



Research article

Exploring effective sampling design for monitoring soil organic carbon in degraded Tibetan grasslands



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ABSTRACT

The effects of climate change and human activities on grassland degradation and soil carbon stocks have become a focus of both research and policy. However, lack of research on appropriate sampling design prevents accurate assessment of soil carbon stocks and stock changes at community and regional scales. Here, we conducted an intensive survey with 1196 sampling sites over an area of 190 km² of degraded alpine meadow. Compared to lightly degraded meadow, soil organic carbon (SOC) stocks in moderately, heavily and extremely degraded meadow were reduced by 11.0%, 13.5% and 17.9%, respectively. Our field survey sampling design was overly intensive to estimate SOC status with a tolerable uncertainty of 10%. Power analysis showed that the optimal sampling density to achieve the desired accuracy would be 2, 3, 5 and 7 sites per 10 km² for lightly, moderately, heavily and extremely degraded meadows, respectively. If a subsequent paired sampling design with the optimum sample size were performed, assuming stock change rates predicted by experimental and modeling results, we estimate that about 5–10 years would be necessary to detect expected trends in SOC in the top 20 cm soil layer. Our results highlight the utility of conducting preliminary surveys to estimate the appropriate sampling density and avoid wasting resources due to over-sampling, and to estimate the sampling interval required to detect an expected sequestration rate. Future studies will be needed to evaluate spatial and temporal patterns of SOC variability.

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1. Introduction

Monitoring natural resources over space and time is expected to promote a better understanding of ecosystem processes, and to provide information to inform decision making for resource

protection and management (Lark, 2009). Soil organic carbon (SOC) is of particular importance in building soil fertility for sustainable development and in reducing atmospheric CO₂ concentration through carbon (C) sequestration (Lal, 2004). However, due to typically large spatial and small temporal variability relative to SOC stocks, efficient estimation of SOC stocks and their change remains a challenge in monitoring programs (Allen et al., 2010). Research can inform the design of cost-effective sampling schemes to achieve monitoring objectives.

Generally, sampling schemes are based on one of two

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contrasting philosophies of statistical investigation: the design- and the model-based approach (Allen et al., 2010; de Gruijter et al., 2006). The essential difference between the two approaches is whether locations are chosen at random or purposively (Brus and de Gruijter, 2012; de Gruijter et al., 2006). Studies estimating the global or regional mean status of SOC stocks often select design-based sampling, as fewer observations and fewer strong assumptions are required (Brus and de Gruijter, 2012; Lark, 2009). For estimating the mean C stock and monitoring its change, sampling design must consider both space and time dimensions (Brus and de Gruijter, 2011). de Gruijter et al. (2006) distinguished and evaluated several types of space-time designs for monitoring regional trends. None of these designs scored best on both status and trend measures (Allen et al., 2010; Brus and de Gruijter, 2013). The applicability of these designs depends on the aim of soil monitoring. Heim et al. (2009) demonstrated that stratified sampling of parent materials reduced the error of SOC estimates in a forest site. Theoretical considerations and empirical studies indicate that a paired sampling scheme is likely to be the most efficient for estimating change in a soil variable (Heim et al., 2009; Lark, 2009).

The Tibetan plateau is a large C reservoir (Yang et al., 2008). Significant warming and anthropogenic disturbance due to overgrazing have led to widespread grassland degradation on the plateau in the past five decades, which has caused rapid C loss (Chen et al., 2013). On the other hand, ecological restoration programs can significantly promote grassland recovery and increase C sequestration (Wang et al., 2011). Policy makers and environmental managers have a pressing need for detailed information about the status of and change in SOC stocks on the plateau. Unfortunately, existing SOC inventories are characterized by large uncertainties that stem from insufficient sample size or lack of a suitable sampling design (Chang et al., 2014a; Yang et al., 2008).

