lichens (220 m, Gilliam County, Oregon).

Fig. 3: *Psora decipiens* (salmon pink) and *Toninia sedifolia* (blue-gray) on the calcareous soil pictured in **Fig. 2**.

Fig. 4: *Circinaria (Aspicilia) hispida* on the calcareous soil pictured in **Fig. 2**.

Fig. 5: White stains on basalt rock indicate localized calcium-rich deposits (397 m, Sherman County, Oregon).

Fig. 6: Canyon rim habitat above the John Day River (438 m Sherman County, Oregon) supports *Texosporium sancti-jacobi* and *Ochrolechia turneri*. The dense vegetation downslope and along the river support lower cover and diversity of biotic soil crusts.

Fig. 7: *Texosporium sancti-jacobi* in the silty bunchgrass habitat surrounding the calcareous area in **Fig. 2**.

Fig. 8: Well-developed bunchgrass habitat near the Columbia River, 261 m in Gilliam County, Oregon; this site supported *Trapeliopsis bisorediata, T. steppica, Texosporium sancti-jacobi, Acarospora schleicheri* and *Rhizocarpon diploschistidina*.

Fig. 9: *Fuscopannaria cyanolepra* in a bunchgrass habitat at 424 m in Sherman County, Oregon.

Fig. 10: *Acarospora schleicheri* in the bunchgrass habitat shown in **Fig. 8**.

Fig. 11: Surveyors should crawl and carefully inspect soils with a hand lens to best observe the biotic crusts.

Fig. 12: On the flat portions of this site just above Highway 84 along the Columbia River (184 m, Gilliam County, Oregon), *Gutierrezia sarothrae* shrubs are common, and biotic crust development is quite limited. Among the basalt rocks, several species of biotic crust survive, including *Texosporium sancti-jacobi*.

Fig. 13: This area at 1364 m in Deschutes County, Oregon, has sandy soils and abundant *Chrysothamnus viscidiflorus*; it supports poor biotic crust habitat except directly under the shrubs

Fig. 14: Poorly drained soil where water pools in winter and spring show shrink-swell cracks. In our experience, these do not support diverse biotic soil crust lichens or bryophytes (1350 m, Deschutes County, Oregon).

Fig. 15: Steep, unstable loose talus slopes typically provide little stable soil habitat for biotic soil crusts (1350 m Crook County, Oregon).

Fig. 16: Properly curated soil crusts lichens should be glued to archival cards with small boxes or pieces of foam to protect them from damage. Herbarium quality labels should document the locality, date, collector and collection number. Notes inside the packet or on the label may include thin layer chromatography (TLC) results, observations of spores or cell structures, and annotations by other scientists. Photos by B. McCune.