SPATIAL HETEROGENEITY IN KEY SOIL CHARACTERISTICS FOLLOWING PIPELINE RECLAMATION IN WYOMING¹

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Abstract: Reclamation efforts across Wyoming have been successful in reestablishing vegetative biomass, but restoration of a diverse vegetative community has proven difficult. We hypothesize that the lack of diverse plant species establishment is due, in part, to the homogenization of the soil environment occurring with disturbance and reclamation. Spatial heterogeneity of vegetation and soil characteristics using geospatial statistics has not been widely examined in wildland systems, particularly in relation to post-disturbance recovery of soils and vegetation. The purpose of this project was to quantify the spatial variability of key soil characteristics on a recently installed and reclaimed pipeline and adjacent undisturbed reference site in south central Wyoming. Soils (0-5 cm) were sampled on a grid design at a small spatial scale (10 - 1000 cm). Soils were analyzed for soil moisture, pH, electrical conductivity, and major microbial group abundance. In general, reclaimed soils support more biomass of vegetation and soil microbes than undisturbed soils. We found weak spatial dependence of most soil properties measured, and the response of the property to disturbance and reclamation varied by property. Spatial variability of soil pH, bacteria abundance, and AM fungi abundance appear to be most affected by soil disturbance and reclamation, but further investigation into the spatial patterns of soil and vegetation properties is warranted.

Additional Key Words: geospatial data, geospatial statistics, spatial variability, phospholipid fatty acid (PLFA) analysis, microbial community, sagebrush steppe

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Introduction

Research conducted on soils subject to surface mining and reclamation in Wyoming has indicated that many soil chemical, physical, and biological components are restored within 15 -30 years following reclamation (Regula, 2007; Wick, 2007; Dangi, 2008; Wick et al., 2009). Reclamation efforts across Wyoming have been successful in reestablishing vegetative biomass (often exceeding that of undisturbed sites); however, plant species diversity is often lower in reclaimed soils compared to reference sites (Bowen et al., 2005; Wick, 2007). The inability of reclaimed areas to support plant community diversity observed on reference sites has important implications for meeting bond release standards determined by the Wyoming Department of Environmental Quality (WYDEQ, 2001). Undisturbed soils exhibit vast spatial heterogeneity in structure and function, which allows for supporting diverse plant communities via resource partitioning and species coexistence (summarized by Ostfeld et al., 1997; Fitter et al., 2000). Disruption of the soil with tillage has been found to have a homogenizing effect on soil nutrients and microorganisms (Robertson et al., 1993; Fraterrigo et al., 2005). We would expect that soil handling associated with soil disturbance and reclamation would have similar effects. Trends in soil and vegetation data previously collected from reclaimed and undisturbed reference areas in Wyoming suggest undisturbed areas have more variability than reclaimed sites as indicated by larger coefficients of variation (calculated as standard deviation divided by the mean), a rough indicator of variability (Table 1, Stahl and Huzurbazar, unpublished data).

We hypothesize that the actions of soil disturbance and reclamation decreases variability of soil properties across space, and a homogenous, low diversity plant community initially accompanies these soil effects. Spatial variability has been perceived as a nuisance in soil ecology research (Ettema and Wardle, 2002). However, it is likely important to assessing recovery of soil structure and function and perhaps an important influence on plant community composition (Palmer et al., 1997). The purpose of this research was to quantify spatial patterns and variability of key soil characteristics in a recently reclaimed soil disturbance and adjacent undisturbed reference site in a Wyoming rangeland. This objective will facilitate further investigations into how these spatial patterns relate to plant community diversity.

Descriptive measures of center and dispersion (means and standard deviations) allow us to compare sample data between treatments. While this approach is useful, the ability to model and

| Soil Property | Reclaimed | | Undisturbed | |
|---------------------------|-----------|---------|-------------|---------|
| | 0-5 cm | 5-15 cm | 0-5 cm | 5-15 cm |
| рН | 3 | 2 | 6 | 4 |
| Electrical Conductivity | 24 | 26 | 41 | 36 |
| Bulk Density | 6 | 9 | 25 | 10 |
| Organic Carbon Content | 59 | 76 | 97 | 79 |
| Total Carbon Content | 35 | 64 | 43 | 79 |
| Total Nitrogen Content | 7 | 35 | 37 | 54 |
| Microbial Biomass Content | 31 | 42 | 58 | 56 |
| AM Fungi Biomass Content | 38 | 58 | 64 | 116 |
| Collembola sp. Numbers | 150 | 91 | 157 | 57 |
| Nematode Numbers | 70 | 63 | 71 | 74 |

