

Thirty-year Changes in Mineral Soil C in a Cumberland Plateau Forest as
Influenced by Inorganic-N, Soil Texture, and Topography

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ABSTRACT

Increases in atmospheric C have resulted in concerns about global warming and interest in finding means to sequester atmospheric C through land management strategies. The purpose of this study was to (i) compare changes in mineral soil C after a 30-year interval and (ii) examine the role of inorganic-N, soil texture, and topography in these changes. Soil samples were collected at permanently identified points on the Camp Branch Watershed, a second growth oak forest on the Cumberland Plateau in central Tennessee, in July of 1976 and archived. These points were re-sampled in July of 2006 and both archived and new samples of the 0 to 10 cm increment of the mineral soil were analyzed for C and N using the same procedures. Paired comparisons revealed changes in C and N were distinct to each of the 8 soil series. Comparison of 2006 samples to 1976 samples indicated changes in C concentration ranged from -13.1% to +12.0%. Changes in C mass ranged from -11.3% to +8.3%. Increases in C were most closely associated with increases in the C/total-N ratio. C was positively correlated to exchangeable inorganic-N in 1976 ($r^2 = 0.387$) and 2006 ($r^2 = 0.107$). Regression analysis revealed C increased with increasing azimuth and decreasing elevation in 1976 ($r^2 = 0.140$). C was predicted only by clay content in 2006 ($r^2 = 0.079$) and exhibited a negative relationship. Since topography was no longer a predictor of mineral soil C in 2006, we speculate that changes in forest cover also influenced changes in mineral soil C.

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Chapter 1. INTRODUCTION

Rationale

Rising levels of atmospheric CO₂ are well documented (Keeling et al., 1995; Whorf and Keeling, 1998; Keeling and Whorf, 2005). This rise in atmospheric CO₂ has resulted in concerns about global warming and in finding means to sequester this carbon (C). Changes in above-ground biomass under elevated CO₂ have been less than expected suggesting that the “missing C” has been stored in the soil (Allard et al., 2006). Jastrow et al. (2005) support this claim as they found that experimental CO₂ enrichment in a temperate deciduous forest led to a 24% increase in net primary production (NPP). Of this 24%, 76% was due to fine root production in the 0 to 5 cm depth. However, the ability of evergreen plantations (Johnson et al., 2003; Jackson et al., 2005) and recently established, fast growing forests (Nelson et al., 2000) to sequester C in the soil has been questioned. Aggrading temperate deciduous forests may play a key role in sequestering C in the soil (Gaudinski et al., 2000; Paul et al., 2002; Jia and Akiyama, 2005; Kelly and Mays, 2005).

Terrestrial C cycling is largely controlled by belowground processes (Ryan and Law, 2005). Perhaps most important, soil C has a longer mean residence time (MRT) compared to that contained in the leaf litter and above-ground biomass (Garten et al., 1999; Guo and Gifford, 2002). The ability of forest soils to sequester C remains uncertain due to a lack of long term studies addressing the issue. It is suggested that the use of a paired sampling approach over extended periods of time will offer clarity (Richter et al., 1999; Yanai et al., 2003).

The Camp Branch Experimental Watershed is located in Fall Creek Falls State Park on the Cumberland Plateau in Central Tennessee (35°38' N lat.; 85°18' W long.). The watershed contains 94 ha of second-growth oak forest dominated by scarlet oak (*Quercus coccinea* L.) (Ramseur and Kelly, 1981). This aggrading forest has been largely undisturbed since the park was established in the late 1930s. In a preliminary study, soils across the watershed exhibited an increase in the mean mineral soil C concentration in the 0 to 10 cm soil depth when samples from 1976 and 2002 were compared (Kelly and Mays, 2005). Availability of archived samples from 1976 for re-analysis presents a valuable opportunity to compare soil C values from paired samples

from a variety of sites over an extended period of time (30-years) and to examine factors contributing to soil C sequestration.

The study conducted in 1976 established permanent sample locations at the intersections of a 100 by 100 m grid spanning the watershed. Soil samples were taken at 73 locations and split after oven-drying. A reference sample from each location was placed in storage. In the preliminary study, 11 permanent locations were re-sampled in July of 2002 and analyzed along with the archived samples from the same location (Kelly and Mays, 2005). A 73% increase in mineral soil C from 1976 to 2002 was detected. However, the sub-sampling approach only allowed a detection of change at the watershed level. Based on the provocative results from the 2002 study, the watershed was revisited in July of 2006 and re-sampled at all 73 locations for analysis and comparison with the archived samples.

Objectives and Hypotheses

The purpose of this study was to quantify changes in mineral soil C content and provide information on variation in mineral soil C as influenced by mineral soil inorganic-Nitrogen (N); soil physical and chemical properties; and forest characteristics such as disturbance regime and topography. Mineral soil C was compared between 1976 and 2006 to provide an assessment of the ability of an aggrading deciduous forest to sequester C in the mineral soil. The goal of our research is to aid in defining effective land management strategies that sequester atmospheric C. In order to successfully define these strategies, it is necessary to quantify changes in mineral soil C content and examine changes in mineral soil C on spatial scales with high resolution.

The overall objective of this study was to determine if change in mineral soil C could be detected after a 30-year interval. Differences in mineral soil C concentration and mass between several different soil mapping units and topographical locations were examined in order to provide a specific assessment of mineral soil C storage in the 0 to 10 cm depth on the watershed. Additionally, information is provided on the role of mineral soil N forms in C sequestration. Our underlying hypothesis is that an increase in mineral soil C concentration would be detected in the 0 to 10 cm soil depth. This hypothesis was based on the results of the preliminary study (Kelly and Mays, 2005). Furthermore, others suggest undisturbed conditions favor mineral soil C accumulation

(Jastrow, 1996; Nelson et al., 2000; Six et al., 2000).

Studies of mineral soil C in central and southern Appalachian forests have identified soil moisture and N availability as factors controlling mineral soil C accumulation (Garten, 1993; Trettin et al., 1999). These factors regulate mineral soil C through their effects on decomposition and respiration rates and belowground C allocation. On the watershed, topography is anticipated to have detectable effects on these factors that regulate mineral soil C. As a result, change in mineral soil C and N is also anticipated to be distinct to each soil series.

Xeric sites are characterized by a topographic position that is generally well-drained with significant periods of soil water deficit. These soils are typically found on southern aspects, ridges, and side-slope positions and exhibit a greater proportion of total biomass C allocated belowground (Ramseur and Kelly, 1981). Xeric sites also exhibit less N mineralization (Garten, 1993) suggesting moisture conditions limit organic matter decomposition. Mesic sites typically exhibit sustained decomposition (Trettin et al., 1999), greater N mineralization (Garten, 1993), nitrification, and a greater proportion of total biomass C allocated aboveground (Ramseur and Kelly, 1981). However, NPP of mesic sites has been found to be greater than NPP of xeric sites (Ramseur and Kelly, 1981).

The first objective was to determine if changes in mineral soil C concentration and mass could be detected after a 30-year interval. The second objective was to define the relationship of mineral soil C to mineral soil inorganic-N, soil texture, and topography. Using the same procedures, both the archived and the new samples were analyzed for C, total-N, and exchangeable inorganic-N. Comparisons examined 30-year changes in mineral soil C and N across the soils of the watershed. These comparisons also examined changes for the two sampling years by soil series in order to detect differences in C and N dynamics distinct to each soil series. We hypothesize that: (i) an increase in mineral soil C concentration and mass will be detected; (ii) C will be negatively correlated to inorganic-N; and (iii) C concentration will be predicted by topographic factors.

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Chapter II. LITERATURE REVIEW

Soil and Site Factors

Mineral soil C accumulation is dependent on the rate of inputs minus the rate of decomposition. Organic matter is gradually transferred and stored in the mineral soil until equilibrium or near-equilibrium is reached (Paul et al., 2002). Trettin et al. (1999) suggest equilibrium of soil organic matter is controlled by chemical and physical soil properties, biomass production and allocation, nutrients, and ambient conditions. Sources of soil organic matter inputs include litter, roots and root exudates, and microbial and fungal products. Aber et al. (1985) suggest an understanding of root dynamics is essential to understanding C and N dynamics in forest ecosystems. This claim is supported by Ryan and Law (2005) and Gregory (2006). Mean residence time (MRT) of mineral soil C is dependent upon the extent of protection against decomposition (Paul et al., 2003). Protected C was found to comprise 53% of total soil organic C in an Appalachian hardwood forest (Tan et al., 2004). The rate of decomposition also regulates mineral soil C accumulation. Factors relating to mineral soil C protection and decomposition rate include mineral soil N forms; soil physical and chemical properties; and forest characteristics such as disturbance regime, topography, and species influences.

The disturbance regime of a forest may contribute to mineral soil C accumulation. Nelson et al. (2000) suggest the rate at which C is sequestered in the soil depends on the age of the forest and the time since disturbance. Jastrow (1996) also suggests that soil C increases with time since disturbance. Decomposition at the interface of the Oa layer and the mineral soil was found to be 3 to 5 times greater in clear-cut stands than in un-cut stands (Binkley, 1984). In a comparison of conventional and no-till methods, a lack of disturbance allowed the gradual development of soil microaggregates within macroaggregates (Six et al., 2000) resulting in an accumulation of C protected from decomposition. Particulate organic matter (POM) may also be of great importance to mineral soil C accumulation in forests as it was found to comprise 52% of total organic matter in the A-horizon of an acidic silt loam (John et al., 2005).

It is suggested that soil texture affects C storage through a combination of direct and indirect effects. For example, storage of C was not found to relate directly to increases in clay and silt (Plante et al., 2006). Rather, increases in clay and silt appeared

to affect storage of C through their effects on aggregate formation and other storage mechanisms; and not through their effects on mineral surface area. Olk and Gregorich (2006) concluded that organic matter in silt fractions is more stable than that in clay fractions. They propose this is due to dominance of microbial residues in clay fractions and to dominance of plant aromatics in silt fractions. Plante et al. (2006) argue that direct evidence for the role of texture on C storage is mostly obtained from soil database correlations. Considering the spatial heterogeneity of soils, texture evaluation of individual samples is necessary to obtain the most precision.

Soil texture influences C storage as organic matter can accumulate on the surface of silt and clay size particles (Sollins et al., 2006). According to Paul et al. (2002), soil C accumulation was greatest in loam soils as aggregates are created during drying and wetting cycles. In loams, the cohesive nature of clay particles promotes aggregate formation (Oades, 1993). Additionally, Adu and Oades (1978) suggest aggregates in loams have greater stability as more CO₂ is evolved from clay soils than from loam soils. This is due to a greater disruption of aggregates in clays by drying and wetting cycles. C contained in soil aggregates is protected from decomposition as it is rendered inaccessible (Jastrow, 1996; McInerney and Bolger, 2000). Fungi have been found to have a significant positive effect on aggregate formation through their deposition of polysaccharides which increase the resistance of aggregates to changes due to wetting (Denef et al., 2001). Denef et al. (2001) also found the contribution of bacteria to aggregate formation to be insignificant. It has been shown that bacterial respiration decreases as pH decreases ($r^2 = 0.71$) allowing fungi to dominate (Baath and Anderson, 2003). Martin (1971) suggests that acidity and low base saturation increase the binding effect of polysaccharides on soil particles in aggregate formation.

Species composition may contribute to soil C and N concentrations. For example, hardwood foliage can contain 12% more N than conifer foliage (Augusto et al., 2002) and nitrification rates have been found to be greater under maple stands than under oak stands (Finzi et al., 1998; Templer and Dawson, 2004). Giardina et al. (2001) suggest litter with high N and lignin content decomposes more slowly. However, Kulmatiski et al. (2004) suggests mineral soil C does not vary with species composition in a southern New

England deciduous forest and that land use, texture, topography, and drainage class may be more influential.

In an earlier study on the Camp Branch Watershed, Ramseur and Kelly (1981) found that while total biomass was greater in mesic hardwood communities (332 Mg ha⁻¹) than total biomass in upland oak-mixed hardwood communities (163 Mg ha⁻¹), fine roots (<2 mm) comprised 27% (44.0 Mg ha⁻¹) of total biomass in upland oak-mixed hardwood communities and 12% (39.8 Mg ha⁻¹) of total biomass in mesic hardwood communities. Johnson and Risser (1974) reported that roots comprised 16% of total biomass in a post oak (*Quercus stellata* L.) and blackjack oak (*Quercus marilandica* L.) stand. Davis et al. (2004) found fine root mass to be greatest in low elevation oak and lowest in cove hardwoods.

