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# Variability in soil properties at different spatial scales (1 m–1 km) in a deciduous forest ecosystem ☆

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## Abstract

The purpose of this research was to test the hypothesis that variability in 11 soil properties, related to soil texture and soil C and N, would increase from small (1 m) to large (1 km) spatial scales in a temperate, mixed-hardwood forest ecosystem in east Tennessee, USA. The results were somewhat surprising and indicated that a fundamental assumption in geospatial analysis, namely that variability increases with increasing spatial scale, did not apply for at least five of the 11 soil properties measured over a 0.5-km$^2$ area. Composite mineral soil samples (15 cm deep) were collected at 1, 5, 10, 50, 250, and 500 m distances from a center point along transects in a north, south, east, and westerly direction. A null hypothesis of equal variance at different spatial scales was rejected ($P \leqslant 0.05$) for mineral soil C concentration, silt content, and the C-to-N ratios in particulate organic matter (POM), mineral-associated organic matter (MOM), and whole surface soil. Results from different tests of spatial variation, based on coefficients of variation or a Mantel test, led to similar conclusions about measurement variability and geographic distance for eight of the 11 variables examined. Measurements of mineral soil C and N concentrations, C concentrations in MOM, extractable soil $NH_4$-N, and clay contents were just as variable at smaller scales (1–10 m) as they were at larger scales (50–500 m). On the other hand, measurement variation in mineral soil C-to-N ratios, MOM C-to-N ratios, and the fraction of soil C in POM clearly increased from smaller to larger spatial scales. With the exception of extractable soil $NH_4$-N, measured soil properties in the forest ecosystem could be estimated (with 95% confidence) to within 15% of their true mean with a relatively modest number of sampling points ($n \leqslant 25$). For some variables, scaling up variation from smaller to larger spatial domains within the ecosystem could be relatively easy because small-scale variation may be indicative of variation at larger scales.


*Keywords:* Spatial analysis; Soil C; Soil N; Soil C-to-N ratio; Particulate organic matter; Mineral-associated organic matter

## 1. Introduction

Like many other aspects of nature, soils are characterized by high spatial variation at multiple scales, ranging from point measurements (centimeters or less) to global scales. Other authors (Parkin, 1993; Heuvelink and Webster, 2001; Ettema and Wardle, 2002) have reviewed various aspects of spatial variation in soil properties and processes and soil biota. These reviews convey a general appreciation for the high degree of natural variation that can sometimes hamper or preclude the precise quantification and scaling of soils measurements to the resolution necessary for various analyses. For example, Lin et al. (2005) concluded that there can be substantial variation in soil properties, like depth of A-horizon and pH, over relatively short distances (meters). Other studies, over similar scales (centimeters to meters), indicate that spatial heterogeneity in soil properties affects variability in soil microbial community structure (Franklin and Mills, 2003).

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At even smaller scales (centimeters), there can still be considerable variability in soil measurements, like fungal and bacterial biomass (Morris, 1999), and microbial community structures (Mummey and Stahl, 2003). Microbial communities and activities have been found to be spatially variable even at a millimeter scale (Grundmann and Debouzie, 2000).

A better understanding of spatial variation in soils has both practical and theoretical ramifications. Practically, we need to understand spatial variation in order to more precisely quantify soil properties and processes at all scales. The natural variability associated with a measurement determines how precisely we can estimate its true value with a given set of soil samples. Theoretically, one of the more important and current research problems in landscape ecology involves understanding how to scale up soil properties and processes measured at small domains (e.g., plots) to larger spatial domains (e.g., ecosystems). This becomes increasingly important as we increase reliance on remote sensing technologies to monitor long-term changes in ecosystems. Remote sensing data are usually verified by on-the-ground measurements at scales appropriate to what satellite-based sensors "see" (i.e., meters to kilometers), however the degree of variability present within such scales could have dramatic effects on the measurement strategies applied when verifying such data.

