Sampling Intensity Required to Adequately Describe Soil Variations at Three Danish Ecosystems: Heather, Oak and Spruce

Søren M. Kristiansen[A], Maria Knadel[B], Jennifer Rowland[C], Per Nørnberg[A]

[A]Department of Earth Sciences, University of Aarhus, Høgh-Guldbergs Gade 2, DK-8000 Aarhus, Denmark Email smk@geo.au.dk
[B]Department of Agroecology and Environment, University of Aarhus, P.O. Box 50, DK-8830 Tjele, Denmark
[C]Department of Geography, University of Reading, Whiteknights, Reading, United Kingdom

Abstract
The objective of this study was to determine the effect of spatial variability on basic soil properties and the necessary number of samples to describe this adequately in the laboratory. This was done at a Danish heathland site, Hjelm Hede, where all soil forming factors except vegetation are comparable. Surface soil (0-10 cm) underneath Heather (*Calluna Vulgaris*), Oak (*Quercus robur*) and Spruce (*Picea* sp.) were sampled in a simple random sampling procedure to obtain 10 subsamples at each ecosystem. Physical and chemical analyses included bulk density, pH, total organic C, organic N, and measurement by Near Infrared (VIS/NIR) spectrophotometer of organic N and C were included to compare different analytical techniques and the soil variability. Presuming that all samples were derived from normal distributions, 10 samples from each ecosystem yielded an error on the determined means of 30-40 % at a 95% confidence level. For an error on the determined mean of 10 % and a 95% confidence level generally 120 to 170 samples would be needed from each sampling unit. Bulk density and pH required least samples, while chemical determinations of C and N and VIS/NIR predictions of C and N required a comparable number of samples for the same level of precision. The main conclusions for the three ecosystems are that the needed number of samples to describe soil property means depend on the property being examined. A precision of 40 % allowable error of the means obtained by approximately 10 subsamples from each ecosystem is nevertheless considered acceptable when resource allocation for soil sampling is included as a parameter.

Key Words
Soil variability, soil sampling, VIS/NIR Spectroscopy, replicates

Introduction
Variability of soil properties is a well known constrain on proper interpretation of soil test results, and quantification of soil spatial variability across multiple scales is thus highly important in many different aspects of soil science. Especially when information on average soil properties are required soil variability associated with micro-scale heterogeneity can be addressed with spatially sampling designs and appropriate sample volumes or replicated samples (Stein & Ettema, 2003). Numerous previous studies has hence investigated the variability of e.g., soil map units and soil properties at multiple scales, and recently geostatistics has been presented as a useful tool for quantifying soil variation and for interpreting spatial soil patterns (e.g., Beckett & Burrough, 1971; Webster & Oliver, 2001).

Site assessment describing soils at any level always rely on properties which especially are known to vary in space. Especially some soil properties and soil ecosystems require more intensive sampling and often have less predictive value for site assessment purposes, e.g. shallow soils (e.g., Hitz *et al*., 2002). The number of samples required to achieve a desired level of precision for estimation of soil properties within a sampling unit has since the 1980s often been obtained by variograms from geostatistical analysis. However, such approaches designed for soil mapping typically require >100 analysis of a soil property. A more simple practice to assess if a soil mapping unit is good or badly defined is that the within-class variance which should be lower than the total variance (e.g., Webster & Oliver, 2001). Variance of soil properties within a soil unit should hence be smaller than their variability in the landscape at large. Several parameters have been used for estimating the uniformity of soil properties within mapping units (e.g., Beckett & Burrough, 1971), while the number of samples necessary to obtain the mean value of a property within a soil mapping unit with a specified allowable error rarely have been assessed (e.g., Amponsah *et al*., 2000).

The aim of the present study was thus to investigate consequences of soil in-homogeneity on soil sampling intensity by specifically examining differences in variances of soil physical and chemical properties when no information on spatial dependency is known from geostatistics.