Roots contribute greatly to soil organic matter and soil N. In a study on the Coweeta Basin in the southern Appalachians, Monk and Day (1985) found that the greatest proportion of biomass N (44%) was contained in roots. They also attributed 41% of net primary production (NPP) and 27% of total biomass to roots. Root turnover has been found to be related to soil N availability. Burton et al. (2000) found that root turnover was greatest on sites with low available soil N in a northern hardwood forest.

Relationships between mycorrhiza diversity and species composition in southern Appalachian forests have been identified (Newman and Reddell, 1988; Walker et al. (2005). Mycorrhiza exert a strong influence on mineral soil C and N through excretion of organic acids and enzymes that accelerate organic matter decomposition. Hyphae of ectomycorrhiza contribute to soil organic matter inputs. Walker et al. (2005) reported mycorrhiza diversity was lower at a drier, high elevation oak forest site compared to a low elevation, mesic cove hardwood forest site. Newman and Reddell (1988) found a strong positive relationship ($r^2 = 0.77$) between the abundance of trees capable of forming vesicular-arbuscular mycorrhiza (VAM) (i.e. *Acer rubrum*) and herb species richness.

Role of Mineral Soil N Forms

Accumulation of C in the mineral soil appears to be linked to N accumulation. Kaye et al. (2003) propose most N in forest soils is held in a stable, rapidly accumulating organic pool not readily accessible to microbial mineralization. According to Paul et al. (2003), C sequestration is dependent on N accumulations in soil organic matter. Resh et

al. (2002) also suggest that increases in soil N lead to increases in soil C. Allard et al. (2006) propose C and N dynamics are strongly linked through soil organic matter decomposition. For example, Berg (2000) has shown that increased N can lower the C/N ratio leading to decreased decomposition of organic matter, faster humus formation and therefore, a lesser amount of CO₂ evolved. Increased N also appears to slow the decay of humus (Yanai et al., 2003). Jastrow (1996) and Kemmitt et al. (2006) suggest that a low C/N ratio is indicative of greater soil C sequestration. In contrast, Trettin et al. (1999) found that increases in soil C in a southern Appalachian forest were associated with a C/N gain of 7.

Decomposition pathways for organic-N include de-polymerization to monomers and separation from organic compounds by mineralization (Schimel and Bennett, 2004). Plants can utilize both monomer and inorganic-N (Schimel and Bennett, 2004). Soil N may also enter nitrification and de-nitrification processes. N can also remain in organic compounds due to resistance to mineralization. This resistance is attributable to C that is not a readily available energy source to microbes such as that with high lignin content (Giardina et al., 2001). Van Miegroet et al. (1990) suggest heterotrophic activity depends on available C energy sources. Readily decomposed hardwood litter components are broken down quickly (Giardina et al., 2001). The resulting components transferred to the mineral soil may be composed mostly of bound humic acids and alkyl compounds resistant to further decomposition (Qualls 2004).

In forest soils, NH₄-N is typically the predominant form of inorganic-N (Malagoli et al., 2000). Factors that contribute to the dominance of NH₄-N include the inhibiting effects of low pH on nitrification bacteria (Kemmitt et al., 2006), binding of NH₄-N to soil particles (Laakso et al., 2000) and the greater mobility of nitrate (NO₃⁻) (Binkley, 1984) contributing to a greater loss by leaching. Also, NH₄-N has been found to become stable by reacting with soil organic matter (Kaye et al., 2003). In eastern hardwood forests that exhibit NH₄-N domination of inorganic-N, no relationship was found between mineralization and fine root turnover (Aber et al., 1985). However, NO₃-N domination of inorganic-N results in increases in root substrate quality (Hendricks et al., 2000) suggesting greater decomposition rates.

Due to the volatile nature of N, treatment of soil samples prior to chemical

analysis must be considered. Samples are typically homogenized by grinding and sieving. Drying is also used to prepare samples for storage until chemical analysis can be performed. Binkley and Hart (1989) suggest exchangeable inorganic-N pools are increased by air-drying and grinding. N does not change further after air-drying and storage for up to one year (Binkley and Hart, 1989). Although air-drying increases N mineralization compared to field-moist soils, values for air-dried soils were highly correlated ($r^2 = 0.80$) with those of field-moist soils (Binkley and Hart, 1989).

Sample preparation effects on mineral soil N are dependent on soil type (Ma et al., 2005) and sampling depth (Gilliam and Richter, 1985). In a comparison of field-moist and air-dried clayey soils, increases in acid extractable inorganic-N were greatest in layers with the highest concentrations of organic matter (0-5 cm depth) (Gilliam and Richter, 1985). Air-drying increased extractable $\text{NH}_4\text{-N}$ by 100% and $\text{NO}_3\text{-N}$ by 700% in the 0 to 10 cm depth. Gilliam and Richter (1985) propose air-drying breaks H^+ bonds in humic substances exposing previously inaccessible organic surfaces and results in acidification of clay minerals. In a study by Ma et al. (2005) comparing samples of arable clayey soils (0-20 cm depth) kept field-moist; air-dried in a greenhouse; and oven-dried at 20 °C and 40 °C; samples air-dried in a greenhouse (30-35 °C) exhibited the largest increase (300-4,000%) in salt extractable $\text{NH}_4\text{-N}$. Samples oven-dried at 20 °C and 40 °C exhibited similar increases of 150-2000% and 160-4000%. The wide range in percent increase was due to time of sampling in this cropped soil. The increase in $\text{NO}_3\text{-N}$ was much less dramatic at around 100-150% for all drying treatments. In a sandy soil, salt extractable $\text{NH}_4\text{-N}$ increased 300% as drying temperature increased from 20 °C to 105 °C (Van Erp et al., 2001). This increase in drying temperature did not significantly increase extractable $\text{NO}_3\text{-N}$.

Central and Southern Appalachian Forests

Studies of mineral soil C in central and southern Appalachian forests have identified soil moisture and N availability as factors controlling mineral soil C accumulation (Garten, 1993; Trettin et al., 1999). These factors affect mineral soil C through their effects on decomposition rates and belowground C allocation.

Ridge soils in a southern Appalachian forest exhibited periods of drought slowing organic matter decomposition (Trettin et al., 1999). Sustained decomposition was

identified in cove soils due to consistently high soil moisture (Trettin et al., 1999). In a study of the 0 to 15 cm soil depth in an elevation gradient of southern Appalachian forest communities, fine root mass was greatest in low elevation oak and lowest in cove hardwoods (Davis et al., 2004). Fine root length was greatest on xeric ridges. Variation in these root properties and belowground C allocation was attributable to topographic position and not elevation. This suggests soil moisture conditions affected belowground C allocation. Variation in soil moisture conditions due to topography may also influence soil respiration rates. In an oak-hickory forest in Ohio, soil respiration was related to topographic position (McCarthy and Brown, 2006).

Aspect can influence mineral soil C storage as southwest slopes in a Cumberland Plateau forest have been shown to contain 25% less C than northeast slopes (Thompson and Kolka, 2005). Northeast slopes in a southern Appalachian forest have been found to contain 60% more C mass in the A-horizon (Miller et al., 2004). Aspect most likely influences mineral soil C through effects on soil temperature and moisture which in turn influence organic matter decomposition.

Storage of C in the mineral soil may also be the result of the influence of topography on soil N. N can vary with topographic position as the surface mineral soil (0 - 7 cm) of valley bottoms has been found to contain double the amount of extractable NH_4^+ compared to slopes and ridges (Garten, 1993). On N deficient slopes and ridges, N immobilization may be favored over nitrification (Garten, 1993).

Physical protection against decomposition may also be important to mineral soil C accumulation in Appalachian forests as Garten et al. (1999) found that 71% to 83% of mineral soil C was protected.

Temperate Deciduous and Plantation Forests

Research conducted in temperate deciduous and plantation forests in the United States, Australia, Canada, Europe and Japan has reached similar conclusions as to factors affecting mineral soil C accumulation. Zushi (2006) suggests topographical characteristics that influence the soil moisture regime affect mineral soil C and N storage in a Japanese cedar (*Cryptomeria japonica*, D. Don) plantation. Jia and Akiyama (2005) also propose soil C storage varies with topography in a Japanese temperate deciduous forest.

In a study of a second growth temperate deciduous forest in Japan, Yanagisawa and Fujita (1999) concluded that variation in rooting patterns resulted from differences in soil moisture between topographic positions. Furthermore, upper and crest slope positions experienced frequent severe droughts independent of rainfall suggesting drainage to be the main control on soil moisture. In another Japanese temperate deciduous forest, soil N availability was found to decrease up-slope (Tateno et al., 2004). This suggests less N mineralization on up-slope positions and hence, less organic matter decomposition. Furthermore, N availability was negatively correlated to belowground/aboveground NPP. As N availability decreased, belowground NPP increased. As a result, up-slope sites exhibited less organic matter decomposition and greater belowground fine root production. These results suggest up-slope sites will accumulate more mineral soil C.

Under experimental drought conditions in a Norway spruce (*Picea abies*, L.) plantation in Germany, a 23% decline in soil CO₂ emissions was observed (Borken et al., 1999). In a similar study at the Harvard Forest, drought reduced soil respiration by 70 to 85% through effects on temperature and moisture (Borken et al., 2006). Another study at the Harvard Forest supported the conclusion that drought reduces soil respiration through effects on soil moisture (Davidson et al., 1998). In a study of a balsam fir (*Abies balsamea*, L.) forest in Canada, soil respiration declined 25 to 50% in response to modest water stress with most of this decline attributable to root respiration (Lavigne et al., 2004).

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Chapter III. THIRTY YEAR CHANGES IN MINERAL SOIL C AS INFLUENCED BY INORGANIC-N, SOIL TEXTURE, AND TOPOGRAPHY

Abstract

Increases in atmospheric C have resulted in concerns about global warming and interest in finding means to sequester atmospheric C through land management strategies. The purpose of this study was to (i) compare changes in mineral soil C after a 30-year interval and (ii) examine the role of inorganic-N, soil texture, and topography in these changes. Soil samples were collected at permanently identified points on the Camp Branch Watershed, a second growth oak forest on the Cumberland Plateau in central Tennessee, in July of 1976 and archived. These points were re-sampled in July of 2006 and both archived and new samples of the 0 to 10 cm increment of the mineral soil were analyzed for C and N using the same procedures. Paired comparisons revealed changes in C and N were distinct to each of the 8 soil series. Comparison of 2006 samples to 1976 samples indicated changes in C concentration ranged from -13.1% to +12.0%. Changes in C mass ranged from -11.3% to +8.3%. Increases in C were most closely associated with increases in the C/total-N ratio. C was positively correlated to exchangeable inorganic-N in 1976 ($r^2 = 0.387$) and 2006 ($r^2 = 0.107$). Regression analysis revealed C increased with increasing azimuth and decreasing elevation in 1976 ($r^2 = 0.140$). C was predicted only by clay content in 2006 ($r^2 = 0.079$) and exhibited a negative relationship. Since topography was no longer a predictor of mineral soil C in 2006, we speculate that changes in forest cover also influenced changes in mineral soil C.

Introduction

Aggrading deciduous forests are numerous throughout the southeastern United States as land used for agriculture has been abandoned. The extent of these aggrading forests provides tremendous potential for atmospheric C sequestration. However, the ability of these forests to sequester atmospheric C remains uncertain. In order to accurately assign C sequestration credits for aggrading deciduous forests, changes in mineral soil C pools must be quantified. Quantifying changes in these pools requires calculation of mineral soil C content expressed as mass C. Providing an assessment of the mineral soil C sequestration potential of an aggrading deciduous forest requires

measurement of mineral soil C over an extended period of time and the use of paired samples (Richter et al., 1999; Yanai et al., 2003).

Studies of mineral soil C in central and southern Appalachian forests have identified soil moisture and N availability as factors controlling mineral soil C accumulation (Garten, 1993; Trettin et al., 1999). These factors regulate mineral soil C through their effects on decomposition and respiration rates and belowground C allocation. On the watershed, topography is anticipated to have detectable effects on these factors that regulate mineral soil C. As a result, change in mineral soil C and N is also anticipated to be distinct to each soil series.

Xeric sites are characterized by topographic position that is generally well drained with significant periods of soil water deficit. These soils are typically found on southern aspects, ridges, and side-slope positions and exhibit a greater proportion of total biomass C allocated belowground (Ramseur and Kelly, 1981). Xeric sites also exhibit less N mineralization (Garten, 1993) suggesting moisture conditions limit organic matter decomposition. Mesic sites typically exhibit sustained decomposition (Trettin et al., 1999), greater N mineralization (Garten, 1993), nitrification, and a greater proportion of total biomass C allocated aboveground (Ramseur and Kelly, 1981). However, NPP of mesic sites has been found to be greater than NPP of xeric sites (Ramseur and Kelly, 1981).