In this study, we conducted an intensive sampling campaign in two communities on the eastern Tibetan plateau. We investigated the variability of SOC in grasslands at different levels of degradation. Our objectives were to: (1) estimate the optimum sample size to meet a desired SOC stock estimate with an uncertainty of 10%, and (2) estimate the minimum detectable change in C stock with the optimum sample size. We then predicted the time interval between soil inventories for detecting a specific sequestration rate with the desired statistical confidence (95%) and power (0.80). On the basis of this analysis, we recommend a sampling framework for estimating the status of and trend in SOC stocks on the community scale.

2. Materials and methods

2.1. Study area

The study area is located in Tangde and Xiala villages in Zeku County, Qinghai province, China (Fig. 1). It covers roughly 190 km², with elevations ranging between 3400 and 4100 m above sea level. The site is characterized by a continental plateau climate. The mean annual temperature is -2 to 2.3 °C and mean annual precipitation is 460 mm. Under-developed gravel soils are relatively uniform, which are classified as Typic Cryoboroll in the US soil taxonomy. Alpine meadow occupies over 90% of the study area, which is dominated by *Kobresia pygmaea*, *Kobresia humilis* and *Kobresia tibetica*. Grassland degradation has taken place across the study area due to overgrazing in past decades. Degraded alpine meadows have native vegetation coverage of less than 85%, and can be further characterized as lightly degraded (70–85% cover), moderately degraded (50–70% cover), heavily degraded (30–50% cover) or extremely degraded (<30% cover) (Ma et al., 2002). According to an unpublished vegetation survey, lightly, moderately, heavily and

extremely degraded grasslands account for 41.6, 31.4, 13.5 and 13.5% of the study area, respectively.

2.2. Field sampling and analysis

Our intensive sampling survey was stratified by degradation status. A total of 1196 sites were sampled between August–September 2009 and 2010, with 591, 204, 121 and 280 sampling sites for lightly, moderately, heavily and extremely degraded grasslands, respectively. At each site, five 20 cm deep soil cores were taken within a 25 m² sampling plot and bulked to form a composite soil sample. Bulk density was sampled with a 100 cm³ (5.04 cm in diameter) metal core. When rock fragments prevented the insertion of the core in soil, another position within the sampling plot was chosen. Aboveground biomass at each site was measured by clipping in one 0.25 m² quadrat located at the center of each sampling plot. One soil core (8 cm in diameter) was sampled to determine root biomass. Root samples were then carefully washed through a 0.25 mm mesh sieve. All plant tissue was dried at 60 °C until a constant mass was achieved.

Composite soil samples were air-dried, sieved (2 mm mesh), handpicked to remove fine roots, and then ground in a ball mill. SOC concentration was determined by dry combustion analysis with a Shimadzu carbon analyzer (TOC-5000, Shimadzu Corp., Kyoto, Japan). Soil texture was determined by a particle size analyzer (MasterSizer, 2000) after removal of organic matter and calcium carbonates. Samples for bulk density determination were dried at 105 °C for 48 h, and passed through a 2 mm sieve to obtain the fine earth fraction. Soil rock content was measured by dry sieving the stones and measuring the dry weights of stones. For each site, SOC density was determined as the product of SOC concentration, bulk density, and sampling depth, and corrected for rock fragments.

2.3. Statistical analysis

The distribution of SOC densities was tested for normality by the Kolmogorov-Smirnov test. One-way ANOVA was used to compare soil and plant variables of different degradation strata using SPSS version 16.0 (SPSS Inc. Chicago, Illinois, USA). To explore the role of elevation, we additionally analyzed SOC in relation to elevation.