Commonalities of parent material, vegetation, and micro-climate would lead to the common sense conclusion that soil samples collected in close proximity to one another are more similar in their soil properties and processes than those separated by greater distances. Indeed, one of the basic assumptions of geospatial approaches to describe spatial variation in soils is that "near points are more similar to one another on average than ones further apart" (Heuvelink and Webster, 2001). For example, Grigal et al. (1991) found that coefficients of variation for some forest floor and mineral soil properties, like loss on ignition and N concentrations, increased when samples were collected over five spatial scales ranging from 2.5 m to 1000 km. However, spatial variation in soils has not been given sufficient attention to determine if an assumption about greater variation with increasing spatial scale is universally valid and how often this premise might be violated.

The purpose of this research was to test the hypothesis that variability in 11 surface, mineral soil properties would increase from small (1 m) to large (1 km) spatial scales within a single ecosystem/forest stand, in this case a temperate, mixed-hardwood forest in east Tennessee, USA. The soil measurements were: (1) mineral soil C concentration, (2) N concentration, and (3) C-to-N ratio; (4) extractable soil $NH_4$-N; (5) C concentration in particulate organic matter (POM), and (6) mineral-associated organic matter (MOM); (7) fraction of soil C in POM; (8) POM C-to-N ratio; (9) MOM C-to-N ratio; (10) silt, and (11) clay content. The findings were somewhat surprising and indicated that increasing variability with increasing spatial scale, did not apply over a 0.5-$km^2$ area for at least five of the 11 soil properties measured in a deciduous forest ecosystem.

## 2. Methods and materials

### 2.1. Study site and sampling design

The study site was located in a deciduous, mixed-species forest on the Oak Ridge Reservation, near Oak Ridge, TN, USA. Oaks (*Quercus* spp.), maples (*Acer* spp.), yellow poplar (*Liriodendron tulipifera*), and hickory (*Carya* spp.) were commonly occurring canopy trees throughout the study area. Based on maps compiled by the USGS and the USDA Forest Service from remote sensing data,[1] the forest cover at this location is classified as "oak-hickory". Mean annual precipitation on the Oak Ridge Reservation, based on a 50-year record, is 135 cm and mean annual temperature is 14.2 °C (Hanson et al., 2003). The forest soils were primarily loam, with varying amounts of forest floor development based on aspect and slope position, and are predominantly acidic (pH = 4–5). The sampling design involved random selection of a center point (35.921 °N latitude and 84.264 °W longitude) in the forest. Soil sampling sites were then placed at 1, 5, 10, 50, 250, and 500 m distances from the center point along transects that extended in a north, east, south, and westerly direction. At the maximum distance from the center, samples in opposite directions were separated by 1 km. There was ≈80 m difference in elevation between the lowest (244 m) and highest (323 m) sampling sites. The total area defined by the sampling points was ≈0.5 $km^2$ (707 × 707 m).

### 2.2. Soil sampling

Four soil samples (15 cm deep) were collected with a bucket auger in the immediate vicinity of each sampling point after removing forest floor organic matter. In the field, fresh, mineral soil samples were pooled, sieved (6 mm), and thoroughly mixed in a plastic bag to yield a single, homogenous sample from each sampling point. All soil samples were taken on the same day (May 16, 2005) and transported to the laboratory in a cooler. Samples were refrigerated (≈10 °C) prior to sample processing, which commenced within 10 days of the field collections.

### 2.3. Soil measurements

Approximately 25% of each fresh soil sample was air dried (21 °C), in a room equipped with a dehumidifier, to determine the dry mass-to-fresh mass conversion factor. Representative subsamples from the air-dry soils were sieved to remove gravel and rocks and part of the <2 mm portion was ground and homogenized in a ball mill and

[1] http://www.nationalatlas.gov/mld/foresti.html

stored in an air-tight glass bottle prior to elemental analysis.

Extractable $NH_4$-N was determined by shaking 5 g of fresh soil in 25 mL of 2 M potassium chloride for 2 h. The supernatant was cleared by centrifugation, removed using a pipette, and analyzed for $NH_4$-N by digital colorimetry with a Bran + Luebbe AutoAnalyzer III. Blanks were included to correct for trace amounts of N in reagents. Results were expressed as extractable $\mu g\ NH_4\text{-}N\ g^{-1}$ dry soil.