Belowground biomass is a key contributor to soil organic matter and varies with community species composition. In an earlier study on the Camp Branch Watershed, Ramseur and Kelly (1981) found that while total biomass was greater in mesic hardwood communities (332 Mg ha^{-1}) than in upland oak-mixed hardwood communities (163 Mg ha^{-1}), fine roots ($<2 \text{ mm}$) comprised 27% (44.0 Mg ha^{-1}) of total biomass in upland oak-mixed hardwood communities and 12% (39.8 Mg ha^{-1}) of total biomass in mesic hardwood communities.

The purpose of this study was to quantify changes in mineral soil C content and provide information on variation in mineral soil C as defined by mineral soil inorganic-N; soil physical and chemical properties; and forest characteristics such as disturbance regime and topography. The goal of our research is to aid in defining effective land management strategies that sequester atmospheric C. The first objective was to

determine if changes in mineral soil C concentration and mass could be detected after a 30-year interval. The second objective was to define the relationship of mineral soil C to mineral soil inorganic-N, soil texture, and topography. Using the same procedures, both the archived and the new samples were analyzed for C, total-N, organic-N, and inorganic-N. Comparisons examined 30-year changes in mineral soil C and N across the soils of the watershed. These comparisons also examined changes for the two sampling years by soil series in order to detect differences in C and N dynamics distinct to each soil series. We hypothesize that: (i) an increase in mineral soil C concentration and mass will be detected; (ii) C will be negatively correlated to inorganic-N; and (iii) C concentration will be predicted by topographic factors.

Site Description

The Camp Branch Experimental Watershed is located in Fall Creek Falls State Park on the Cumberland Plateau in Central Tennessee (35°38' N lat.; 85°18' W long.). The watershed contains 94 ha of second-growth oak forest dominated by scarlet oak (*Quercus coccinea* L.) (Ramseur and Kelly, 1981). This aggrading forest has been largely undisturbed since the park was established in the late 1930s. Prior to park establishment, the site was primarily subjected to woodland grazing with a small area under row-crops. Elevation ranges from 516 m to 598 m with a mean of 552 m. Slopes range from a minimum of 0.01% to a maximum of 50.4%. An earlier study of the soils of the Camp Branch Watershed (Mays et al., 1991) identified Ultisols, Inceptisols, and an Entisol as the dominant soil orders (Table 1). These soils are acidic, having a pH between 3.8 and 4.8 and are well-drained with the exception of the Entisol.

A species gradient is found on the watershed as described by Kelly (1979) and Mays et al. (1991). In general, mesic hardwood communities are found in coves with upland oak-mixed hardwood communities covering the rest of the watershed. An exception to this is a small area dominated by Virginia pine (*Pinus virginiana* Mill.).

Table 1. Soil series, classification, dominant forest cover type, and topographic position for each of the eight soils sampled in 1976 and 2006 on the Camp Branch Watershed.

Soil Series	Classification	Forest Cover	Topographic Position
Atkins	fine-loamy, mixed, mesic Typic Fluvaquents	mesic mixed hardwoods	concave drainageways
Clifty taxadjunct	fine-silty, mixed, mesic Fluventic Dystrochrepts	mesic mixed hardwoods	upland drainageways
Wallen/Ramsey	loamy-skeletal, siliceous, mesic Typic Dystrochrepts - loamy, siliceous, mesic Lithic Dystrochrepts	xeric upland oak - <i>Kalmia latifolia</i> L. understory	steep side slopes
Beersheba	fine-loamy, siliceous, mesic Typic Hapludults	oak-hickory	gentle slopes, narrow ridges, and benches
Lily	fine-loamy, siliceous, mesic Typic Hapludults	upland oak-mixed hardwoods	gentle slopes, narrow ridges, and benches
Sequoia taxadjunct	clayey, mixed, mesic Typic Hapludults	upland oak-mixed hardwoods	gently sloping uplands
Clarkrange	fine-silty, siliceous, mesic Typic Fragiudults	upland oak-mixed hardwoods	broad, smooth plateaus and hilltops
Clarkrange taxadjunct	fine-loamy, siliceous, mesic Typic Fragiudults	upland oak-mixed hardwoods	broad, smooth plateaus and hilltops

Methods

Field Sampling and Processing

The study conducted in 1976 (Kelly, 1979) established permanent sample locations at the intersections of a 100 by 100 m grid. Sample locations were marked with a metal stake. Soil samples were taken at 73 locations and split after oven-drying at 105 °C. A reference sample was taken from each sample location and sealed in a glass jar. The glass jars were stored in an unheated building subject to fluctuations in temperature. Since a preliminary study by Kelly and Mays (2005) detected a significant increase in mineral soil C in the 0 to 10 cm depth in a comparison of 1976 and 2002 samples, the watershed was revisited in July of 2006 and re-sampled at the 73 permanent locations. Two samples of the mineral soil were taken at each location in the 0 to 10 cm depth; one for chemical and particle size analysis and the other for determination of bulk density. Depth of the A-horizon was determined after excavating a small pit to 10 cm depth.

In July of 2006, samples of the mineral soil were taken at 0 to 10 cm in depth for chemical analysis. Samples were placed in plastic bags until arrival at the laboratory when they were transferred to paper bags and air-dried in a greenhouse for approximately six days. Samples were then ground to pass a 2 mm sieve. Each sample was then split with half of the sample sealed in a plastic jar after air-drying and the other half sealed in a plastic jar after oven-drying at 105 °C for 48 hours. The purpose of this treatment was to provide a means to estimate the amount of C and N that might have been lost or changes in the form of N brought about by oven-drying in 1976.

Bulk density samples were collected in 2006 by widening the auger hole of the sample to be used for chemical analysis. A 4.7 d x 5.2 h cm core was driven horizontally into the profile approximately midway in the 0 to 10 cm depth. The sample was placed in a tin for transport and oven-dried at 105 °C for 48 hours.

Chemical and Physical Analysis

Each of the 73 air- and oven-dried samples from 2006 and the 65 oven-dried archived samples from 1976 (the number of samples from 1976 was reduced due to empty and missing sample jars) were analyzed in duplicate for total C and N concentration using a Vario MAX CNS analyzer (Elementar, Hanau, Germany). Additionally, 7 samples from 2002 were re-analyzed for C for comparison with the same

sample subset from 1976. This was performed to compare results of our chemical analysis with results obtained by Kelly and Mays (2005).

Since air- and oven-dried samples from 2006 represented one sample that was split, we felt it was valid to compare exchangeable inorganic-N among drying treatments. Determination of exchangeable inorganic-N was performed by extraction with 2M KCL using the method described by Bremner (1965). Exchangeable inorganic-N was determined for each of the 73 air- and oven-dried samples. Exchangeable inorganic-N was also determined for the 65 samples from 1976 to be used in regression analyses. Extracts were analyzed in duplicate for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentration using a TRAACS 2000 analytical console (Bran & Luebbe, Norderstedt, Germany) and corrected for moisture content. Soil texture for the 0 to 10 cm increment of the mineral soil was determined using the hydrometer method adapted from Bouyoucos (1936), Kilmer and Alexander (1949), and Day (1953).

Forest Cover Plots: Measurement and Analysis

Twenty-four 10 by 20 m plots were established as part of the study in 1976 (Kelly, 1979). Species composition and diameter measurements were taken in these plots to determine species composition, basal area, relative basal area, density, relative density, importance value, and dominance. These plots were re-measured in August of 2006. Species and dbh for each woody plant with $\text{dbh} \geq 2.5$ cm was recorded. Data were used to determine changes in species composition, basal area, relative basal area, density, relative density, importance value, and dominance for each of the 24 plots over the 30-year interval. Since plots were established under an earlier soil map of the watershed, we were unable to relate changes in the plots to changes in soil C and N. Instead, re-measurement of the plots was conducted to provide an estimate of changes in forest cover.

GIS Mapping

In order to obtain topographic factors to be used as predictors of mineral soil C in regression analyses, data were collected to create a map of the watershed in ArcGIS v.9.2 (ESRI Inc., Redlands, CA, USA). Location of the 73 permanent soil sampling locations was recorded in UTM coordinates using Trimble GeoXT GPS (Trimble Navigation Ltd., Sunnyvale, CA, USA) to an accuracy of 3 m in November of 2006. Digital correction of

the coordinates was performed using TerraSync v.2.52 (Trimble Navigation Ltd., Sunnyvale, CA, USA). A digital elevation model (DEM) for the watershed was obtained from the U.S. Geological Survey (EROS, Sioux Falls, SD, USA) to be used to generate these topographic factors for each sampling location: elevation, azimuth, slope, plan curvature, profile curvature, and flow accumulation.

Statistical Analysis

Since assumptions of normality in mean distribution were not met, the bootstrap method was used to calculate mean difference and 95% confidence intervals. At each step of the bootstrap procedure, a random sample with replacement of 65 points for the 1976 oven-dried samples and 73 points for the 2006 oven-dried samples (same as sample size) was chosen to calculate a sample mean for mineral soil C, total-N, exchangeable inorganic-N (2006 samples only), and the C/total-N ratio using SAS v.9.1 (SAS Institute Inc., Cary, NC, USA). This procedure was also performed on C and N values of the air-dried samples. For watershed level comparisons, 10,000 sample means were generated to increase the sample size of each year to 10,000. For soil series comparisons, 10,000 sample means were generated for each of the two sampling years for each soil series. Confidence intervals were established by eliminating the lowest 2.5% and the highest 2.5% of the sample means. Mean difference was significant if the confidence interval values were either strictly positive or strictly negative. Mean difference and 95% confidence intervals were determined for C, total-N, and the C/total-N ratio between 1976 and 2006 across all samples on the watershed. Also, these comparisons examined changes between years by soil series to determine if changes across the watershed were attributable to individual soil series. The bootstrap method was also used to calculate mean difference and 95% confidence interval for mineral soil C between 1976 and 2002 for comparison with the results of Kelly and Mays (2005). This method was also used to test for differences in C and N among air- and oven-dried samples.

Mineral soil C and N content in kg ha^{-1} for each sampling location was calculated as mineral soil C concentration (g kg^{-1}) * bulk density (g cm^{-3}) * $1 \times 10^8 \text{ cm}^3 \text{ ha}^{-1}$ * $1/1,000 \text{ kg g}^{-1}$ * $1/1,000 \text{ kg g}^{-1}$ * sampling depth (10 cm). Bulk density determined in 2006 was used for calculation of 2006 mineral soil C and N content. Bulk density values from the 1980's, where available, (Mays et al., 1991) were used to calculate C and N content for

1976 to estimate possible changes in C and N due to changes in bulk density. Statistical analysis of mineral soil C and N content was conducted with the same method used to compare mineral soil C concentration.

For the 1976 and 2006 oven-dried samples, the relationship of mineral soil C concentration to exchangeable inorganic-N concentration (Model 1) was examined using regression analyses (PROC REG, SAS v.9.1, SAS Institute Inc., Cary, NC, USA). Additionally, multiple regression analyses (PROC REG, SAS v.9.1, SAS Institute Inc., Cary, NC, USA) were performed to examine the relationship of C concentration to depth of the A-horizon, clay, sand, silt, elevation, azimuth, slope, plan curvature, profile curvature, and flow accumulation (Model 2) (PROC REG, SAS v.9.1, SAS Institute Inc., Cary, NC, USA). The 1976 multiple regression model omitted depth of the A-horizon and particle size distribution as this was not determined for the 1976 samples. In the multiple regression analyses, collinearity of predictors was tested by examining variance inflation. Silt and sand were identified as covariates. Multiple regression model refinement was performed with a stepwise procedure ($\alpha = 0.15$ to enter).

Model 1 - 1976 and 2006

C = exchangeable inorganic-N

Model 2 - 1976

C = elevation + azimuth + slope + plan curvature + profile curvature + flow accumulation

Model 2 - 2006

C = depth of A-horizon + clay + silt + elevation + azimuth + slope + plan curvature + profile curvature + flow accumulation

Results

Paired Comparisons

Significant changes in exchangeable inorganic-N and the C/total-N ratio were detected when comparing air- and oven-dried samples from 2006 (Table 2). Ammonium (NH₄-N) and NO₃-N for both drying treatments were pooled into inorganic-N as NO₃-N was detected in only 7 of the 146 samples and was <0.001 mg kg⁻¹ in each of the samples. Oven-drying at 105 °C for 48 hours increased mean exchangeable inorganic-N by approximately 12.4 mg kg⁻¹ (155%) over air-drying. The C/total-N ratio of oven-dried samples decreased by 2% compared to air-dried samples. Air- and oven-dried values for exchangeable inorganic-N were not well correlated ($r^2 = 0.36$).