Materials and Methods
Study area and soil sampling design
The study area Hjelm Hede in NW Jutland, Denmark (56 ^{O} 24’ N; 8^{O} 54’ E) is a unique site where sampling

from three different vegetation types in the same parent material and climate is possible. The type of vegetation was heather (*Calluna Vulgaris*) since at least 2,000 years. However, over the past 50-70 years unmanaged parts of the area has turned into oak (*Quercus robur*) woodland, and parts have been planted with spruce (*Picea abies or P. sitchensis*). These vegetation shifts in parts of the area has resulted in major changes to the organic layers and soils chemistry (e.g., Madsen & Nørnberg, 1995). Ten representative composite soil samples were randomly collected from each of the three ecosystems at 0- to 10-cm depth, using a 10x10x10 cm sharpened steel box.

Laboratory Methods
Fresh vegetation was removed and soil samples were subsequently air-dried at 50°C. Prior to analysis, all samples were put through a grinding and homogenizing machine to pass <2 mm and split by a rotary divider. Analysis of bulk density, C, N, pH, and Visible Near Infrared spectrophotometry (VIS/NIR) was performed on all samples. The pH was determined with a glass-calomel electrode in 1:2.5 soil:water suspensions (w/w). Total organic C was determined by dry combustion. Total nitrogen was determined by the Kjeldahl method.

VIS/NIR measurements were acquired using a shank based spectrophotometer system (Veris Technologies, USA). The system includes two spectrometers measuring soil reflectance in the VIS/NIR regions (350-1000 and 1100-2200 nm). A calibration process involved the correlation of total organic C and N of the 30 samples with their spectral data, and calibration equations were calculated using the raw spectral data (log 1/R). Calibrations from spectral data were developed using the segmented cross-validation method on centred data. Prior to calibrations soil spectra were pre-processed. To improve the model spectra were reduced to eliminate the noise near the edges of each spectrum, and Savitzky-Golay smoothing averaging algorithms and the first derivative were calculated on spectral data.

Statistical Analysis
Statistical treatment of the 30 subsamples from the three ecosystems included calculations of mean, SD, CV, and maximum and minimum values for each property. To determine the number of samples necessary to obtain the mean value of a property within a specified allowable error and confidence level, an iterative procedure using the calculated CVs of the properties and the number of samples required for allowable error of 10% at 95% confidence level was used (see e.g., Amponsah *et al*., 2000). Due to the low number of samples, data was not transformed to normality prior to analysis, even that this may bias interpretation of the pH which is on a logarithmic scale. The calculation was performed using eq. 1:

Eq. 1: $$N = \frac{CV^2 xt(\frac{\alpha}{2}, n-1)}{AE^2}$$

N is the number of sample units needed to estimate the mean with a specific allowable error and probability, t(α/2,n-1) is the value of the student's t-distribution with n-1 degrees of freedom, CV is the coefficient of variation (%) and AE is the allowable sampling error expressed as a percentage of the mean. Student's t-test was used to reveal significant differences between the three ecosystems.

To test whether the chosen sampling units, i.e. ecosystems, gives more precise statements on soil properties than without them, an inter-class correlation coefficient has been calculated according to Beckett & Burrough (1971) using eq. 2:

Eq. 2: $$RV = \frac{pooled\ within-unit\ \text{var}iance}{total\ \text{var}iance}$$

Where the 'pooled' is intra-sampling unit variance and 'total' is the variance for the total dataset.

Results and Discussion
The analyzed properties from the three ecosystems reflect major changes in topsoil properties after heather was replaces by either oak woodland or spruce plantation since *c.* 50-70 years (Table 1 and 2). Especially the oak trees resulted in significant increases in soil pH relative to the original heathland (Table 1). Bulk densities are significantly different between the three ecosystems with topsoil below oak having the densest surface soil (mean of 0.88 g cm^{-3}) and spruce the least dense (mean of 0.38 g cm^{-3}). This is probably a result of organic layer thickness, which is thickest under spruce and very thin under oak (Madsen & Nørnberg, 1995).