Dry, sieved (<2 mm), soil samples were separated into POM and MOM (Cambardella and Elliott, 1992). Dispersion was accomplished by shaking a 20 g sample overnight in a 100 mL solution of sodium hexametaphosphate ($5\ g\ L^{-1}$). The mixture was wet sieved through a 0.053 mm sieve. POM ($\geqslant$0.053 mm) was recovered by back-washing the sieve, filtration (Whatman 541), and oven drying (70 °C). MOM that passed the 0.053 mm sieve (i.e., silt and clay) was also weighed after oven drying. The POM and MOM part from each soil sample was ground in a ball mill and stored in an airtight glass vial prior to elemental analysis. Soil C in POM (g POM–C $g^{-1}$ soil) or MOM (g MOM–C $g^{-1}$ soil) was calculated by multiplying the dry mass of POM or MOM (g part $g^{-1}$ soil) by the respective C concentration (g C $g^{-1}$ part). The fraction of soil C in POM ($F_p$) was calculated as follows: (g POM–C $g^{-1}$ soil)/(g POM–C $g^{-1}$ soil + g MOM–C $g^{-1}$ soil). The silt and clay content for each soil sample was also determined during the foregoing procedure using methods described by Kettler et al. (2001).

Whole mineral soils, POM, and MOM fractions were analyzed for total C and N concentrations (combustion methods) using a LECO CN-2000 elemental analyzer (LECO Corporation, St. Joseph, MI). The instrument was calibrated using LECO standards traceable to the National Institute of Standards and Technology (NIST), Gaithersburg, MD.

### 2.4. Statistical analysis

There were four soil samples at each corner of six different distances (1, 5, 10, 50, 250, and 500 m) from the forest center point. We first tested for equal variances at different distances (six groups of four samples) using Bartlett's test. This test corresponded to the null hypothesis (H1) of no differences in variance at different spatial scales of soil sampling. Bartlett's test is sensitive to deviations from a normal distribution, so we conducted a second test for associations between variance and spatial scale by examining the nonparametric, Spearman rank correlation ($r$) between coefficients of variation (100 × S.D./mean) and distance from the center point. The latter test corresponded to the null hypothesis (H2) of no association between variance and different spatial scales of soil sampling. A nonparametric test of association was chosen because the functional relationship (i.e., linear versus nonlinear) between variance and sampling distance was unknown.

A Mantel and partial Mantel test were also used to uncover associations between pairs of measurements in consideration of spatial autocorrelation (geographic distance). Although semivariogram analysis is the preferred method for spatial analysis of soil properties (Burrough, 1993), attempts at semivariogram analysis indicated the distribution of sampling points made binning difficult, thus results were unstable, and in most cases they did not reach sill. The Mantel test with geographic distance is, in this case, a replacement for semivariogram analysis because measurements in the current study are still within the range of spatial autocorrelation. The Mantel test is a nonparametric test for simultaneous comparisons between two dissimilarity matrices of same dimension and provides a correlation coefficient ($r_M$) and probability value based on a permutation test (Mantel, 1967; Legendre and Legendre, 1998). It tests the hypothesis of spatial autocorrelation in that soil samples collected in close proximity to one another are more similar than samples that are spatially removed from one another. Two codes were written in *R* (v.2.2.0, ww.r-project.org) for this analysis using functions Mantel and Mantel Partial (vegan package (version 1.7-67, cc.oulu.fi/~jarioksa)) to automate multiple calculations.

Although not directly related to the main hypothesis, principal components analysis (PCA) was also performed to uncover associations between the 11 soil measurements at the scale of the study, without correction for spatial autocorrelation. Principal components were derived from a correlation matrix (Table 1). Variables were transformed to $z$-scores prior to PCA. The final component structure was unrotated and included only principal components with eigenvalues greater than unity. The purpose of the PCA was to determine which soils measurements were related over the 0.5-$km^2$ sampling area. PCA was used to interpret some results with respect to spatial variability.