Table 2. Mean soil C, total-N, exchangeable inorganic-N, and C/total-N in 2006 for the 0-10 cm depth derived from analysis of 73 (2006) oven- and air-dried samples. Oven-dried and estimated air-dried values for 1976 samples also shown. Bootstrap with replacement technique used to generate 10,000 means for each drying treatment. Value in parentheses is the standard error of the mean. Mean change and confidence interval (95%) values based on bootstrap analysis. For a comparison to be significant, the confidence interval values (not shown) must be either strictly positive or strictly negative.

Soil Property	2006			1976	
	Drying treatment		Comparison	Oven	Estimated Air
	Oven	Air	Oven - Air		
			Mean difference		
C (g kg ⁻¹)	23.7 (0.01)	23.9 (0.01)	-0.16	23.6 (0.01)	23.8
Total-N (g kg ⁻¹)	1.46 (<0.01)	1.43 (<0.01)	0.03	1.38 (<0.01)	1.35
Exchangeable Inorganic-N (mg kg ⁻¹)	20.4 (0.01)	8.0 (<0.01)	12.4 *	66.9 (0.03)	26.2
C/total-N (g kg ⁻¹ /g kg ⁻¹)	16.4 (<0.01)	16.7 (<0.01)	-0.3 *	17.1 (<0.01)	17.4

* Indicates oven-dried and air-dried values differ from one another (95% confidence interval).

The 0.4% increase in mineral soil C concentration across the watershed was not significant (Table 3). Atkins, Clifty, Lily, and Wallen/Ramsey soils exhibited significant decreases in C concentration ranging from -13.1% to -6.5%. Beersheba and Clarkrange taxadjunct soils exhibited significant increases in C concentration of 12.0% and 5.7%, respectively. Mineral soil C content across the watershed changed by 0.30 Mg ha^{-1} , but the increase was not significant (Table 4). Significant decreases were exhibited by Atkins and Lily soils. Changes in C content for these series were -3.3 Mg ha^{-1} and -1.3 Mg ha^{-1} , respectively. Beersheba, Clarkrange taxadjunct, Clifty, and Sequoia soils exhibited significant increases in C content ranging from $+1.2 \text{ Mg ha}^{-1}$ to $+1.7 \text{ Mg ha}^{-1}$.

Total-N concentration across the watershed exhibited a significant increase of 5.8% (Table 5). Clarkrange soils exhibited the only significant decrease in total-N concentration at -3.1%. Total-N concentration for Beersheba, Clarkrange taxadjunct, and Wallen/Ramsey soils increased significantly. Total-N content across the watershed increased significantly by 0.10 Mg ha^{-1} (Table 6). Beersheba, Clarkrange taxadjunct, Clifty, and Wallen/Ramsey soils exhibited significant increases in total-N content ranging from $+0.04 \text{ Mg ha}^{-1}$ to $+0.33 \text{ Mg ha}^{-1}$. Total-N content of Clarkrange soils decreased significantly by 0.06 Mg ha^{-1} .

The C/total-N ratio on the watershed decreased significantly by 4.1% (Table 7). Beersheba soils exhibited the only significant increase in the C/total-N ratio of 3.6%. Atkins, Clifty, and Wallen/Ramsey soils exhibited significant decreases in the C/total-N ratio of -17.1%, -14.4%, and -11.4%, respectively. These changes in C and total-N concentration and content and the C/total-N ratio are summarized in Table 8.

Table 3. Mean soil series C concentration for the 0-10 cm depth derived from re-analysis of oven-dried samples collected in 1976 and analysis of oven-dried samples collected in 2006. Bootstrap with replacement technique used to generate 10,000 means for each year. Value in parentheses is the standard error of the mean. Mean change and confidence interval (95%) values based on bootstrap analysis. For a comparison to be significant, the confidence interval values must be either strictly positive or strictly negative.

Soil Series	1976	2006	Change	Mean difference	95% CI
	g kg^{-1}			g kg^{-1}	
Atkins †	29.7 (0.03)	25.9 (0.03)	-12.8%	-3.8	-5.0, -2.8 *
Clifty taxadjunct †	26.7 (0.03)	23.2 (0.03)	-13.1%	-3.5	-3.9, -0.4 *
Wallen/Ramsey †	30.7 (0.02)	28.7 (0.02)	-6.5%	-1.9	-3.1, -0.1 *
Beersheba †	20.0 (0.01)	22.4 (0.01)	12.0%	2.4	2.3, 2.7 *
Lily †	24.4 (0.02)	22.7 (0.01)	-7.0%	-1.8	-4.0, -0.4 *
Sequoia taxadjunct †	23.2 (0.01)	24.3 (0.01)	4.7%	1.1	-0.04, 2.1
Clarkrange †	25.3 (0.04)	24.1 (0.03)	-4.7%	-1.2	-5.3, 1.3
Clarkrange taxadjunct †	21.1 (0.01)	22.3 (0.01)	5.7%	1.2	1.0, 1.7 *
All samples ‡	23.6 (0.01)	23.7 (0.01)	0.4%	0.1	-0.2, 0.5

* Indicates 2006 value is different from the 1976 value (95% confidence interval).

† Bootstrap technique performed on samples of each soil series.

‡ Bootstrap technique performed on all samples.

Table 4. Mean soil series C mass for the 0-10 cm depth derived from re-analysis of oven-dried samples collected in 1976 and analysis of oven-dried samples collected in 2006. Bootstrap with replacement technique used to generate 10,000 means for each year. Value in parentheses is the standard error of the mean. Mean change and confidence interval (95%) values based on bootstrap analysis. For a comparison to be significant, the confidence interval values must be either strictly positive or strictly negative.

Soil Series	1976	2006	Change	Mean	95% CI
	Mg ha ⁻¹			difference	
Atkins †	30.0 (0.03)	26.6 (0.03)	-11.3%	-3.3	-4.2, -2.3 *
Clifty taxadjunct †	24.8 (0.03)	26.0 (0.03)	4.8%	1.2	0.7, 3.9 *
Wallen/Ramsey †	29.1 (0.03)	26.7 (0.01)	-8.2%	-2.3	-6.3, 0.5
Beersheba †	23.4 (0.01)	25.1 (0.01)	7.3%	1.7	1.2, 2.2 *
Lily †	24.4 (0.02)	23.2 (0.02)	-4.9%	-1.3	-3.4, -0.1 *
Sequoia taxadjunct †	23.0 (0.01)	24.9 (0.01)	8.3%	1.9	0.8, 2.8 *
Clarkrange †	29.4 (0.05)	27.6 (0.01)	-6.1%	-1.8	-7.5, 3.2
Clarkrange taxadjunct †	24.4 (0.01)	25.7 (0.01)	5.3%	1.2	1.0, 1.8 *
All samples ‡	25.1 (0.01)	25.4 (0.01)	1.2%	0.3	-0.1, 0.6

* Indicates 2006 value is different from the 1976 value (95% confidence interval).

† Bootstrap technique performed on samples of each soil series.

‡ Bootstrap technique performed on all samples.

Table 5. Mean soil series total-N concentration for the 0-10 cm depth derived from re-analysis of oven-dried samples collected in 1976 and analysis of oven-dried samples collected in 2006. Bootstrap with replacement technique used to generate 10,000 means for each year. Value in parentheses is the standard error of the mean. Mean change and confidence interval (95%) values based on bootstrap analysis. For a comparison to be significant, the confidence interval values must be either strictly positive or strictly negative.

Soil Series	1976	2006	Change	Mean difference	95% CI
	g kg^{-1}			g kg^{-1}	
Atkins †	1.84 (0.003)	1.88 (0.002)	2.2%	0.04	-0.18, 0.26
Clifty taxadjunct †	1.67 (0.001)	1.68 (0.002)	0.6%	0.02	-0.05, 0.23
Wallen/Ramsey †	1.75 (0.001)	1.92 (0.002)	9.7%	0.17	0.12, 0.27 *
Beersheba †	1.20 (<0.001)	1.31 (<0.001)	9.2%	0.10	0.01, 0.11 *
Lily †	1.40 (0.001)	1.38 (0.001)	-1.4%	-0.02	-0.08, 0.02
Sequoia taxadjunct †	1.25 (<0.001)	1.33 (0.001)	6.4%	0.08	-0.08, 0.21
Clarkrange †	1.27 (0.001)	1.23 (0.001)	-3.1%	-0.04	-0.12, -0.03 *
Clarkrange taxadjunct †	1.22 (0.001)	1.26 (0.001)	3.3%	0.05	0.04, 0.05 *
All samples ‡	1.38 (<0.001)	1.46 (<0.001)	5.8%	0.081	0.076, 0.088 *

* Indicates 2006 value is different from the 1976 value (95% confidence interval).

† Bootstrap technique performed on samples of each soil series.

‡ Bootstrap technique performed on all samples.

Table 6. Mean soil series total-N mass for the 0-10 cm depth derived from re-analysis of oven-dried samples collected in 1976 and analysis of oven-dried samples collected in 2006. Bootstrap with replacement technique used to generate 10,000 means for each year. Value in parentheses is the standard error of the mean. Mean change and confidence interval (95%) values based on bootstrap analysis. For a comparison to be significant, the confidence interval values must be either strictly positive or strictly negative.

Soil Series	1976	2006	Change	Mean difference	95% CI
	Mg ha ⁻¹			Mg ha ⁻¹	
Atkins †	1.86 (0.003)	1.94 (0.002)	4.3%	0.08	-0.13, 0.31
Clifty taxadjunct †	1.55 (0.001)	1.88 (0.001)	21.3%	0.33	0.26, 0.52 *
Wallen/Ramsey †	1.66 (0.001)	1.80 (0.001)	8.4%	0.14	0.11, 0.19 *
Beersheba †	1.41 (<0.001)	1.47 (<0.001)	4.3%	0.06	0.04, 0.09 *
Lily †	1.40 (0.001)	1.42 (0.001)	1.4%	0.02	-0.02, 0.03
Sequoia taxadjunct †	1.24 (<0.001)	1.37 (0.001)	10.5%	0.13	-0.04, 0.39
Clarkrange †	1.47 (0.002)	1.41 (0.001)	-4.1%	-0.06	-0.22, -0.05 *
Clarkrange taxadjunct †	1.41 (0.001)	1.45 (0.001)	2.8%	0.04	0.035, 0.051 *
All samples ‡	1.47 (<0.001)	1.57 (<0.001)	6.8%	0.095	0.093, 0.097 *

* Indicates 2006 value is different from the 1976 value (95% confidence interval).

† Bootstrap technique performed on samples of each soil series.

‡ Bootstrap technique performed on all samples.

Table 7. Mean soil series C/total-N ratio for the 0-10 cm depth derived from re-analysis of oven-dried samples collected in 1976 and analysis of oven-dried samples collected in 2006. Bootstrap with replacement technique used to generate 10,000 means for each year. Value in parentheses is the standard error of the mean. Mean change and confidence interval (95%) values based on bootstrap analysis. For a comparison to be significant, the confidence interval values must be either strictly positive or strictly negative.

Soil Series	1976	2006	Change	Mean difference	95% CI
	$\text{g kg}^{-1} / \text{g kg}^{-1}$			$\text{g kg}^{-1} / \text{g kg}^{-1}$	
Atkins †	16.4 (0.01)	13.6 (0.01)	-17.1%	-2.8	-3.2, -2.5 *
Clifty taxadjunct †	16.0 (0.01)	13.7 (0.01)	-14.4%	-2.3	-3.3, -2.0 *
Wallen/Ramsey †	17.5 (0.01)	15.5 (0.01)	-11.4%	-2.1	-2.3, -2.0 *
Beersheba †	16.5 (0.004)	17.1 (0.004)	3.6%	0.54	0.52, 0.59 *
Lily †	17.5 (0.01)	16.3 (0.004)	-6.9%	-1.2	-3.1, 0.1
Sequoia taxadjunct †	18.5 (0.01)	18.4 (0.01)	-0.5%	-0.1	-0.8, 0.6
Clarkrange †	19.7 (0.01)	19.5 (<0.001)	-1.0%	-0.2	-2.0, 1.6
Clarkrange taxadjunct †	17.4 (0.01)	17.5 (0.003)	0.6%	0.2	-0.4, 0.9
All samples ‡	17.1 (0.003)	16.4 (0.002)	-4.1%	-0.67	-0.76, -0.61 *

* Indicates 2006 value is different from the 1976 value (95% confidence interval).