2.3.1. Statistical power for SOC stock estimates

We evaluated the efficacy of the current sampling scheme, i.e. whether the number of sampling sites was sufficient or not. Previous studies have presented detailed descriptions of procedures for power analysis (Allen et al., 2010; Kravchenko and Robertson, 2011). Here we summarize the key points relevant to the power analysis conducted in this study. First, we calculated the individual mean and variance for each degradation stratum. Then, we set a tolerable uncertainty of 10% above and below the sample mean. Once those parameters are specified, the probability (statistical power) to detect a deviation greater than the tolerable uncertainty with 95% confidence (using a two-tailed test) can be calculated for different sample sizes. In our experience, the mean and variance of the light degradation (591 samples) were $\mu = 9.75$ kg C m⁻² and $s^2 = 1.32$ (kg C m⁻²)², respectively. A significant deviation lies outside the interval $\mu \pm 1.96 s$, which corresponds to $\mu < 7.50$ and $\mu > 12.01$. The mean at the limit of tolerance was $\mu + 0.1\mu = 10.73$. For $\mu = 12.01$ the normal deviate is $z_u = (12.01 - 10.73)/s = 1.11$. For $\mu = 7.50$ the normal deviate is $z_l = (7.50 - 10.73)/s = -2.81$. The probability associated with these deviates are $P(z_l) < z_l \approx 0$ and $P(z_u) > z_u = 0.999$. These quantities are summed to yield the statistical power to detect a deviation greater than the tolerable uncertainty with 95% confidence (two-tailed test). Power analysis