Table 1
Principal components extracted from a correlation matrix based on measurements of forest soil properties over a 0.5-$km^2$ area in a deciduous forest ecosystem on the Oak Ridge Reservation

| Variable | Principal components | | |
|---|---|---|---|
| | I | II | III |
| Mineral soil C concentration | **+0.90** | +0.31 | −0.24 |
| POM C concentration | **+0.86** | +0.24 | −0.09 |
| MOM C-to-N ratio | **+0.80** | −0.54 | +0.05 |
| Mineral soil C-to-N ratio | **+0.71** | **−0.68** | +0.13 |
| Fraction of whole soil C in POM ($F_p$) | **+0.70** | −0.01 | −0.35 |
| MOM C concentration | **+0.56** | +0.51 | −0.22 |
| Mineral soil N concentration | +0.31 | **+0.86** | −0.30 |
| Initial extractable soil $NH_4$-N | +0.16 | **+0.77** | +0.44 |
| POM C-to-N ratio | +0.55 | **−0.69** | +0.30 |
| Silt content | +0.28 | +0.29 | **+0.85** |
| Clay content | −0.20 | −0.26 | **−0.76** |
| Percent variance explained | 36.6 | 28.8 | 17.6 |

Values in the table are the correlation of each variable with each principal component. Bold values indicate the strongest associations.

Finally, we calculated the sample size required to estimate the mean of each measurement to within 5%, 10%, or 15% of its true value, with 95% confidence, over the 0.5-km$^2$ area represented by the 25 sampling points (including the center point). The sample size ($n$) was calculated using an iterative process described by Mollitor et al. (1980) and the formula

$$n = \frac{t^2 S^2}{d^2},$$

where $t$ is the value of Student's-$t$ for $n-1$ degrees of freedom and $\alpha = 0.05$, $S$ is the standard deviation, $d$ is 5%, 10%, or 15% of the mean, and $n$ is the number of soil samples.

Statistical analysis was performed using StatView® software (SAS Institute, Carry, NC) and GraphPad InStat® (GraphPad Software, San Diego, CA).

## 3. Results

### 3.1. Simple tests of spatial variation

The null hypothesis of equal variance at different spatial scales within the studied ecosystem (i.e., Bartlett's test or H1) was rejected ($P \leqslant 0.05$) for five soil properties: silt content; mineral soil C concentration; and the C-to-N ratios in POM, MOM, and whole mineral soil (Table 2). These five measurements were characterized by significant heterogeneity of variance at different spatial scales of soil sampling. The six remaining measurements (mineral soil N concentration, extractable soil $NH_4$-N, clay content, POM and MOM C concentrations, and $F_p$) did not differ significantly in their variance at spatial scales ranging from 1 to 500 m from the center point.

The null hypothesis of no association between variability and sampling distance (H2) was rejected for three soil properties: mineral soil C-to-N ratio ($r = 0.89$; $P \leqslant 0.05$), MOM C-to-N ratio ($r = 0.94$; $P \leqslant 0.05$), and $F_p$ ($r = 0.89$; $P \leqslant 0.05$). For these three measurements, the Spearman rank correlation coefficients were statistically significant and coefficients of variation increased from smaller to larger spatial scales (Table 2). For eight remaining variables (mineral soil C and N concentrations, extractable soil $NH_4$-N, clay content, silt content, POM and MOM C concentrations, and POM C-to-N ratio), there was no significant association between coefficients of variation and geographic distance. These eight soil properties were just as variable (in some cases more variable) at smaller scales (1–10 m from the center point) than at larger scales (50–500 m from the center point).

### 3.2. Mantel test

Unlike the preceding tests, the Mantel test considers associations between measurements and geographic distance at all distances within the sampling matrix (i.e., overall spatial autocorrelation), and thus is a more comprehensive way of examining spatial variation over the entire study area. With a Type I error rate of 0.05, six of the 11 measurements (i.e., C-to-N ratios in mineral soil, POM, and MOM; silt content; POM C concentrations; and $F_p$) were spatially autocorrelated over the sampling area (Table 2). Three measurements (mineral soil C-to-N ratio, $F_p$, and MOM C-to-N ratios) that were determined to be spatially variable by a simpler test (i.e., H2) were among the six indicated to be spatially autocorrelated by the Mantel test. The Mantel test also indicated that three remaining soil properties (silt content, POM C concentration, and POM C-to-N ratios) were spatially autocorrelated, but the Spearman rank correlation (i.e., test H2) indicated that the measurement variability was unrelated to