† Bootstrap technique performed on samples of each soil series.

‡ Bootstrap technique performed on all samples.

Table 8. Summary of changes in mineral soil C and total-N concentration and content and the C/total-N ratio from 1976 to 2006.

Soil Series	C		Total-N		C/total-N
	g kg ⁻¹	Mg ha ⁻¹	g kg ⁻¹	Mg ha ⁻¹	g kg ⁻¹ /g kg ⁻¹
Atkins	-	-	ns	ns	-
Beersheba	+	+	+	+	+
Clarkrange	ns†	ns	-	-	ns
Clarkrange tax.	+	+	+	+	ns
Clifty tax.	-	+	ns	+	-
Lily	-	-	ns	ns	ns
Sequoia tax.	ns	+	ns	ns	ns
Wallen/Ramsey	-	ns	+	+	-
All samples	ns	ns	+	+	-

† Change not significant (95% confidence interval).

Forest Cover Plots

Analysis of forest cover plots revealed that while change in basal area across all plots was very small (0.07 m²), notable changes in density, species composition, and species richness occurred (Tables 9 and 10). The sum of individual trees in all plots decreased from 917 to 727. Species richness increased from 19 in 1976 to 23 in 2006. The most notable changes from 1976 to 2006 were the large gains in basal area and importance value by red maple (*Acer rubrum* L.) (+48%, +137%) and sourwood (*Oxydendrum arboreum* L.) (+123%, +51%). The largest decreases in basal area and importance value were exhibited by post oak (*Quercus stellata* L.) (-68%, -70%) and scarlet oak (-29%, -47%).

Regression Analyses

1976

Model 1

Regression analysis revealed a significant positive relationship between C and inorganic-N (Table 11 and Fig. 1). Exchangeable inorganic-N concentration explained 38.7% of the variability in C concentration. The model predicting C concentration as a function of inorganic-N concentration is shown below.

$$C \text{ (g kg}^{-1}\text{)} = 10.6 + 0.194 \text{ inorganic-N (mg kg}^{-1}\text{)}$$

Model 2

Multiple regression analysis revealed azimuth and elevation as significant predictors explaining 14.0% of the variability in C concentration (Table 11 and Figs. 2 and 3). The model predicting C concentration as a function of azimuth and elevation is shown below.

$$C \text{ (g kg}^{-1}\text{)} = 163 + 0.019 \text{ Azimuth (degrees)} - 0.262 \text{ Elevation (m)}$$

Table 9. Basal area, relative basal area, density, relative density, and importance value for twenty four 10 by 20 m forest cover plots measured in 1976.

Species	Basal area m ²	Relative basal area	Density	Relative Density	Importance value
Scarlet oak (<i>Quercus coccinea</i> L.)	2.89	22.97	107	11.7	34.6
Post oak (<i>Quercus stellata</i> L.)	1.75	13.96	45	4.9	18.9
Red maple (<i>Acer rubrum</i> L.)	1.36	10.79	102	11.1	21.9
Southern red oak (<i>Quercus falcata</i> L.)	1.32	10.49	102	11.1	21.6
White oak (<i>Quercus alba</i> L.)	1.30	10.36	94	10.3	20.6
Sourwood (<i>Oxydendrum arboreum</i> L.)	0.91	7.27	125	13.6	20.9
Virginia pine (<i>Pinus virginiana</i> Mill.)	0.84	6.70	32	3.5	10.2
Yellow poplar (<i>Liriodendron tulipifera</i> L.)	0.69	5.52	8	0.9	6.4
Black gum (<i>Nyssa sylvatica</i> L.)	0.35	2.77	78	8.5	11.3
Dogwood (<i>Cornus florida</i> L.)	0.34	2.68	95	10.4	13.0
Hickory (<i>Carya spp.</i> Nutt.)	0.23	1.83	37	4.0	5.9
Blackjack oak (<i>Quercus marilandica</i> L.)	0.21	1.66	7	0.8	2.4
Chestnut oak (<i>Quercus prinus</i> L.)	0.13	1.02	4	0.4	1.5
Black oak (<i>Quercus velutina</i> L.)	0.12	0.93	14	1.5	2.5
Sassafras (<i>Sassafras albidum</i> Nutt.)	0.09	0.76	53	5.8	6.5
Eastern red cedar (<i>Juniperus virginiana</i> L.)	0.02	0.20	1	0.1	0.3
Mountain laurel (<i>Kalmia latifolia</i> L.)	0.01	<0.10	11	1.2	1.2
American chestnut (<i>Castanea dentata</i> Marsh.)	<0.01	<0.10	1	0.1	0.1
Black cherry (<i>Prunus serotina</i> Ehrh.)	<0.01	<0.10	1	0.1	0.1
Total	12.57		917		

Table 10. Basal area, relative basal area, density, relative density, and importance value for twenty four 10 by 20 m forest cover plots measured in 2006.

Species	Basal area m ²	Relative basal area	Density	Relative Density	Importance value
Red maple (<i>Acer rubrum</i> L.)	2.07	16.39	259	35.6	52.0
Scarlet oak (<i>Quercus coccinea</i> L.)	2.05	16.19	17	2.3	18.5
Sourwood (<i>Oxydendrum arboreum</i> L.)	2.03	16.08	113	15.5	31.6
White oak (<i>Quercus alba</i> L.)	1.46	11.54	50	6.9	18.4
Yellow poplar (<i>Liriodendron tulipifera</i> L.)	0.97	7.69	27	3.7	11.4
Virginia pine (<i>Pinus virginiana</i> Mill.)	0.78	6.16	14	1.9	8.1
Post oak (<i>Quercus stellata</i> L.)	0.56	4.46	8	1.1	5.6
Blackjack oak (<i>Quercus marilandica</i> L.)	0.53	4.16	13	1.8	5.9
Southern red oak (<i>Quercus falcata</i> L.)	0.45	3.54	9	1.2	4.8
Northern red oak (<i>Quercus rubra</i> L.)	0.40	3.15	14	1.9	5.1
Black gum (<i>Nyssa sylvatica</i> L.)	0.37	2.95	84	11.6	14.5
Black oak (<i>Quercus velutina</i> L.)	0.26	2.02	9	1.2	3.3
Chestnut oak (<i>Quercus prinus</i> L.)	0.21	1.67	5	0.7	2.4
Sassafras (<i>Sassafras albidum</i> Nutt.)	0.15	1.22	26	3.6	4.8
Hickory (<i>Carya spp.</i> Nutt.)	0.14	1.07	10	1.4	2.4
Dogwood (<i>Cornus florida</i> L.)	0.06	0.47	22	3.0	3.5
Sweet gum (<i>Liquidambar styraciflua</i> L.)	0.05	0.43	5	0.7	1.1
Eastern White Pine (<i>Pinus strobus</i> L.)	0.04	0.29	1	0.1	0.4
Mountain laurel (<i>Kalmia latifolia</i> L.)	0.03	0.24	20	2.8	3.0
Eastern hemlock (<i>Tsuga canadensis</i> L.)	0.01	0.12	3	0.4	0.5
American holly (<i>Ilex opaca</i> Ait.)	0.01	<0.10	6	0.8	0.8
Sparkleberry (<i>Vaccinium arboreum</i> Marsh.)	0.01	<0.10	7	1.0	1.0
Black cherry (<i>Prunus serotina</i> Ehrh.)	<0.01	<0.10	5	0.7	0.7
Total	12.64		727		

Table 11. Regression coefficients, coefficient standard error, root mean square error, r^2 , and p values intervals for regressions of 1976 oven-dried samples.

Parameter	Coefficient	Standard Error		r^2	p
		Coefficient			
Response - C					
Intercept	10.6	2.2		0.387	<0.001
Inorganic-N	0.194	0.031			
Root MSE	5.8				
Response - C					
Intercept	163.0	58.3		0.140	0.01
Azimuth	0.019	0.009			
Elevation	-0.262	0.107			
Root MSE	7.0				

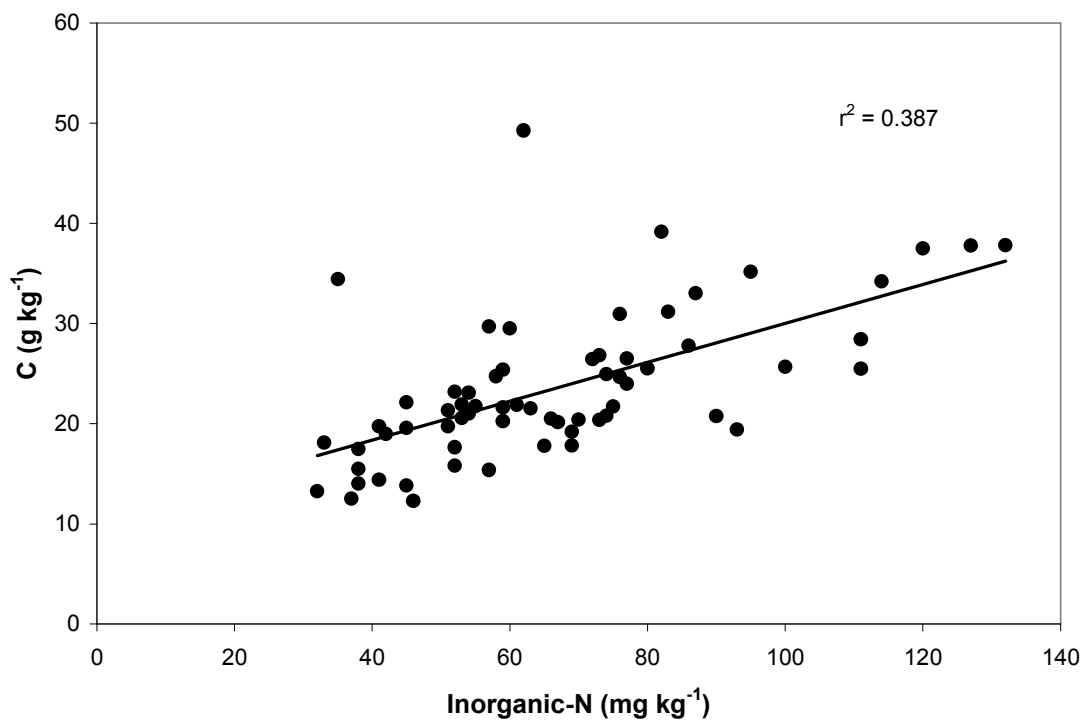


Fig. 1. Scatter-plot depicting 1976 C concentration as a function of 1976 inorganic-N concentration. Linear regression line and r^2 also shown.

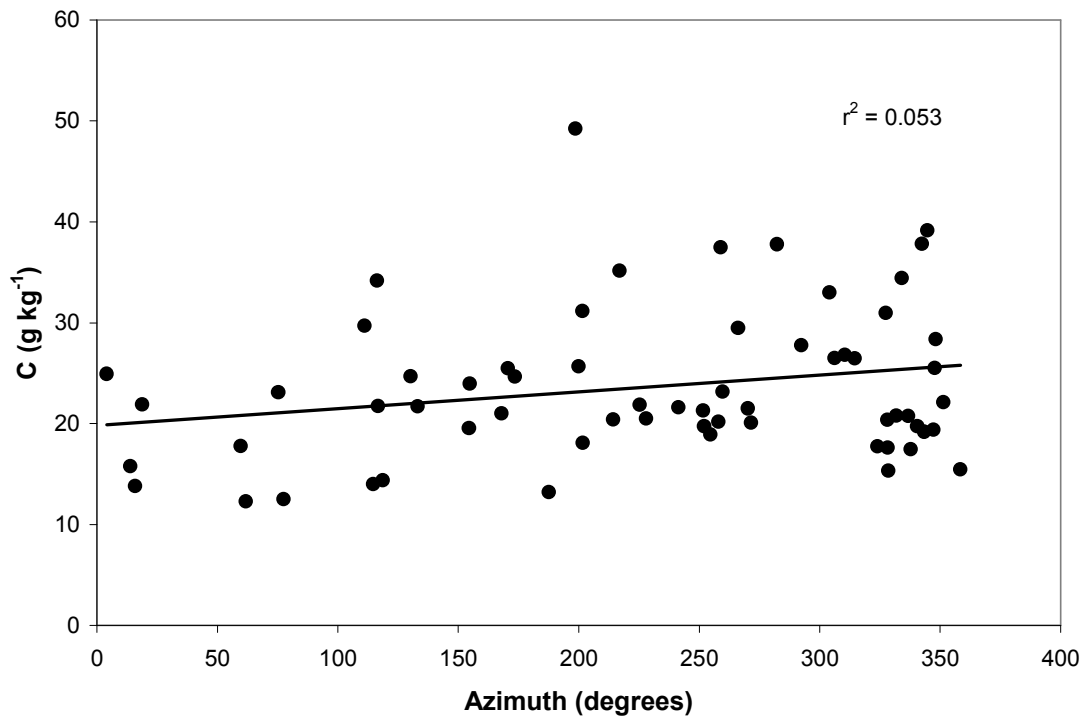


Fig. 2. Scatter-plot depicting 1976 C concentration as a function azimuth. Linear regression line and r^2 also shown.