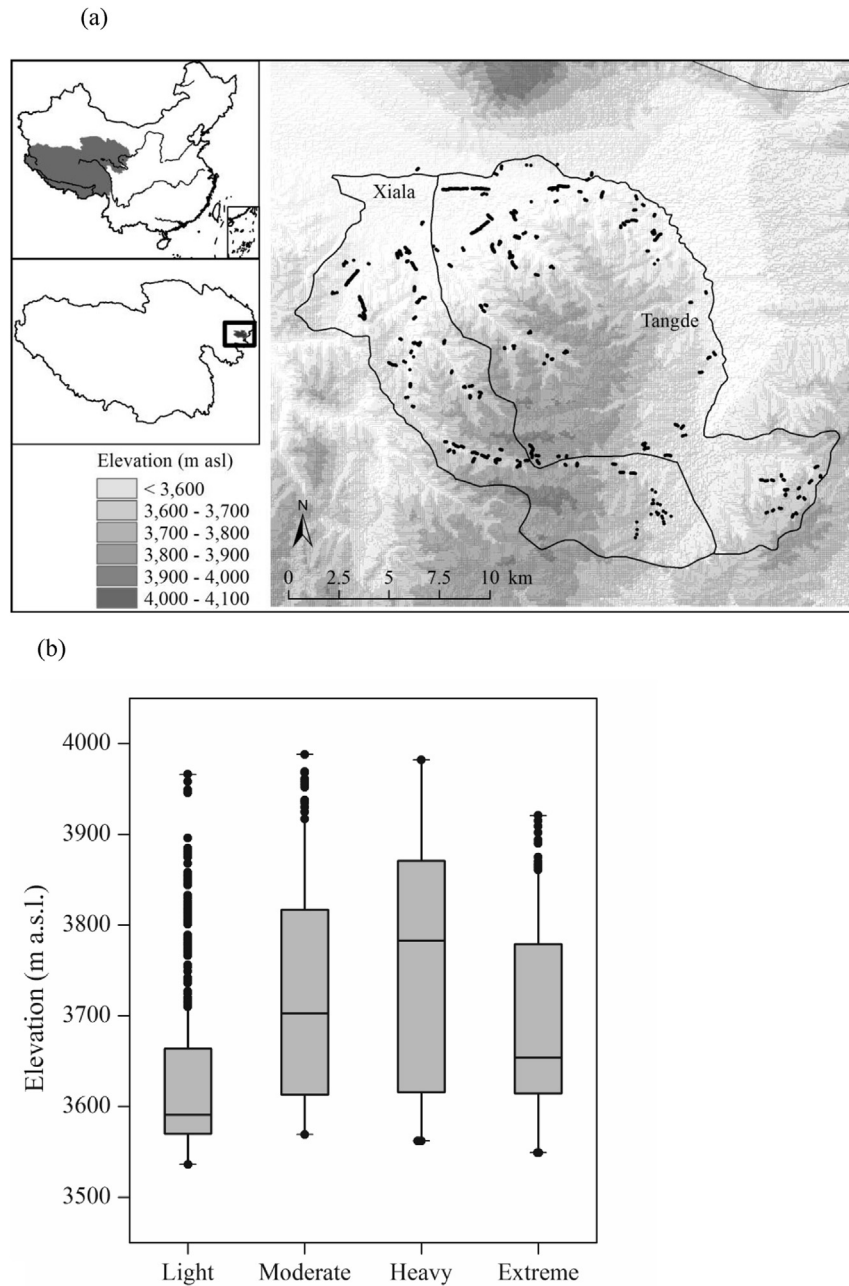


Fig. 1. (a) Location of the study area on the eastern Tibetan plateau and the location of sampling sites. (b) Distribution by elevation (m.a.s.l.) of lightly, moderately, heavily and extremely degraded sites. Boxes have lines at the medians (50th), lower (25th), and upper (75th) quartiles, with the whiskers extended to 1.5 times the interquartile ranges. Outliers are displayed as black dots.

was conducted using the PROC MIXED procedure of SAS software version 9.2 (SAS Institute, 2008).

2.3.2. Estimation of minimum detectable difference

The minimum detectable difference (MDD) represents the smallest detectable difference between the two most different means (Zar, 1999). In the present study, the estimate of the MDD was based on the assumption of a paired sampling design, i.e. a repeated inventory at exactly the same sampling locations as previous samples. In this case, because the future SOC stock change is not known, variance in the estimate of the difference in SOC stocks between paired samples (i.e., baseline and resampling observations at the

same site) was assumed to equal the variability of current (baseline) SOC stocks. The MDD of SOC stock for each degradation stratum was determined for various sample sizes as follows (Zar, 1999):

$$MDD = \sqrt{\frac{s^2}{n}} (t_{\alpha(2),\nu} + t_{\beta(1),\nu})$$

where s^2 is the degradation stratum variance, n is the number of samples, t is the t -statistic at a given significance level (α) and power ($1-\beta$) (using $\alpha = 0.05$ and $\beta = 0.20$), considering a two-sided (2) test with ν degrees of freedom.

3. Results

3.1. Change in plant biomass and soil properties

Plant biomass decreased significantly with increasing degradation level (Table 1). Compared to lightly degraded meadow, aboveground biomass in the moderately, heavily and extremely degraded strata were 18.6%, 39.3% and 37.2% lower, respectively. The decrease in belowground biomass was greater than that of aboveground biomass. Soil texture was significantly different between different degradation strata. Specifically, a higher degree of degradation was associated with increased soil sand content, and with lower clay content. Soil bulk density was lower for lightly and moderately degraded strata than for heavily and extremely degraded strata. Grassland degradation also decreased both SOC concentration and stocks. Although sampling sites varied widely in elevation (Fig. 1b), the spatial distribution of SOC was unrelated to elevation (Fig. 2). Overall, spatial variability was particularly high for plant biomass, while soil properties exhibited a low spatial variability (Table 1).

3.2. Estimation of sample size

In our field survey campaign, the sample sizes for each degradation stratum levels were more than the optimum sizes to obtain the desired accuracy with an uncertainty of 10% in SOC stocks. Power analysis showed that 14, 19, 12 and 17 sites are required for lightly, moderately, heavily and extremely degraded alpine meadows, respectively (Fig. 3). To account for the different proportions of the total study area covered by each degradation stratum, we calculated the sampling density by dividing the required sample size by the area of each degradation stratum. Results indicate that the required sampling density is 2, 3, 5 and 7 sampling sites per 10 km² for lightly, moderately, heavily and extremely degraded alpine meadows, respectively.

3.3. Minimum detectable differences

If using a paired sampling regime is implemented with the current survey and resampling sites at the same locations, MDD values of 0.10, 0.21, 0.19 and 0.18 kg C m⁻² were obtained for lightly, moderately, heavily and extremely degraded strata, respectively, which represent about a 1–3% change in C stock with a statistical power of 0.80 and a 0.05 level of significance. Compared to lightly