Table 2
Coefficients of variation (%) in soil properties based on measurements at varying distances from a center point in a deciduous forest ecosystem on the Oak Ridge Reservation

| Measurement | Distance (m) from center point ($n = 4$) | | | | | | H1 test | H2 test | Mantel test |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 5 | 10 | 50 | 250 | 500 | | | |
| Mineral soil N concentration | 11.1 | 16.5 | 39.0 | 6.6 | 16.9 | 16.4 | ns | ns | ns |
| Extractable soil $NH_4$-N | 33.3 | 12.0 | 54.8 | 24.8 | 20.3 | 64.5 | ns | ns | ns |
| Clay content | 18.8 | 20.1 | 25.0 | 27.8 | 15.4 | 20.6 | ns | ns | ns |
| MOM C concentration | 9.1 | 18.4 | 30.1 | 16.0 | 6.2 | 20.9 | ns | ns | ns |
| POM C concentration | 25.0 | 21.9 | 45.4 | 12.6 | 30.8 | 48.9 | ns | ns | * |
| POM C-to-N ratio | 4.4 | 4.1 | 12.0 | 6.3 | 22.7 | 19.6 | * | ns | * |
| Silt content | 6.2 | 4.8 | 8.8 | 13.0 | 7.8 | 27.0 | * | ns | * |
| Mineral soil C concentration | 9.3 | 15.6 | 28.5 | 8.2 | 12.0 | 35.0 | * | ns | ns |
| $F_p$ | 5.0 | 4.6 | 15.4 | 10.3 | 15.8 | 22.7 | ns | * | * |
| Soil C-to-N ratio | 2.3 | 2.1 | 15.3 | 7.1 | 28.6 | 34.4 | * | * | * |
| MOM C-to-N ratio | 1.2 | 2.7 | 12.4 | 7.3 | 25.9 | 43.7 | * | * | * |

ns: not statistically significant.
H1: tests homogeneity of variance, H2: tests association between variance and distance from center point. The Mantel statistic tests the null hypothesis that the observed relationship between variability and geographic distance over the study area was random.
*$P \leqslant 0.05$.

geographic distance from the center point at the study location.

### 3.3. Principal components analysis

Three principal components, that explained 83% of the variation in the data, were extracted from the correlation matrix based on 11 soil properties (Table 1). The first principal component was related to soil C and accounted for 37% of the common variance, independent of other factors. Component I was correlated with C concentrations in whole mineral soil, POM, and MOM; C-to-N ratios in mineral soil and MOM; and the fraction of soil C in the POM. Principal component II was related to soil N and was correlated with mineral soil N concentrations, initial extractable soil $NH_4$-N, and C-to-N ratios in whole soil and POM. Principal component III was related to soil texture and exhibited a contrasting association with soil silt and clay content. No other soil properties were strongly correlated with PC III.

### 3.4. Partial Mantel test

Results from the partial Mantel test (Table 3), that quantified associations between all pairs of measurements while controlling for the effect of spatial autocorrelation among them, were often similar to patterns revealed by the PCA. For example, consistent with PC I, patterns within the matrix of partial correlations indicated strong associations ($r > 0.70$) between C-to-N ratios at the level of whole soils, POM, and MOM. Consistent with PC II, mineral soil N concentrations were significantly correlated with extractable soil $NH_4$-N. However, no significant partial correlations were found between silt or clay content and other mineral soil properties. This finding was consistent with results from the PCA that indicated soil texture (PC III) was not associated with various measures of mineral soil C or N at the study site.

### 3.5. Minimum required sample size

Calculations presented in Table 4 indicate that soil measurements within the study site, with the exception of extractable soil $NH_4$-N, could be estimated to within 15% of the mean, with 95% confidence, using a relatively small number of sampling points (generally 3–20). Doubling the number of samples would permit estimation of the mean to within 10%. At our location, intensive sampling designs involving $\geqslant 70$ soil samples would be required to estimate the mean of some forest ecosystem soil attributes, including mineral soil C and N concentrations, to within 5% of their true value.