Table 1: Coefficients of variation for soil properties at two depths in reclaimed mine soils and undisturbed reference soils. (Stahl & Huzurbazar, unpublished data).

describe the behavior of variability across space provides more information about environmental heterogeneity, which aligns with our objective. Geospatial statistics are useful in addressing our purpose because they provide an analysis framework for quantifying and visualizing spatial relationships and patterns (Cressie, 1993). These methods are often used to determine minimum spacing requirements for meeting sample independence assumptions. However, we can also use geospatial statistics to quantify the behavior of variability of key soil properties across space. Semi-variograms are graphical representations and models of correlation in two-dimensional data (Mulla and McBratney, 2000). Semi-variance is conceptually expressed as (Equation 1):

$$\gamma(h) = \frac{1}{2} \operatorname{Var} [Z(s) - Z(t)]$$
 (1)

where " γ (h)" is the semi-variance of property "Z," at a given lag distance "h," or distance between two datapoints, "s" and "t." The semi-variogram is plotted as the lag distance (x-axis) by semi-variance (y-axis). As lag distance increases, the semi-variance values typically increase, but then plateau, at which point, spatial independence occurs between two datapoints. The semivariance value corresponding to the plateau is denoted as " σ^2 ", the sill, and the lag distance corresponding to the plateau is denoted " ϕ ," the range. A measure of variability at a lag distance of zero is designated as " τ^2 ," the nugget. Collectively, these parameters and the semi-variance model can be combined with a mean model, or surface trend, to describe the patterns of variation (the residuals) across space.

The range (ϕ) parameter of the fitted model indicates the distance at which the given property achieves spatial independence. A small value for the range parameter indicates high heterogeneity (patchiness) in the measured property across space, whereas a large range parameter indicates low heterogeneity across space. The sill (σ^2) parameter is a correlation function, so its magnitude will reflect the magnitude of the measured values. The extent to which the data values vary around the sill value is reflected by the sample standard deviation. The nugget (τ^2) parameter, an indication of measurement error and inherent variability of a property, would change according to observer or instrument error as well as the characteristics of the property being measured. A "pure nugget" model describes a property whose variance lacks spatial dependence, in which case, a mean surface trend (regression) would sufficiently describe the observed property across space. If disturbance and reclamation has a homogenizing effect on a soil property, we would expect to observe a smaller standard deviation, a larger range parameter, and/or perhaps a smaller nugget value in reclaimed soils compared to undisturbed soils for a given property.

Defining the spatial scale of interest is important to sampling design and analysis of geospatial data. Spatial heterogeneity of soil properties has been examined at the fine scale (influenced by aggregation, roots, and organic matter particles), plot scale (influenced by vegetation, burrows, and microtopography), and large scale (influenced by soil texture, topography, and vegetation communities), and these levels of scale are nested (Trangmar et al., 1985; Ettema and Wardle, 2002). Our work has attempted to examine soil spatial variability at the plot and field, scale, because we hope to make inferences about the relationships between soil properties and individual plants and small vegetation patches. Reclamation success is often measured in terms of plant community structure, which we are attempting to tie to belowground patterns. By understanding how belowground patterns influence plant community structure, and vice versa, we may be able to identify ecosystem characteristics that, when manipulated, can enhance reclamation success by promoting plant community diversity.

Materials and Methods

Field Site Description, Sampling Design

The field sampling location for this project was approximately one mile south of Wamsutter, WY (41° 41' 17.11" N, 107° 58' 24.41" W, elevation = 2051.6 m (6731 ft)). This site lies within Wyoming's Red Desert Basin and receives an estimated 180 mm (7 in) of precipitation per year (Western Regional Climate Center, 2009). The Red Desert is dominated by vegetation associated with Big Sagebrush (*Artemisia tridentata*) and Greasewood (*Sarcobatus vermiculatus*).

Two sampling plots were established, one on a site in which a pipeline had been installed and reclaimed in the summer of 2008, and one on an adjacent, undisturbed sagebrush steppe vegetation type. The total distance between the plots was approximately 20 m (66 ft). An equilateral triangular grid (Fig. 1) was established on both the reclaimed pipeline as well as the undisturbed control. The equilateral triangular grid allows for efficiency in determining directional dependency in measured properties (Cressie 1993). Two spatial scales were sampled so that the distance between sample points (the lag) ranged from 10 cm to 1000 cm (10 m) within each plot (Fig. 1). In June, 2009, the top five cm of soil was sampled at each of the grid points using a step probe. Soil was kept frozen until laboratory analysis.