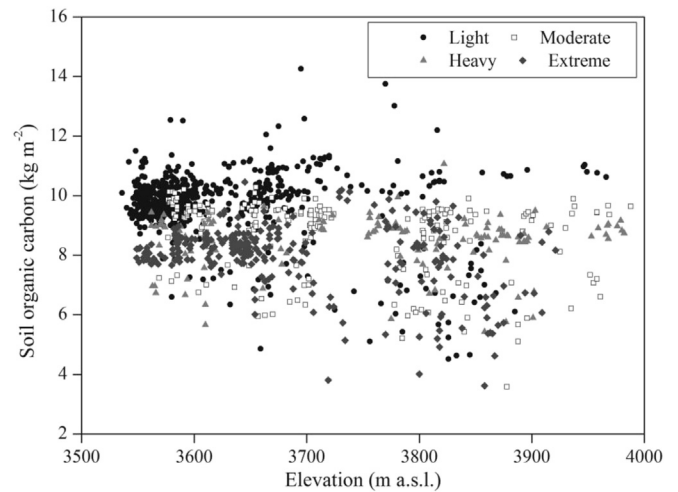


Fig. 2. Variability of SOC stocks with elevation.

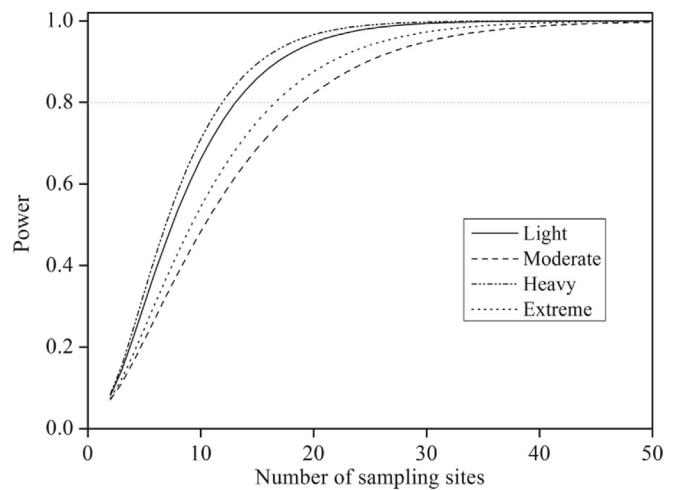


Fig. 3. Statistical power to detect a deviation within 10% of the sample mean of SOC stocks with a 95% confidence (two-tailed test) for grassland at different degrees of degradation. The dotted line represents a statistical power of 0.8.

degraded grassland, the MDD in moderately, heavily and extremely

Table 1
Grassland degradation in the study area, the distribution of sampling sites, plant biomass and soil properties. Values represent means with standard error in parentheses. A different letter indicates that the means are significantly different ($P < 0.05$) between degradation levels.

		Light	Moderate	Heavy	Extreme
Sampling sites		591	204	121	280
Aboveground biomass	Mean (g m ⁻²)	134.8 (75.6) ^a	109.1 (58.1) ^b	81.4 (43.0) ^c	84.2 (43.6) ^c
	CV (%)	56.1	53.3	52.8	51.8
Belowground biomass	Mean (g m ⁻²)	2625.0 (781.7) ^a	1626.5 (205.1) ^b	908.8 (169.6) ^c	423.4 (770.5) ^d
	CV (%)	29.8	12.6	18.7	182.0
Clay	Mean (%)	32.3 (2.3) ^a	29.2 (1.9) ^b	21.2 (2.3) ^c	16.3 (3.9) ^d
	CV (%)	7.1	6.5	10.8	23.9
Silt	Mean (%)	39.9 (4.0) ^c	43.9 (4.8) ^a	41.6 (3.3) ^b	27.3 (6.3) ^d
	CV (%)	10.0	10.9	7.9	23.1
Sand	Mean (%)	27.8 (4.0) ^c	26.8 (5.4) ^c	37.2 (4.3) ^b	56.5 (9.8) ^a
	CV (%)	14.4	20.1	11.6	17.3
Bulk density	Mean (g m ⁻³)	0.88 (0.07) ^b	0.86 (0.10) ^c	0.91 (0.08) ^a	0.91 (0.09) ^a
	CV (%)	8.0	11.6	8.8	9.9
SOC concentration	Mean (g C kg ⁻¹)	55.1 (4.3) ^a	50.3 (3.6) ^b	46.1 (3.9) ^c	44.0 (4.1) ^d
	CV (%)	7.8	7.2	8.5	9.3
SOC stock	Mean (kg C m ⁻²)	9.75 (1.15) ^a	8.68 (1.28) ^b	8.43 (0.94) ^b	8.00 (1.09) ^c
	CV (%)	11.8	14.7	11.2	13.6