The organic matter properties (C, N, and C/N ratio) underneath the spruce generally is comparable with the original heather vegetation (Table 2), as also found in previous studies on soil chemistry between these two ecosystems (e.g., Mossin *et al*., 2001). Mean content of total organic C increases from oak to heather to spruce as a response of both increasing content of C in A-horizons and thicker organic layers. Nitrogen contents increases in the same way but heather and spruce have comparable C/N ratios (Table 1) reflecting that soil organic matter in the oak ecosystem is more decomposed.

Table 1: Significant differences (Student's t-test) of soil (0-10 cm) under heather, oak and spruce in Denmark (n=10 for all). *, ** * is significant differences at the 0.001, 0.01 and 0.05 significance level respectively; n.s. not significant.**

	Bulk Density	Organic Carbon	Organic Nitrogen	C/N ratio	pH (1:2.5 in H_2O)
Heather vs. spruce$	***	*	*	n.s.	n.s.
Heather vs. oak	**	**	n.s.	***	***
Spruce vs. oak	***	**	**	**	***

Comparison of means and standard deviations of C and N determined by chemical methods and by VIS/NIR are in good agreement (Table 2). This is probably a partial result of the chemical data being used for calibration of the VIS/NIR method. However, underneath oak the VIS/NIR predicted N and C means (and standard deviation) deviates from the measured total organic carbon content probably as a response of the lower absolute contents underneath oak. For the two other ecosystems the VIS/NIR predicted C and N contents had lower standard deviations relative to the measured total organic C.

Table 2. Soil physical and chemical properties and estimated number of sample units to estimate the means with a specific allowable error (in % of the mean) using the calculated coefficients of correlations (CV). Soil samples (0-10 cm) under heather, oak and spruce in Denmark (n=10 for all).

Ecosystem/	Summary statistics			Required no. of samples (95% confidence)			
Parameter	Mean	Standard deviation	CV (%)	mean ± 10%	mean ± 20%	mean ± 30%	mean ± 40%
Oak							
Bulk Dens. (g cm^{-3})	0.88	0.22	25	123	31	14	8
Carbon (g kg^{-1})	45	23	52	153	38	17	10
Predicted C (g kg^{-1})	32	51	160	370	92	41	23
Nitrogen (g kg^{-1})	2.02	0.86	42	141	35	16	9
Predicted N (g kg^{-1})	1.49	1.63	109	243	61	27	15
pH (1:2.5 in H_2O)	4.42	1.28	29	127	32	14	8
Heather							
Bulk Dens. (g cm^{-3})	0.68	0.19	28	125	31	14	8
Carbon (g kg^{-1})	109	53	48	148	37	16	9
Predicted C (g kg^{-1})	116	44	38	136	34	15	9
Nitrogen (g kg^{-1})	3.10	1.34	43	142	36	16	9
Predicted N (g kg^{-1})	3.38	0.71	21	119	30	13	7
pH (1:2.5 in H_2O)	3.98	1.14	29	126	32	14	8
Spruce							
Bulk Dens. (g cm^{-3})	0.30	0.18	59	161	40	18	10
Carbon (g kg^{-1})	267	173	65	170	42	19	11
Predicted C (g kg^{-1})	273	129	47	147	37	16	9
Nitrogen (g kg^{-1})	7.94	5.07	64	169	42	19	11
Predicted N (g kg^{-1})	8.17	3.28	40	139	35	15	9
pH (1:2.5 in H_2O)	3.86	1.11	29	126	32	14	8

The statistical estimate of required number of samples to adequately describe the soil variation with some allowable error and confidence interval showed some general aspects of soil properties in the three ecosystems. In all cases, it was presuming that all samples were derived from normal distributions and a 95 % confidence interval (two-tailed) as this often is considered acceptable in laboratory studies. Within the 95 % confidence interval the 10, 20, 30 and 40 % allowable error are shown in Table 2. For an allowable error on the determined mean of 10 % and a 95% confidence interval generally 120 to 170 subsamples would be needed from each ecosystem. However, if an error on 40 % of the soil property's mean derived from the field sampling was allowed between 7 and 11

subsamples were needed. Only two VIS/NIR predicted contents below oak was above this estimate. The Bulk density generally required the least number of samples for an adequately determination, while both chemical determinations and VIS/NIR predictions of C and N required a comparable number of samples for the same level of allowable error.