In addition to the soil samples taken from within the grid plot, six soil cores were taken adjacent to the plot to assess soil particle size (0-5 cm), bulk density (0-5 cm), and root biomass (0-15 cm). We also established three one m^2 quadrats adjacent to each plot, wherein vegetation was clipped to the ground, dried at 65°C to a constant weight, and weighed.

Laboratory Analysis Methods

Spatial soil samples were analyzed for gravimetric percent soil moisture of field moist soil. Soil pH was determined using a slurry of 10 g of air-dried soil and 10 mL deionized water (modified from McLean, 1982) measured with an Accumet AP62 pH/mV probe (Fisher Scientific, Pittsburgh, PA). The supernatant of the slurry was analyzed for electrical conductivity (EC) using an Accumet AR50 pH/ion/conductivity meter (Fisher Scientific, Pittsburgh, PA) (Rhoades, 1982). Relative abundance of major microbial groups was measured by phospholipid fatty acid analysis (PLFA) (Bligh and Dyer, 1959) as modified by

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(A) (B)
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Figure 1: Spatial sampling point locations at two scales: (A) small scale sampling grid with 10 cm point spacing in the equilateral triangular grid design; (B) large scale sampling grid with 100 cm (1 m) point spacing in the equilateral triangular grid design. Points with cross hatch indicate soil sample locations (N = 99).

Frostegard et al. (1991) and Buyer et al. (2002). Fatty acid identity and abundance was analyzing using an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) and Sherlock software (MIDI Inc., Newark, DE). Fatty acid signatures were identified to major microbial group as in Fierer et al. (2003) and Allison et al. (2005). The PLFA analysis provides relative abundance of major soil microbial groups including bacteria, arbuscular mycorrhizal (AM) fungi, and saprotrophic fungi. Because lipids rapidly degrade upon cell death, PLFA analysis provides a snapshot of the living soil microbial community and has been shown to be a superior method for detecting treatment effects on soil microbial community structure (Ramsey et al., 2006). Hydrometer mechanical particle size analysis was conducted according to Gee and Bauder (1986) and bulk density was measured according to Blake and Hartge (1986). Root biomass samples were soaked overnight in water, dried at 105 degrees centigrade to a constant weight, and dry roots were elutriated and weighed.

Statistical Analysis

General soil property and vegetative biomass means were compared between reclaimed and undisturbed plots using a Mann-Whitney test (nonparametric comparison of means) with an alpha level of 0.001. Sample semi-variograms were computed and fit with semi-variogram models using restricted maximum likelihood (REML) analysis. The REML method of model fitting utilizes the data and initial estimates of the parameters to maximize a likelihood function, or to represent the "most likely" model for the data (Zimmerman and Zimmerman, 1991). This method is preferred over least squares methods because it minimizes bias in covariance estimation given the sample data (Zimmerman and Zimmerman, 1991). Of the total number of points in the reclaimed plot (88) and the total number of points in the undisturbed plot (97), a minimum of 30 point pairs at a given lag were required to be included in the analysis. This precaution ensures sufficient replication of point pairs within a sample plot (Journel and Huijbregts, 1978). All statistical analysis, parameter estimates, and figure generation were conducted using basic and "geoR" packages in R (The R Project for Statistical Computing, http://www.r-project.org/).

Results

General soil and vegetative biomass properties are presented in Table 2. Bulk density did not differ between reclaimed and undisturbed soils, at least in the top five cm sampled. Soil texture was a loam for both plots sampled, but the reclaimed soils contained 6% less clay than the undisturbed soils. Vegetation structure was extremely different between the two plots, with reclaimed soils having about 60% more root biomass and 84% more leaf and stem biomass than undisturbed soils. The species composition was also different, with reclaimed soils dominated by annual forbs and undisturbed soils dominated by perennial grasses and shrubs (data not included).

reclaimed plots. Different superscripts indicate significantly different values between treatments. **Bulk Density** Sand Silt Clay **Root Biomass** Leaf & Stem $(g \text{ cm}^{-3})$ (kg m^{-3}) Biomass $(g m^{-2})$ % % % 1.14^{A} 45^{A} 24^{A} 31^{A} 12.59^{A} 428.35^{A} Reclaimed