degraded strata were almost twice as high, which illustrates that the ability of the paired sampling approach to detect statistically significant change in C stocks in lightly degraded meadows is relatively low. If we assume that the average change in SOC in each degradation stratum is 10% of its initial SOC stock, sample sizes of 10–20 sites per degradation stratum would be required to detect such a change with a paired sampling design (Fig. 4).

4. Discussion

4.1. SOC loss in grassland degradation

The observed decrease in SOC stocks in degraded grasslands was consistent with commonly observed patterns (Huang et al., 2010), even when using equivalent soil mass correction. We found that changes in SOC stock due to soil compaction were negligible (–2 to 3%), as there were only slight differences in bulk density between different degradation strata (Table 1). Therefore, the observed differences in soil C stocks among degradation states appear to be primarily driven by the negative effects of overgrazing. Overgrazing often results in vegetation degradation and consequent soil C loss. Studies have demonstrated that overgrazing reduces graminoids and palatable legumes (Wang et al., 2012), and lowers plant production and consequent C inputs to alpine meadows on the Tibetan plateau (Bagchi and Ritchie, 2010; Wang et al., 2012). In addition, a decrease in vegetation cover is accompanied by an increase in bare ground, thereby accelerating soil erosion (Golluscio et al., 2009; Steffens et al., 2008). Deterioration of soil physical properties with grassland degradation (i.e. coarsening of the soil) can potentially further decrease soil infiltrability and nutrient availability (Golluscio et al., 2009) and reduce grassland capacity to accumulate and store SOC (McSherry and Ritchie, 2013).

4.2. Implications for SOC sampling design

Spatial heterogeneity is an important issue affecting soil C stock inventories. In addition, SOC stock changes are usually expected to be small compared to the size of SOC stocks (Allen et al., 2010). Therefore, an intensive sampling effort is often necessary to attain a meaningful level of precision. Our survey sample sizes were about fifteen times more than the optimum sample sizes required to

detect a deviation of 10% from the sample mean of SOC stocks. The intensive sampling undertaken was sufficient, but is not recommended when the labour and financial costs required for such an inventory are considered. For monitoring programs, besides sample size, choice of sampling design in space and time i.e. deciding where and when to make observations, is a key issue (Brus and de Gruijter, 2011, 2013). Studies have provided evidence that a paired sampling scheme is more efficient than independent random sampling for estimating change in SOC (Lark, 2009). If the optimal sample sizes and locations for measuring baseline SOC status were revisited for resampling, the MDD would be 0.89, 0.85, 0.83 and 0.79 kg C m⁻² for lightly, moderately, heavily and extremely degraded soils, respectively. Wang et al. (2011) estimated that enclosure of degraded grasslands from grazing over 3–28 years leads to an annual average C sequestration of 80.1 g C m⁻² yr⁻¹ in northern China's grassland soils (0–20 cm). About 10 years would be needed to detect such a sequestration rate of this magnitude. Chang et al. (2014b) modelled C stocks and stock changes for degraded grasslands in the same study area as the present study, and predicted annual C sequestration rates of 60–107, 192–200, 188 and 193 g C m⁻² yr⁻¹ in lightly, moderately, heavy and extremely degraded soils, respectively. These expected changes could be detected after 9–15 years for lightly degraded soils, and about 5 years for soils at higher levels of degradation.