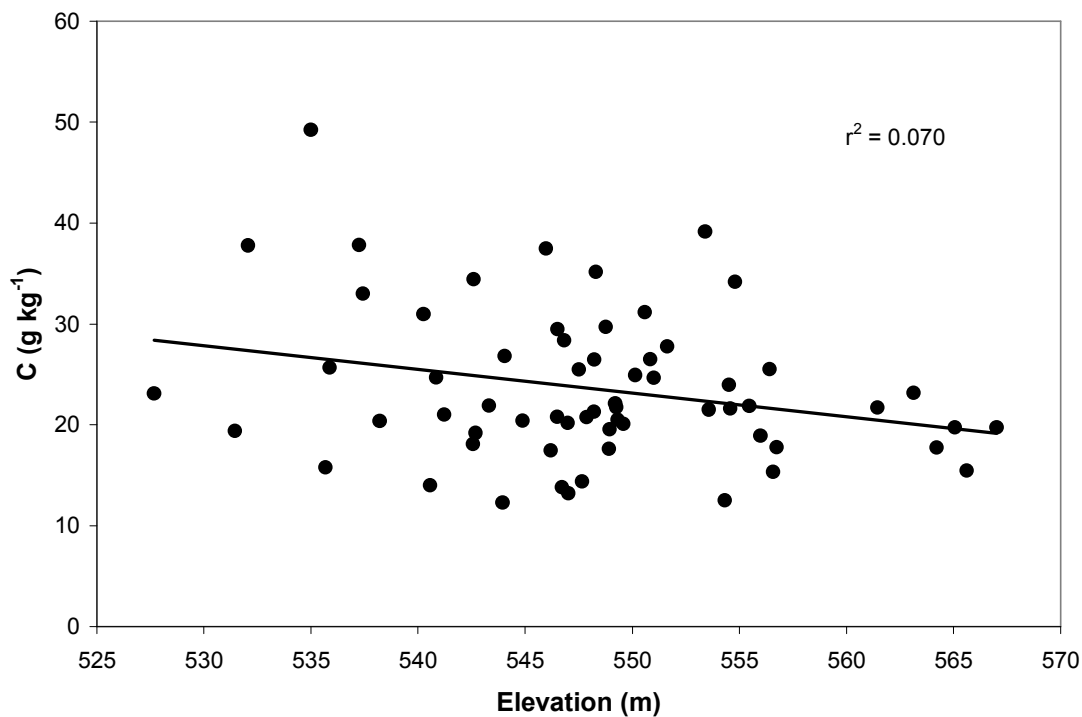


Fig. 3. Scatter-plot depicting 1976 C concentration as a function elevation. Linear regression line and r^2 also shown.

2006

Model 1

Regression analysis revealed a significant positive relationship between C and inorganic-N (Table 12 and Fig. 4). Exchangeable inorganic-N concentration explained 10.7% of the variability in C concentration. The model predicting C concentration as a function of inorganic-N concentration is shown below.

$$C \text{ (g kg}^{-1}\text{)} = 17.8 + 0.291 \text{ inorganic-N (mg kg}^{-1}\text{)}$$

Model 2

Multiple regression analysis revealed percent clay as the only significant predictor explaining 7.9% of the variability in C concentration (Table 12 and Fig. 5). The model predicting C concentration as a function of percent clay is shown below.

$$C \text{ (g kg}^{-1}\text{)} = 30.3 - 1.05 \text{ Clay (\%)}$$

Table 12. Regression coefficients, coefficient standard error, root mean square error, r^2 , and p values intervals for regressions of 2006 oven-dried samples.

Parameter	Coefficient	Standard Error		r^2	p
		Coefficient			
Response - C					
Intercept	17.8	2.2		0.107	0.005
Inorganic-N	0.291	0.010			
Root MSE	6.1				
Response - C					
Intercept	30.3	2.8		0.079	0.016
Clay	-1.05	0.43			
Root MSE	6.2				

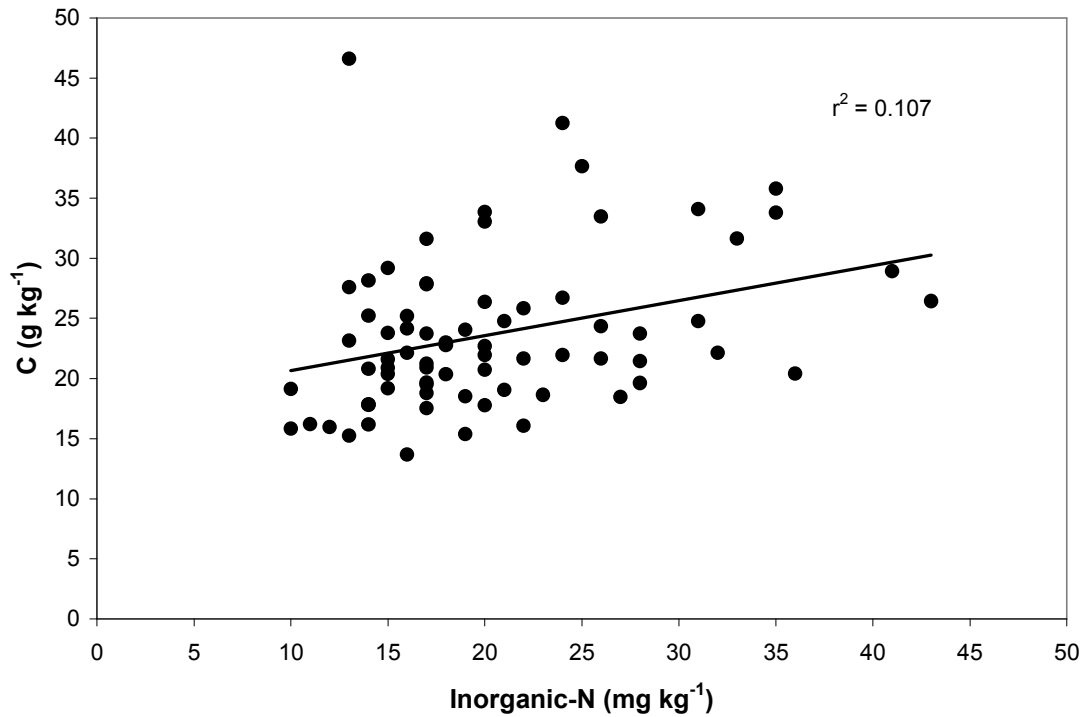


Fig. 4. Scatter-plot depicting 2006 C concentration as a function of 2006 inorganic-N concentration. Linear regression line and r^2 also shown.

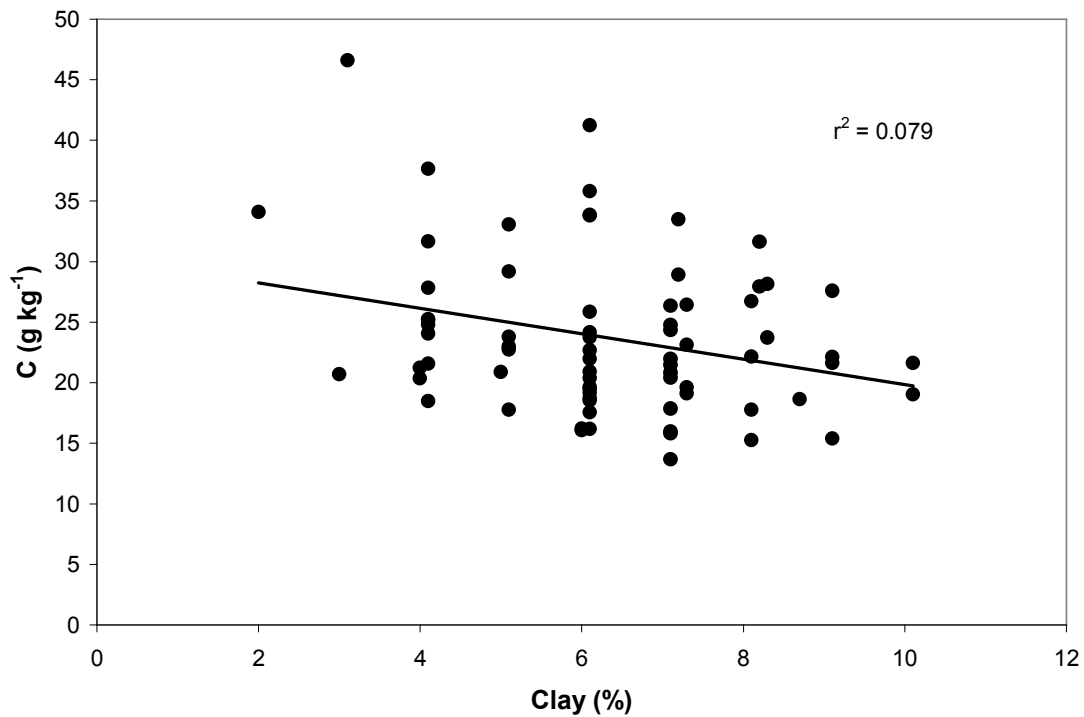


Fig. 5. Scatter-plot depicting 2006 C concentration as a function of 2006 percent clay content in the 0 to 10 cm depth. Linear regression line and r^2 also shown.

Discussion

Soil Sampling

An area of concern when sampling the 0 to 10 cm depth of the mineral soil is the contamination of the sample with portions of the O-horizons. This contamination could be large enough to mask differences in C concentration among paired samples. In other words, contamination of the sample with O-horizon material at one sampling event and not in the other could result in detecting false change in C concentration. We investigated whether or not the magnitude of change in C concentration in this study was large enough to overcome possible inclusions of O-horizon material in our samples of the 0 to 10 cm mineral soil depth. Significant differences in C concentration (Table 3) ranged from -3.8 to $+2.4$ g kg^{-1} . To determine the quantity of O-horizon material that would need to be included in our samples of the mineral soil to detect false change, C concentration of Oe and Oa-horizons of a southern Appalachian forest was obtained from the literature (Clinton et al., 1996). Mean C concentration of three southern Appalachian sites was reported at 370 g kg^{-1} . We determined the volume of soil sampled at each location in 2006 to be approximately 3000 cm^3 . The smallest significant increase in mineral soil C we detected was exhibited by the Clarkrange taxadjunct series at $+1.2$ g kg^{-1} . At a bulk density of 1.0 g cm^{-3} , approximately 10 g of Oe and Oa horizon material would need to be included in the 3 kg sample of the Clarkrange taxadjunct samples to increase C concentration by 1.2 g kg^{-1} . It is unlikely that 10 g of O-horizon material was included in any of our 3 kg samples as great care was taken not to include this material in our samples of the mineral soil. We also considered a dilution effect brought about by sampling beyond the 0 to 10 cm depth. C concentration of the 10 to 30 cm depth in 1976 was taken from a previous study on the Camp Branch Watershed (Kelly and Mays, 2005). Mean C concentration was reported at 9.0 g kg^{-1} . The smallest significant decrease in mineral soil C concentration we detected was exhibited by the Lily series at -1.8 g kg^{-1} . We determined that including approximately 350 g of soil beyond the 10 cm depth was necessary to dilute C concentration of the Lily series by 1.8 g kg^{-1} . Once again, it is very unlikely that 350 g of soil beyond the 10 cm sampling depth was included in our samples. Therefore, we assume the significant differences we detected in mineral soil C concentration are real differences and not due to sampling errors.