 18^{B}

 45^{A}

 1.22^{A}

Undisturbed

 37^{B}

 7.88^{B}

 70.63^{B}

Mean values for general soil and vegetation properties in undisturbed and Table 2:

Exponential semi-variance models were fit to all measured properties within each treatment. Matern and Gaussian semi-variance models were also fit to the sample semi-variograms, but the exponential models had lower AIC and BIC values (model fit measures, where a lower number indicates better model fit) provided by the software. The exponential semi-variance is calculated as (Equation 2):

$$\gamma(\mathbf{h}) = \sigma^2 \left(1 - \exp\left(-\mathbf{h} / \phi\right)\right) + \tau^2 \mathbf{1}(\mathbf{h} \neq 0) \text{ where } \sigma \ge 0, \phi \ge 0$$
(2)

Semi-variograms and model parameters for each measured property in reclaimed and undisturbed soils are presented in Fig. 2 - 7.



Figure 2: Sample semi-variograms for percent soil moisture in reclaimed and undisturbed plots (distance is in cm). Mean, standard deviation, and semi-variogram parameter values for percent soil moisture in each treatment.



Figure 3: Sample semi-variograms for soil pH in reclaimed and undisturbed plots (distance is in cm). Mean, standard deviation, and semi-variogram parameter values for soil pH in each treatment.



Figure 4: Sample semi-variograms for soil EC (μ S cm⁻¹) in reclaimed and undisturbed plots (distance is in cm). Mean, standard deviation, and semi-variogram parameter values for soil EC in each treatment.



Figure 5: Sample semi-variograms for soil bacteria abundance (nmol fatty acid g⁻¹ soil) in reclaimed and undisturbed plots (distance is in cm). Mean, standard deviation, and semi-variogram parameter values for soil bacteria abundance in each treatment.



Figure 6: Sample semi-variograms for soil AM fungi abundance (nmol fatty acid g⁻¹ soil) in reclaimed and undisturbed plots (distance is in cm). Mean, standard deviation, and semi-variogram parameter values for soil AM fungi abundance in each treatment.



Figure 7: Sample semi-variograms for soil fungi abundance (nmol fatty acid g⁻¹ soil) in reclaimed and undisturbed plots (distance is in cm). Mean, standard deviation, and semi-variogram parameter values for soil fungi abundance in each treatment.

All soil properties measured provided larger mean values of the given property in reclaimed soils than in undisturbed soils. With the exception of soil moisture and soil pH, the standard deviation values were also larger in reclaimed soils—as exhibited by the "spread" of points around the semi-variogram lines.

Reclaimed soils had a slightly larger mean percent of soil moisture than undisturbed soils. The "flat" semi-variograms and large range parameter for each treatment indicate that soil moisture exhibits a very weak correlation in variance across space, at the scale sampled. However, all other soil properties measured demonstrated slight differences in spatial variability between treatments. The semi-variograms for soil pH indicate that reclaimed soils have more homogeneous pH values across space. As with percent soil moisture, the pH in undisturbed soils is weakly spatially dependent, or highly variable across space. Conversely, in reclaimed soils,

the range is well defined at 89 cm. The small sill value and the small nugget value in the reclaimed soils also support low variability in soil pH at the scale sampled. Soil EC semi-variograms demonstrate a similar pattern, with undisturbed soils having constant variability across space compared to reclaimed soils. The EC values in reclaimed soils are much more variable in terms of raw measurement values, but they display stronger spatial correlation as indicated by the smaller range parameter (17 cm).

Soil microbial patterns were also weakly defined, but different between treatments. The sill value associated with bacterial abundance in reclaimed soils is higher due to an increase in abundance in those soils. The values of bacterial abundance in reclaimed soils are more variable, but also spatially dependent at a smaller scale, as indicated by the range parameter (21 cm). Thus, the models here suggest that the bacterial abundance occurs in a more patchy distribution in reclaimed soils than that in undisturbed soils. The AM fungal semi-variogram patterns demonstrate the opposite pattern. Specifically, the smaller range parameter (33 cm) of the AM fungi abundance in undisturbed soils suggests a patchier distribution than in reclaimed soils. While again, the standard deviation of the AM fungi values in the reclaimed soils is higher (along with the abundance), the larger range parameter (141 cm) indicates a larger scale of spatial dependence. Lastly, the saprotrophic fungi demonstrate a "pure nugget" model, or a lack of spatial correlation of variance in both soils. The fit of a no-nugget model and range parameters lower than the smallest lag sampled suggest a lack of spatial dependence with this property.