When quantifying long-term SOC changes over decades, several sampling rounds would need to be planned. The costs of repeat sampling and analysis can make it difficult to revisit all sites in every round, which might reduce feasibility of the paired sampling design. Based on the results of extensive simulations, Brus and de Gruijter (2013) provide insight into the performance of alternative space-time designs and make recommendations in situations with and without the persistence of strong spatial patterns. However, identifying the persistence of spatial patterns is itself complicated. On the one hand, it may be assumed that since the passing of the revised Grassland Law in 2002, relatively uniform restoration strategies and practices have been adopted and promoted on the Tibetan plateau. Following this assumption, a smaller variance of SOC could occur at resampling, and sampling schemes based on baseline variance alone may underestimate the true ability to detect statistically significant change in C stocks. On the other hand, at community scale, there may be high variability in the adoption of management practices by herder households, which may increase the variance in SOC at resampling. Moreover, SOC stock and SOC stock change are different variables, and their variability may differ (Lark, 2009). So, assuming identical variance of the baseline and resampled data is hazardous. Further research on variability in SOC stock changes over time at the landscape scale is required.

4.3. Limitations and the way forward

It must be emphasized that application of our results to field sampling should be treated with caution. Poor road networks and difficult terrain in the study area constrained the ability of field survey teams to conduct a perfectly random sampling, which led to undersampling in some spatial locations (Fig. 1). So, inference for the sample population is a challenge. Demarcating the geographic area containing the sampled population should be useful to properly extrapolate results from a random sample of sites. Second, unaccounted within-site variance increased uncertainty in the evaluation of SOC (Chappell and Rossel, 2013). Degraded grassland is characterized by areas of greater fertility interspersed with bare, infertile soil, which increases landscape spatial variability. In our study, the sampling support of a 25 m² sampling plot in degraded grasslands most likely only captured a small portion of within-site

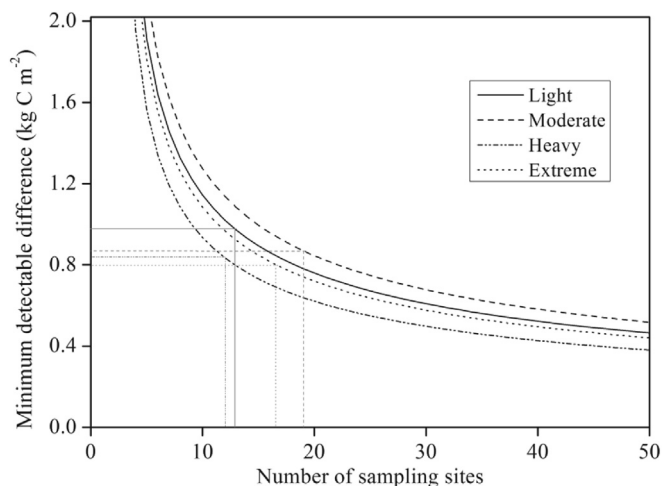


Fig. 4. Relationship between sampling size and minimum detectable difference (MDD) in SOC stocks with a 95% confidence level and a statistical power of 0.80 for grassland at different degrees of degradation using a paired sampling approach. Sample size requirements to detect a 10% change in carbon stocks are plotted by grey lines.

SOC variance, leaving a considerable proportion of SOC variance unaccounted for. This unaccounted site uncertainty would contribute significantly to noise in the explanation of SOC stock change. Exploring the spatial extent of a local support based on adequate information on the variogram of SOC at the small scale would be needed (Chappell and Rossel, 2013; Lark, 2009). This would require an approach such as nested sampling or transect sampling (Liu et al., 2010; Hoffmann et al., 2014). Third, standards and methods for determining degradation status are contested (Harris, 2010). Quantifying degradation through SOC status rather than changes in vegetation provides a direct way to assess the status and trend of soil C (De Baets et al., 2013).

5. Conclusions

Grassland degradation is a geographically extensive phenomenon that has the potential to significantly deplete SOC stocks. Evaluation of SOC stock changes requires exploring an effective sampling design for heterogeneous degraded grasslands that considers sample effort, statistical power and the ability to detect temporal trends in SOC. This study estimated the necessary sample size to obtain a desired level of precision and the soil monitoring interval to detect SOC trends when implementing a paired sampling design on the community scale. Further work is needed to determine the appropriate support area to represent within-site variability in SOC, and to understand variability in stock changes over time at the landscape level.

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