Drying Treatment

Comparison of oven- and air-dried 2006 samples revealed no significant change in mineral soil C. Oven-drying at 105 °C resulted in a 155% increase in exchangeable inorganic-N compared to air-drying. Nearly all of this increase was attributable to NH₄-N. This is consistent with increases of 300% reported in the literature when samples dried at 20 °C and 105 °C were compared (Van Erp et al., 2001). Our results showed oven- and air-dried inorganic-N were not well correlated ($r^2 = 0.36$) compared to the correlation of field-moist and air-dried soils ($r^2 = 0.80$) reported by Binkley and Hart (1989). It appears that as drying temperature increases, differences in substrate may become more pronounced in N transformations. Temperature influences N mineralization differently depending on the source of N (Agehara and Warncke, 2005).

Since significant changes were detected among air- and oven-dried samples, we felt it would be valuable to examine differences in C and N among air- and oven-dried samples and field-moist samples. The watershed was revisited in July of 2007 and 36 of the 73 permanent sampling locations were re-sampled. Samples were kept field-moist with refrigeration. C and total-N were determined using the same chemical analysis procedures used on air- and oven-dried samples. C concentration of field-moist samples was approximately 25% greater than that of air- and oven-dried samples. Total-N concentration and the C/total-N ratio of field-moist samples were approximately 10% and 17% greater than that of air- and oven-dried samples. These results suggest it may be necessary to analyze field-moist samples when quantifying mineral soil C and N. One could also argue that C and N concentrations of dried samples represent more recalcitrant pools. However, differences in C and N that this comparison suggests could be confounded by the time between sampling events (1-year) and the sub-sampling approach.

Re-analysis of Preliminary Study Samples

Comparison of results obtained by Kelly and Mays (2005) and chemical and statistical re-analysis of the same subset of samples were comparable. Kelly and Mays (2005) reported a significant increase in mineral soil C of 10.2 g kg⁻¹. Re-analysis detected a significant increase of 7.5 g kg⁻¹. Comparison of results suggests a sample size of 11, as used in the preliminary study, was not large enough to accurately predict change

in mineral soil C across the 94 ha watershed as our analysis of all sampling locations did not detect significant change. This is most likely the result of considerable micro-site variability in the soils on the watershed and that the relatively few samples used by Kelly and Mays (2005) were not sufficient to represent the variability to be found at a larger scale.

C and N Content

Carbon and N content in 2006 were calculated with bulk density values determined for each sampling location (Table 13). Bulk density for 1976 was only available for 4 of the 8 soil series and was measured at one sampling location. For those soil series without a 1976 bulk density value, mean bulk density of the corresponding soil series in 2006 was used to calculate C and N content. Although the accuracy of our estimate may be questioned, this approach was the only means available to provide an estimate of 1976 C and N content. Since studies with comparable goals have calculated long term changes in C content using bulk density from only one of the sampling years (Trettin et al., 1999; Johnson et al., 2003), we assume changes in bulk density over similar periods of time are not significant enough to warrant determination of bulk density in each of the sampling years. In examining 30-year changes for those soil series with both 1976 and 2006 bulk density values, no consistent trend was found. Bulk density of Atkins, Beersheba, and Lily soils changed very little from 1976 to 2006 (± 0.01 to 0.04 g cm^{-3}) compared to the large change in Clifty soils ($+0.19 \text{ g cm}^{-3}$). We propose that the large difference among 1976 and 2006 bulk density of Clifty soils was the result of 1976 bulk density values being determined from only one sampling location.

Paired Comparisons

The hypothesis that an increase in mineral soil C concentration and content would be detected was accepted since 2 of the 8 soil series exhibited significant increases in C concentration (Beersheba and Clarkrange taxadjunct) and 4 soil series exhibited significant increases in C content (Beersheba, Clarkrange taxadjunct, Clifty taxadjunct, and Sequoia).

Table 13. Bulk density values used to calculate mineral soil C and N content and number of sampling locations used for chemical and physical analysis. Value in parentheses is standard error of the mean.

Soil Series	Mean Bulk Density		Sampling Locations	
	1976 †	2006	1976	2006
	g cm ⁻³		n	
Atkins	1.01	1.02 (0.07)	4	6
Beersheba	1.17	1.13 (0.03)	22	25
Clarkrange	1.16 ‡	1.16 (0.09)	2	2
Clarkrange tax.	1.16 ‡	1.16 (0.04)	13	13
Clifty tax.	0.93	1.12 (0.05)	4	6
Lily	1.00	1.03 (0.05)	9	9
Sequoia tax.	0.99 ‡	0.99 (0.08)	2	3
Wallen/Ramsey	0.95 ‡	0.95 (0.06)	9	9

† Source: Mays et al., 1991.

‡ No value for 1976, 2006 mean values used.

Atkins, Clifty, and Wallen/Ramsey soils contained the greatest concentrations of mineral soil C in 1976 (Table 3). Although these soils exhibited the greatest decline in mineral soil C concentration after 30-years, their C concentrations were still greater or equal to that of the remaining soil series in 2006. In contrast, soils that exhibited the only increases in mineral soil C concentration (Table 3), Beersheba and Clarkrange taxadjunct, contained the smallest C concentrations in 1976. Although Lily soils are located on the same landscape position as Beersheba soils, the C concentration of Lily soils also exhibited a decrease in mineral soil C concentration. We speculate that observed changes in C concentration of Lily soils were driven by changes in biomass and species composition.

Paired comparisons revealed increases in mineral soil C were most closely associated with increases in the C/total-N ratio. This is consistent with results reported by Trettin et al. (1999) in a southern Appalachian forest. Furthermore, decreases in mineral soil C coincided with decreases in the C/total-N ratio. Cove soils (Atkins and Clifty) exhibited a decrease in mineral soil C. Furthermore, upland soils (Lily and Wallen/Ramsey) also exhibited a decrease in mineral soil C. Paired comparisons also

revealed increases in mineral soil C were associated with increases in total-N with the exception of the Wallen/Ramsey soils which exhibited a decrease in C and an increase in total-N.

Changes in Forest Cover

Analysis of forest cover plots indicated little change in aboveground biomass across all plots as basal area increased by only 0.07 m². However, results also suggest that although the overall increase in aboveground biomass was minimal, changes in density, species composition, and species richness were notable. In 1976, basal area of 19 plots was dominated by oaks and in 2006 only 8 plots were still oak dominated (Table 14). This suggests a decline in oak domination on the watershed. The large decrease in density with little change in basal area suggests the forest is now characterized by larger trees with wider spacing. Of course, there are exceptions to this generalization due to blow-downs and losses attributable to southern pine beetle (*Dendroctonus frontalis* Zimmermann). Areas on the watershed that have lost overstory canopy are generally characterized by young, dense stands comprised of early successional species such as red maple, sweet gum (*Liquidambar styraciflua* L.), sassafras (*Sassafras albidum* Nutt.), and eastern white pine (*Pinus strobus* L.).

Changes in forest cover in a similar study by Johnson et al. (2007) were reported as live vegetation biomass. Biomass of live vegetation increased substantially from 1982 (~80 Mg ha⁻¹) to 2004 (~100 Mg ha⁻¹) while changes in species composition were minimal. These changes in forest cover on the Walker Branch Watershed appear to be quite different from those that took place on the Camp Branch Watershed. On Camp Branch, changes in biomass (as basal area) were minimal compared to the increase in biomass on Walker Branch. Additionally, species composition changed substantially on Camp Branch while changes in species composition on Walker Branch were much less pronounced. Differences in change in biomass suggest that the forest on Walker Branch is still aggrading while the forest on Camp Branch has reached equilibrium in terms of biomass.

Table 14. Topographic position and changes in density, basal area, and dominance of forest cover plots from 1976 to 2006.

Plot Number	Topographic Position	Change in		Dominant Species	
		Change in Density	Basal Area		
		1976 to 2006		1976	2006
		n	m ²		
1	Cove	-8	0.30	<i>Quercus coccinea</i> L.	<i>Quercus coccinea</i> L.
3	Cove	-7	-0.28	<i>Quercus falcata</i> L.	<i>Oxydendrum arboreum</i> L.
4	Cove	-11	0.04	<i>Quercus stellata</i> L.	<i>Acer rubrum</i> L.
5	Cove	7	0.01	<i>Quercus stellata</i> L.	<i>Quercus velutina</i> L.
9	Cove	7	-0.41	<i>Liriodendron tulipifera</i> L.	<i>Quercus alba</i> L.
10	Cove	25	0.19	<i>Quercus coccinea</i> L.	<i>Acer rubrum</i> L.
11	Cove	-1	-0.06	<i>Quercus stellata</i> L.	<i>Quercus stellata</i> L.
21	Cove	-20	-0.02	<i>Acer rubrum</i> L.	<i>Acer rubrum</i> L.
22	Cove	-16	-0.10	<i>Pinus virginiana</i> Mill.	<i>Pinus virginiana</i> Mill.
2	Sideslope	-19	-0.05	<i>Pinus virginiana</i> Mill.	<i>Oxydendrum arboreum</i> L.
7	Sideslope	-14	-0.17	<i>Quercus stellata</i> L.	<i>Acer rubrum</i> L.
15	Sideslope	-18	-0.01	<i>Quercus falcata</i> L.	<i>Acer rubrum</i> L.
16	Sideslope	6	-0.05	<i>Quercus coccinea</i> L.	<i>Oxydendrum arboreum</i> L.
17	Sideslope	-8	0.41	<i>Quercus coccinea</i> L.	<i>Quercus coccinea</i> L.
18	Sideslope	-19	-0.55	<i>Quercus coccinea</i> L.	<i>Oxydendrum arboreum</i> L.
8	Footslope	-3	0.00	<i>Quercus coccinea</i> L.	<i>Quercus coccinea</i> L.
12	Footslope	7	0.14	<i>Quercus falcata</i> L.	<i>Oxydendrum arboreum</i> L.
13	Footslope	-6	-0.12	<i>Quercus coccinea</i> L.	<i>Quercus coccinea</i> L.
14	Footslope	-27	0.23	<i>Quercus coccinea</i> L.	<i>Oxydendrum arboreum</i> L.
6	Ridge	-3	-0.32	<i>Quercus coccinea</i> L.	<i>Oxydendrum arboreum</i> L.
19	Ridge	-34	0.14	<i>Pinus virginiana</i> Mill.	<i>Liriodendron tulipifera</i> L.
20	Ridge	-16	0.34	<i>Quercus coccinea</i> L.	<i>Oxydendrum arboreum</i> L.
23	Ridge	-20	0.28	<i>Quercus coccinea</i> L.	<i>Quercus prinus</i> L.
24	Ridge	8	0.11	<i>Quercus coccinea</i> L.	<i>Oxydendrum arboreum</i> L.

Regression Analyses

The hypothesis that mineral soil C would be negatively correlated to inorganic-N was rejected since C was found to be positively correlated to inorganic-N both in 1976 and 2006. Additionally, it is important to note that the relationship of C to inorganic-N was much stronger in 1976 than in 2006. In contrast to our results, research by Tateno et al. (2004) suggested that as N availability increased, belowground NPP decreased. This suggests a greater quantity of organic matter inputs on sites with lower quantities of available N. Furthermore, more available N suggests greater mineralization and organic matter decomposition compared to soils with less available N. Hence, one could anticipate C to be negatively correlated to inorganic-N. However, $\text{NH}_4\text{-N}$ has been found to become stable by reacting with soil organic matter (Kaye et al., 2003) suggesting the relationship of available N to C may also depend on the rate of $\text{NH}_4\text{-N}$ fixation by organic matter. In addition, we examined exchangeable inorganic-N and not N mineralization. It is possible that evaluating the relationship of C to mineralizable N would have produced different results.

The hypothesis that C would be predicted by topographic factors was accepted for the 1976 data and rejected for the 2006 data. In 1976, C was found to increase with increasing azimuth and decreasing elevation. However, in 2006 no topographic factors were significant predictors of C. This suggests that the effects of topography on C have lessened considerably from 1976 to 2006 and that the variability of C is now driven by factors that were not evaluated. Zushi (2006) reported that topographic factors explained ~50% of variation in C storage in a monoculture Japanese cedar (*Cryptomeria japonica* D. Don.) plantation. Kulmatiski et al. (2004) suggests mineral soil C did not vary with species composition in a southern New England deciduous forest and that land use, texture, topography, and drainage class may be more influential. However, we propose that species composition and changes in quantity of inputs may have been important controls on mineral soil C due to topographic factors explaining only 14.0% of the variability in C in 1976 and none of the variability in C in 2006. Species differ in biomass partitioning and substrate quality.