If we are to ignore the semi-variogram lines that were fit to the sample semi-variogram points, it appears that many of the sample points demonstrate a flat line, with little emphasis of shape. So, regardless that the analysis methods employed were capable of fitting these models to the data, it should be acknowledged that the data alone do not reveal strong patterns in variability across space at the scales examined.

Discussion and Conclusions

Our purpose was to quantify the degree of spatial heterogeneity of key soil properties in a recently reclaimed soil and an adjacent undisturbed reference soil. This study has provided some evidence that soil disturbance and reclamation may have a homogenizing effect on some soil properties. Topsoil handling and re-spreading likely results in a more even distribution of salts,

organic matter, and soil structure across space, as is observed in tilled soils (Fraterrigo et al., 2005). Soil disturbance also breaks up patterns of fungal hyphae growth that are dependent on variable pockets of roots, soil structure, and organic matter (Stahl and Christensen, 1992; Buyer et al., 2002; Allison et al., 2005).

The difference in soil moisture between the two treatments is attributed to soil structural alterations with the actions of soil handling. Specifically, soil disturbance is associated with a modification of aggregate size distribution (Wick et al. 2009) and associated pore spaces, which would presumably influence the evaporative dynamics of soil water. The close proximity of the plots ensures that they were both exposed to the same precipitation regimes, and they were both sampled on the same day. While soil pH and soil EC values were slightly higher in reclaimed soils, the increases are likely not biologically significant. The increase in pH and EC could potentially be a result of topsoil mixing with subsoil material, incorporation of organic debris into the soil, and more even distribution of salts across space.

The undisturbed soils contained less microbial abundance than the reclaimed soils, perhaps due to microbial flush associated with carbon and nutrient release and mineralization with the actions of soil handling (Burke et al., 1989, Franzleubbers, 1999). The increased belowground plant biomass in reclaimed soils may also facilitate enhanced carbon and nitrogen mineralization and microbial biomass production via "the rhizosphere effect" (Hook et al., 1991; Buyer et al., 2002). Reclaimed soils also contained more vegetative biomass, which provides more substrate for microbial growth.

As for the spatial distribution of the microbes, the responses of bacteria and AM fungi distribution are not surprising. Bacteria are probably not mechanically perturbed by soil disturbance as are fungi, so their post-reclamation distribution would reflect substrate distribution. Small pockets of labile organic materials revealed through soil disturbance may be influencing the patchy distribution of bacteria that we observed. Conversely, AM fungal hyphae networks are likely broken with the actions of soil disturbance. Their more homogeneous distribution following disturbance may imitate the more continuous distribution of weedy vegetation and roots as opposed to the patchy distribution of vegetation in the undisturbed soil. We might have expected a similar pattern to emerge from the saprotrophic fungi abundance, but

that was not the case. It is difficult to make any conclusions about the spatial distribution of saprotrophic fungi from this data.

Although we were able to fit semi-variogram models to the sample semi-variograms, and we were able to obtain model parameters and compare them between treatments, we do not feel the trends are strong enough to suggest widespread soil homogenization with disturbance and reclamation. Without more information about the nugget variance, most of the sample semi-variogram points lack distinct shape. Additionally, inclusion of larger lag distances may provide stronger trends in spatial variability in these treatments.

The values for the range parameters observed with this data are much smaller than is typically reported in the literature for these soil properties. Most spatial soil research has been conducted in agricultural lands where the scale of interest is much larger. For instance, nematode groups and soil nutrients in active and abandoned agricultural fields exhibit spatial dependence on a scale of 5 m to >100 m (Robertson et al., 1993; Robertson and Freckman, 1995; Robertson et al., 1997; Ettema et al., 1998; Ettema et al., 2000; Fraterrigo et al., 2005).

Future directions of this project will include the investigation of measurement error (nugget variance) associated with each property, incorporation of a larger sampling scale, more pipelines of different installation dates, and incorporation of vegetation and ground surface characteristics. We hope to identify the scales of relevance for successful vegetation establishment, the development of soil spatial patterns over time, and the feedbacks between plants and soil involved in developing and maintaining spatial variability in soils. Given an understanding of these processes, we hope to deduce management implications that enhance establishment of a diverse plant community on these reclaimed soils.

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