Table 3: Relative variance (RV) expressing the within-class correlation between the three sampling units and the total variance, calculated according to Beckett & Burgess (1971). Soil samples (0-10 cm) under heather, oak and spruce in Denmark (n=10 for all).

Relative variance	Spruce	Heather	Oak
Carbon	1.55	0.13	0.03
pH	0.40	0.32	0.56
Bulk density	0.42	0.25	0.18
Nitrogen	1.67	0.13	0.05
Predicted Carbon	0.74	0.05	0.19
Predicted Nitrogen	0.73	0.04	0.24

Table 3 reveals that the heather and oak sampling units are well defined as they have lower within class variance than total variance in all cases (Beckett & Burrough, 1971). The measured C and N contents in the spruce ecosystem have, however, higher variance than the total dataset reflecting that the sampling unit is less well-defined or more spatial variable than the general area. Conclusions on soil sampling intensity under spruce are thus likely less well supported than under oak and heather.

Conclusion

The required soil sampling intensity in the three ecosystems is here evaluated without taking spatial effects into consideration which an initial use of a variogram could have revealed. However, most national guidelines on e.g. soil pollution sampling does not request such an initial geostatitical investigation (Theocharopolus *et al*., 2001). The present study thus yield basic knowledge on the number of field samples needed for an adequately determination within some well-defined sampling units (Danish vegetation types) when no information on soil spatial variation is know in advance.

In order to yield reasonable results with a moderate field precision (here <40 % analytical error at 95 % confidence level) a larger number of replicates (7-11) are required. Considering analytical costs careful bulking of 10 or more soil subsamples after sampling is thus recommendable, and should prior to analysis be followed by representative diving into subsamples suitable for analysis.

References

Amponsah GI, Meyer WL, Murchison HG (2000) Soil sampling size estimates for soils under teak (*Tectona grandis* Linn. F) plantations and natural forests in Ashanti Region, Ghana. *Canadian Journal of Soil Science* **80**, 327-336.

Beckett PHT, Burrough PA (1971) The relation between cost and utility of soil surveys: IV: comparison of the utilities of soil mapds produced by different survey procedures, and at different scales. *Journal of Soil Science* **22**, 465-480.

Hitz C, Egli M, Fitze P (2002) Determination of sampling volumes needed for representative analysis of alpine soils, *Journal of Plant Nutrition and Soil Science* **165**, 326-331.

Madsen HB, Nørnberg P (1995) Mineralogy of four sandy soils developed under heather, oak, spruce and grass in the same fluvioglacial deposit in Denmark, *Geoderma* **64**, 233-256.

Mossin L, Jensen TB, Nørnberg P (2001) Altered Podzolization Resulting from Replacing Heather with Sitka Spruce. *Soil Science Society of America Journal* **65**, 1455-1462.

Stein A, Ettema C, (2003) An overview of spatial sampling procedures and experimental design of spatial studies for ecosystem comparisons. *Agriculture Ecosystems and Environment* **94,** 31–47.

Theocharopoulos SP, Wagner G, Sprengart J, Mohr ME, Desaules A, Muntau H., Christou M, Quevauviller P (2001) European soil sampling guidelines for soil pollution studies. *The science of the total environment* **264**, 51-66.

Webster R, Oliver MA (2001) Geostatistics for enviromental scientists. (John Wiley & Sons, Ltd., England).