In 2006, C was found to be related only to clay content and was negatively correlated to clay content. This may be the result of the influence of clay content on soil

aggregates and source of C. Olk and Gregorich (2006) propose that organic matter of clay fractions is less stable due to the dominance of microbial residues in clay fractions as opposed to the dominance of plant aromatics in silt fractions. Furthermore, Adu and Oades (1978) suggest greater clay content results in greater disruption of aggregates in drying and wetting cycles which results in less physical protection of C. However, the relationship of C to clay content was rather weak and as a result, the effects of clay content on C are anticipated to be small

Species Influences

Since regression analyses revealed topographic factors accounted for a small proportion of variability (14.0%) in C in 1976 and none of the variability in C in 2006, the question arises as to what factors could account for the remaining variability in C. Tree species exhibit distinct differences in litter decomposition rates (Mudrick et al., 1994; Kelly and Beauchamp, 1987), belowground biomass allocation (Harris et al., 1977; Ramseur and Kelly, 1981; Davis et al., 2004) and mycorrhizal associations (Newman and Reddell, 1988; Walker et al., 2005). Belowground biomass allocation also varies with stand age (Walters et al., 2003).

Changes in mineral soil C on the Walker Branch Watershed were reported to be related to estimated CO₂-C respiration from organic matter and leaf litterfall C inputs (Johnson et al., 2007). This suggests changes in mineral soil C on the Camp Branch Watershed may also have been driven by these changes in respired C and C inputs from leaf litterfall. However, changes in forest cover on Camp Branch were dissimilar to those on Walker Branch and suggest that changes in species composition may have been an additional influence on mineral soil C in the Camp Branch Watershed.

Distribution of belowground biomass in the soil profile varies with community species composition. In an earlier study on the Camp Branch Watershed by Ramseur and Kelly (1981), 35% of fine root mass (<2 mm) was located in the 0 to 10 cm depth in upland oak-mixed hardwood communities and 50% in mesic hardwood communities. Total biomass of mesic hardwood communities was found to be 200% greater than that of upland oak-mixed hardwood communities (Ramseur and Kelly, 1981). Considering that mesic hardwood communities have much higher total biomass and a greater percentage of this biomass allocated in the 0 to 10 cm depth, one would expect a greater quantity of

organic matter inputs into the mineral soil. This is reflected in our results as cove soils (Atkins and Clifty) contained higher C concentrations than upland soils in 1976. In addition, although C concentration of cove soils decreased over the 30-year interval, their C concentration is still higher than that of upland soils. The same pattern found in cove soils holds true for Wallen/Ramsey soils; located on steep side-slope positions. It is important to note that Wallen/Ramsey soils are typically under a cover of mountain laurel (*Kalmia latifolia* L.) which should have distinct effects on C of these soils. Since soils that are expected to contain the highest mineral soil C concentrations exhibited a decline in C, it appears that a substantial change may have occurred in quantity of leaf litter inputs, quantity and distribution of fine roots (<2 mm) in the soil profile, or factors influencing rates of decomposition such as substrate quality.

In order to investigate the influence of changes in quantity of soil organic matter inputs, the 24 forest cover plots were classified according to landscape position (Table 14). Net change in density and basal area of cove plots was -24 and -0.33 m², respectively. Net change in density and basal area of side-slope plots was -72 and -0.42 m², respectively. Although net changes in density of plots on ridge (-65) and foot-slope (-29) positions was similar to that of cove and side-slope plots, net change in basal area of ridge and foot-slope plots was +0.55 m² and +0.25 m². This suggests that the quantity of soil organic matter inputs in 2006 may have decreased in cove and side-slope positions relative to the quantity of inputs in 1976. The opposite holds true for foot-slope and ridge positions. This evaluation corresponds to changes in soil series C concentration with the exception of upland soils other than Beersheba and Clarkrange taxadjunct that exhibited decreases in C concentration (Lily) and no significant change (Clarkrange and Sequoia taxadjunct).

The decrease and lack of significant change in C concentration of Lily, Clarkrange, and Sequoia taxadjunct soils may have resulted from changes in species composition. Examination of changes in dominant species of the 24 plots (Table 14) indicated that substantial changes in species composition have occurred from 1976 to 2006. In 1976, 19 plots were dominated by oaks and in 2006 only 8 plots were still oak dominated. Grouping foot-slope and ridge plots indicated that 8 of 9 plots were oak dominated in 1976 and in 2006 the number decreased to 3. Grouping cove and side-slope

plots indicated that 11 of 15 were oak dominated in 1976 and in 2006 oak domination decreased to 5 plots. These results suggest that the substrate quality of soil organic matter inputs has changed on sites that have shifted from oak domination of basal area to domination of basal area by species with less recalcitrant soil organic matter inputs such as red maple and sourwood. This loss of oak domination occurred in all landscape positions suggesting that the decrease in C concentration of cove and side-slope soils, in addition to Lily soils, may also be the result of changes in substrate quality of soil organic matter inputs.

In a similar study on the Walker Branch Watershed where mineral soil C and N were measured at decadal intervals from 1972 to 2004, Johnson et al. (2007) reported that mineral soil C and N pools were variable over time. Mineral soil C in the 0 to 15 cm depth increased from 1972 to 1982, decreased from 1982 to 1993, and then increased from 1993 to 2004. Mineral soil N mostly followed the same trend as mineral soil C. Considering the results obtained by Johnson et al. (2007), the question arises as to whether or not we have measured only temporary fluxes in mineral soil C concentration or if measured changes are representative of a shift in mineral soil C storage patterns over the long term. Since we have measured changes over a period of time similar to the study by Johnson et al. (2007), we have more confidence that the former holds true. In light of this, we must conclude that although mineral soil C pools fluctuate over time, these pools will most likely be greater on mesic sites (Atkins and Clifty soils) and sites with a *Kalmia latifolia* L. understory (Wallen/Ramsey soils) than on other sites within the Camp Branch Watershed over the long term.

Agricultural Soils

Large areas of the southeastern U.S. formerly used for agriculture have been abandoned and allowed to return to native forest cover. Four of 25 Beersheba sampling locations are designated as severely eroded due to past agricultural activities (row-cropping). Evaluation of changes in mineral soil C and N of this subset revealed marked increases in both C and N. C increased by 47% (18.0 to 26.4 g kg⁻¹) and total-N increased by 28% (1.17 to 1.50 g kg⁻¹). The C/total-N ratio increased by 14% (15.2 to 17.3 g kg⁻¹/ g kg⁻¹). Comparison of the C and N concentration of these soils in 1976 with soil series concentrations in 1976 (Tables 3 and 5) indicates that initial C and N

concentration of these former agricultural soils is noticeably lower. Results suggest that former agricultural soils that have been reforested have greater potential for C sequestration through melanization processes as they approach a new equilibrium under forest cover.

Conclusions

The use of paired samples over an extended period of time was effective in detecting 30-year changes in mineral soil C and N. Changes in C and N were distinct to individual soil series. Increases in mineral soil C were associated with increases in the C/total-N ratio and decreases in mineral soil C were associated with decreases in the ratio of C/total-N. Given that topographic factors explained a small proportion of variability C in 1976 and none of the variability in C in 2006, we speculate that change in mineral soil C storage was influenced by changes in species composition, basal area, and stand age and only in part by topography. The watershed is characterized by notable changes in species composition and stand age which creates a gradient of biomass partitioning and substrate quality independent of topography. Decreases in soil series C concentration coincided with net decreases in basal area of forest cover plots located on the same landscape positions. Although both increases and decreases in mineral soil C were observed after a 30-year interval, mineral soil C storage on mesic sites (Atkins and Clifty soils) and sites with a *Kalmia latifolia* L. understory (Wallen/Ramsey soils) was greater than or equal to C storage on other sites within the watershed in both 1976 and 2006 suggesting mineral soil C storage will be greater in Atkins, Clifty, and Wallen/Ramsey soils over the long term. Furthermore, the C/total-N ratio of Atkins, Clifty, and Wallen/Ramsey soils is notably lower than that of the other soils on the watershed suggesting a greater degree of humification of the organic matter in Atkins, Clifty, and Wallen/Ramsey soils.

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Appendix

**Coordinates of Permanent Soil Sampling Grid Locations and Twenty Four Forest
Cover Plots
UTM - Zone 16N**

<u>Location</u>	<u>Northing</u>	<u>Easting</u>
N11E5	3,948,106.45	653,165.76
N11E6	3,948,108.06	653,265.66
N11E7	3,948,113.36	653,365.83
N11E8	3,948,114.71	653,464.52
N11E9	3,948,118.96	653,565.69
N10E4	3,948,003.96	653,070.97
N10E5	3,948,007.57	653,170.68
N10E6	3,948,007.18	653,269.92
N10E7	3,948,011.77	653,369.07
N10E8	3,948,016.97	653,467.60
N10E9	3,948,019.37	653,568.53
N9E3	3,947,899.13	652,973.69
N9E4	3,947,902.99	653,073.41
N9E5	3,947,906.50	653,174.30
N9E6	3,947,910.51	653,274.15
N9E7	3,947,914.76	653,373.74
N9E8	3,947,915.44	653,472.20
N9E9	3,947,919.99	653,573.98
N8E2	3,947,795.67	652,875.69
N8E3	3,947,799.89	652,977.20
N8E4	3,947,802.38	653,075.42
N8E5	3,947,807.62	653,179.19
N8E6	3,947,811.04	653,276.81
N8E7	3,947,814.48	653,376.20
N8E8	3,947,819.28	653,476.47
N8E9	3,947,820.24	653,575.07
N7E2	3,947,697.41	652,879.86
N7E3	3,947,699.82	652,980.41
N7E4	3,947,704.71	653,078.47
N7E5	3,947,707.58	653,180.16
N7E6	3,947,710.29	653,279.02
N7E7	3,947,712.37	653,378.91
N7E8	3,947,714.68	653,482.50
N7E9	3,947,717.63	653,578.43
N6E2	3,947,596.09	652,883.21
N6E3	3,947,599.89	652,984.64
N6E4	3,947,603.83	653,082.77
N6E5	3,947,607.73	653,181.84
N6E6	3,947,609.20	653,283.05
N6E7	3,947,612.15	653,381.50
N6E8	3,947,615.73	653,483.55
N6E9	3,947,617.92	653,581.03

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<u>Location</u>	<u>Northing</u>	<u>Easting</u>
N5E2	3,947,496.85	652,886.88
N5E3	3,947,500.11	652,984.67
N5E6	3,947,505.98	653,287.11
N5E7	3,947,512.06	653,385.04
N5E8	3,947,518.76	653,484.64
N5E9	3,947,517.91	653,586.08
N4E1	3,947,393.77	652,788.78
N4E2	3,947,400.46	652,889.00
N4E3	3,947,402.51	652,990.13
N4E4	3,947,402.51	653,087.11
N4E5	3,947,406.10	653,188.89
N4E6	3,947,408.42	653,289.84
N4E7	3,947,414.15	653,389.30
N4E8	3,947,417.40	653,487.57
N4E9	3,947,418.08	653,587.10
N3E1	3,947,302.55	652,794.95
N3E2	3,947,299.04	652,891.30
N3E3	3,947,301.62	652,990.57
N3E4	3,947,303.77	653,087.71
N3E5	3,947,307.59	653,191.62
N3E6	3,947,310.46	653,290.82
N2E2	3,947,200.38	652,895.33
N2E6	3,947,211.15	653,292.70
N1E3	3,947,103.38	652,995.74
N1E4	3,947,105.23	653,097.47
N1E5	3,947,107.98	653,195.33
N1E6	3,947,109.87	653,298.30
Plot 1	3,948,131.28	653,277.53
Plot 2	3,948,029.20	653,088.18
Plot 3	3,948,036.81	653,381.92
Plot 4	3,947,927.68	653,387.58
Plot 5	3,947,813.53	652,990.12
Plot 6	3,947,811.67	653,095.76
Plot 7	3,947,711.24	652,895.80
Plot 8	3,947,734.16	653,284.43
Plot 9	3,947,728.37	653,504.96
Plot 10	3,947,731.56	653,587.68
Plot 11	3,947,609.71	652,885.62
Plot 12	3,947,509.44	653,001.90
Plot 13	3,947,519.81	653,199.76
Plot 14	3,947,532.13	653,401.13
Plot 15	3,947,412.71	652,803.15
Plot 16	3,947,415.86	653,083.35
Plot 17	3,947,421.71	653,298.70
Plot 18	3,947,423.27	653,601.62

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<u>Location</u>	<u>Northing</u>	<u>Easting</u>
Plot 20	3,947,312.23	653,014.97
Plot 21	3,947,289.69	653,180.55
Plot 22	3,947,215.36	652,907.83
Plot 23	3,947,225.68	653,298.06
Plot 24	3,947,120.08	653,099.06