## 4. Discussion

### 4.1. Measurement variability may or may not increase with increasing spatial scale

For about half of the soil properties (including mineral soil C and N concentrations), we cannot reject the hypothesis of no association between measurement variation and spatial scale at distances ranging from 1 to 1000 m (Table 2). In the ecosystem examined, it appears that variations in mineral soil C and N concentrations, extractable soil $NH_4$-N, clay content, and C concentrations in MOM are just as variable at small scales as they are at larger scales. This finding was supported by both (1) the absence of an association between coefficients of variation and geographic distance and (2) a Mantel test that indicated a random arrangement of these five soil properties over the 0.5-$km^2$ study area. There were some discrepancies between the statistical analysis methods, thus the same conclusion may or may not be true for silt content, POM C concentrations, and POM C-to-N ratios where there was a difference between tests based on coefficients of variation (H2) and the Mantel test. However, agreement between the Mantel test and the simpler tests indicated that three of the 11 soil properties

Table 3
Statistically significant ($P \leqslant 0.05$) pairwise, partial Mantel tests

| Variable | *b* | *c* | *d* | *e* | *f* | *g* | *h* | *i* | *j* | *k* |
|---|---|---|---|---|---|---|---|---|---|---|
| (a) Extractable soil $NH_4$-N | 0.27 | ns | ns | ns | ns | ns | ns | ns | 0.15 | ns |
| (b) Soil N concentration | 1.00 | ns | 0.19 | ns | 0.42 | 0.24 | 0.35 | ns | ns | ns |
| (c) Soil C-to-N ratio | | 1.00 | 0.76 | 0.94 | 0.44 | ns | ns | ns | ns | ns |
| (d) POM C-to N ratio | | | 1.00 | 0.64 | 0.28 | ns | ns | ns | ns | ns |
| (e) MOM C-to N ratio | | | | 1.00 | 0.51 | ns | ns | ns | ns | ns |
| (f) Soil C concentration | | | | | 1.00 | 0.66 | 0.31 | 0.36 | ns | ns |
| (g) POM C concentration | | | | | | 1.00 | 0.31 | ns | ns | ns |
| (h) $F_p$ | | | | | | | 1.00 | ns | ns | ns |
| (i) MOM C concentration | | | | | | | | 1.00 | ns | ns |
| (j) Silt content | | | | | | | | | 1.00 | 0.42 |
| (k) Clay content | | | | | | | | | | 1.00 |

"ns" denotes no significant association between variables.

Table 4
Number of sampling points ($n$) required to estimate the mean to within 5%, 10%, or 15% of its true value, with 95% confidence, for various soil properties at a 0.5-km$^2$ site in a deciduous forest ecosystem on the Oak Ridge Reservation

| Variable | Mean | S.D. | $n$ | | |
|---|---|---|---|---|---|
| | | | 5% | 10% | 15% |
| Mineral soil C concentration (%) | 3.03 | 0.651 | 71 | 20 | 10 |
| Mineral soil N concentration (%) | 0.176 | 0.039 | 75 | 21 | 11 |
| Mineral soil C-to-N ratio | 17.5 | 3.15 | 52 | 15 | 8 |
| Extractable soil $NH_4$-N ($\mu g\,N\,g^{-1}$) | 21.6 | 9.64 | 306 | 77 | 37 |
| Silt content (%) | 39.2 | 8.06 | 65 | 19 | 10 |
| Clay content (%) | 23.9 | 6.72 | 123 | 33 | 16 |
| POM C concentration (%) | 3.00 | 1.06 | 192 | 50 | 24 |
| MOM C concentration (%) | 2.32 | 0.438 | 57 | 16 | 9 |
| POM C-to-N ratio | 25.7 | 3.19 | 26 | 8 | 3 |
| MOM C-to-N ratio | 13.3 | 2.83 | 70 | 20 | 10 |
| Fraction of soil C in POM ($F_p$) | 0.420 | 0.053 | 27 | 8 | 3 |

(i.e., C-to-N ratios in whole soils, MOM C-to-N ratios, and $F_p$) clearly met the geospatial assumption of increasing variability with increasing spatial scale (Table 2). The findings indicate that it is perhaps unwarranted to assume spatial variability in soil properties will always increase with increasing spatial scale within a forest ecosystem. Such relationships may be parameter- and/or site-specific. Additional studies are needed to investigate how often such findings might be encountered in other ecosystems.

In general, the Mantel test is probably more sensitive to detecting differences than the two tests that were based on coefficients of variation because, unlike the simpler tests (H1 and H2), the Mantel test uses all possible pairs of measurements for the analysis, while correcting for interdependencies among different variables. The larger number of possible comparisons in the Mantel test results in greater statistical power, coverage of a larger spatial scale, and thus a more reliable test of association between measurement variance and geographic distance. The Mantel test is also unbiased by anything except the measurements themselves, but tests based on the coefficient of variation can be strongly biased by a few outliers. The differences in these tests could also possibly be affected by the nature of the comparisons. Tests of H1 and H2 evaluated variation among six groups at different distances from a center point (1–500 m). The Mantel test considered all pairwise comparisons within the 0.5 km$^2$ study area. Despite limitations of the simpler tests, the test of H2 led to the same conclusions about variability and geographic distance as the Mantel test for eight of the 11 soil properties examined in this study.

### 4.2. Associations between soil properties and soil texture

Both the PCA and the partial Mantel test indicated no associations between soil silt or clay content and concentrations of C or N in surface mineral soil, POM, or MOM. Aside from relatively strong associations between C-to-N ratios in the whole mineral soil, POM, and MOM, the correlations among various soil properties were relatively weak once corrected for spatial autocorrelation (see Table 3). This suggests that spatial patterns in soil texture were not an underlying reason for observed spatial patterns, or the absence of patterns, between variation in the measured soil properties and geographic distance. It appears that within the system studied that other undetermined factors often govern variability in soil properties and that these factors are independent of spatial autocorrelation.

Some spatial variation at the study site may be due to topographic differences (Garten et al., 1994), but this does not explain why coefficients of variation for some measurements (like soil N concentration or clay content) are sometimes equally variable for samples collected at 5 and 50 m from the center point. Moreover, little topographic variation occurs at the smallest spatial scale (1 m) relative to the largest spatial scale. Differences in tree species composition can contribute to spatial variation in soil properties (see e.g., Aubert et al., 2005), but this effect should be negligible over distances of $\leqslant$10 m and would cause samples collected at small scales to be more similar than those collected at larger scales which is contrary to some observed patterns at our study site. Except for mineral soil C-to-N ratios, MOM C-to-N ratios, and the fraction of soil C associated with POM ($F_p$), we must reject the assumption that soils collected in closer proximity to one another are more similar in their soil properties than those collected farther apart at the spatial scale of this study. In such cases, scaling up variation in soil properties from smaller to larger spatial domains in the ecosystem could be relatively easy because small-scale variation may be indicative of variation at larger scales for such properties.

### 4.3. How spatial variation affects estimation of the mean

The current sampling design was not aimed at representing very fine scale variation ($\leqslant$1 m) but rather the trend in soil properties at larger scales that would be representative of a forest ecosystem. Over the entire extent of the sampling domain (0.5 $km^2$), there was relatively little variation in most of the measured forest soil properties (coefficients of variation were often $\leqslant$25%). Due to a high degree of spatial variation, prohibitively large numbers of samples are sometimes required to estimate soil properties to within a small percentage of the mean (e.g. see Mollitor et al., 1980; Garten and Wullschleger, 1999). With the exception of extractable soil $NH_4$-N, the soil properties that were examined could be estimated (with 95% confidence) to within 15% of their true mean with a relatively modest number of soil sampling points ($n \leqslant 25$). Estimation of silt content, mineral soil C and N concentrations, and mineral soil C-to-N ratios at our location to within 10% of mean would require $\approx$20 soil samples. Mollitor et al. (1980) arrived at similar numbers for estimating these same surface soil properties (to within 10% of the mean with 95% confidence) in northeastern flood plain forests. A summary of soil N concentration and soil organic matter (i.e., loss on ignition) data from multiple forest studies by Grigal et al. (1991) indicates that these measurements typically have coefficients of variation $\leqslant$25%, similar to many measurements at our study site. Collectively, this research indicates that only 10–20 composite samples are required at our location for estimation of forest ecosystem mineral soil C and N concentrations, as well as some other surface soil properties (Table 4), to within 15% of the mean at scales ranging from 1 $m^2$ to 0.5 $km^2$. This result is of considerable importance when it comes to assessing soil changes over time and for verification of technologies that might remotely sense soil attributes.

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