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Soil Variability And Its Influence On Plant Performance

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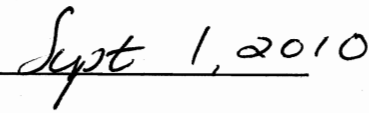
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Soil variability and its influence on plant performance

BY

Jeremy R. Klass

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Abstract

During recorded history, nearly all temperate deciduous forests in eastern North America have seen extensive agricultural land-use conversions. Persistent influences of agricultural disturbance continue to shape the diversity and composition of the plant community long after agriculture has ceased. While some measures of ecosystem functioning may recover relatively quickly from agricultural disturbance, other characteristics such as levels of organic matter, carbon, and phosphorus, may remain different from primary forests for much longer periods. The alteration of the original ecosystem due to agriculture practices has far reaching impacts on biotic and nutrient dynamics along with causing significant shifts in community composition both above and below ground.

Large scale disturbance, such as agriculture, creates the opportunity for invasive species to become established, colonize these disturbed areas and out-compete native vegetation. The potential for plant communities to be influenced through abiotic pathways, such as nutrient availability and competition, has received much attention within the scientific literature. Accumulating evidence has also identified soil biota, mainly through plant-soil feedback mechanisms, as a determinant of species composition. Feedback mechanisms have been shown to increase the rates of non-native species invasions that may impact essential successional transitions. As soils and their associated biotic communities are likely to vary over small spatial scales, the direction and magnitude of biofeedbacks may also vary spatially. However, most studies have not examined spatial variation within soil biofeedbacks.

This study was conducted at the Hutcheson Memorial Forest Center (HMFC; East Millstone, NJ, USA; 40° 30'N, 74° 34'W). This site consists of a mixed-oak old growth forest surrounded by a series of successional fields and forests and a recently abandoned agricultural field. HMFC is also the site of the Buell-Small Succession Study (BSS), a long-term study of succession in abandoned agricultural fields. The BSS consists of 10 replicated post-agricultural fields that were abandoned over several years (1958-1966) and subjected to a variety of pre-abandonment agricultural practices. Utilizing the HMFC and the BSS provided the perfect situation to evaluate soil recovery through the successional trajectory of a system. This study evaluated soil recovery within the 10 mid-successional old-fields using the abandoned agricultural field and the old growth forest as successional endpoints.

The agricultural field and old growth forest significantly differed in nearly every soil characteristic measured, possessing the highest and lowest observed values for nearly all soil characteristics, respectively. A 30% reduction in the average amount of organic matter was observed between the old growth forest and agricultural field. As carbon (C) accrual and sequestration is directly dependent upon organic matter inputs, the agricultural field showed an average of a 50 % decrease in total soil carbon while the old growth forest on average exhibited a 49 % greater soil nitrogen (N) than the agricultural field.

The mid-successional fields have not exhibited full recovery of C and N levels exhibited by the old growth forest soils, however, some recovery of total C and total N has occurred. Old growth soils have become more acidic over time exhibiting a 10 fold decrease in pH levels. Surface compaction at the surface was similar over all study sites,

but as one moves down through the soil profile, soils became less compacted through time. Old-fields exhibited some soil recovery from compaction as they were less compacted than the agricultural field, but were more compacted than the old growth at both 7.5 and 12.5 cm. The abandoned agricultural field showed consistent compaction at all depths that may have significant impacts on biological and soil ecological processes. The 10 mid-successional, old-fields showed remarkable variation between the two successional endpoints, indicating that recovery was variable across the landscape and that lasting effects of agriculture still remain after 50 years. Variation in vegetation that has developed during the old-field succession may have also played a significant role for the observed variation due to plant-soil interactions. Soil succession and vegetation dynamics appear to have interacted to jointly influence the soil recovery that has occurred since abandonment.

Secondly, the role of soil biofeedbacks associated with the major non-native species invasion that has occurred within the BSS was examined in order to assess the effect upon tree regeneration across the site. This study specifically looked at the biotic feedback effects of *Microstegium vimineum* invasion on *Acer rubrum* regeneration in a successional system. A soil inoculum experiment was conducted with replicate soil samples from 10 old fields and an old growth forest to quantify the variability of soils in regulating tree regeneration across a single landscape mosaic. Differences in plant performance were not due to the overall abundances of *Microstegium* or *Acer rubrum* across the site, but products of abiotic differences among sampling locations. Soils taken from within *Microstegium* did not have strong negative effects on the success of *A. rubrum*, but tended to have a net positive effect on seedling growth, although this effect

varied in magnitude across the site. Because of the weak biotic interaction observed and the constant and strong effect of sampling location, it appears that *Microstegium* had a spatially variable facilitative influence on seedling success. Field data also showed that *A. rubrum* seedlings were positively correlated with *Microstegium*. While initial red maple establishment may be enhanced by *Microstegium* invasion, competitive effects appear to dramatically reduce post-establishment survival. Large spatial variation in seedling performance indicates that some areas are more suitable to support *Acer* regeneration and that this variability may be due to a combination of abiotic differences, *Microstegium* invasion, soil feedbacks, and allocational shifts in biomass partitioned to leaf mass, leaf area, and specific leaf area.

Lastly, the effect of soil biofeedbacks associated with *Microstegium* has on the herbaceous forest understory within the BSS was evaluated and explored as a major mechanism for invasion success. Particular to this evaluation of soil biofeedbacks, spatial variation was also examined in context of *Microstegium* invasion within the 10 BSS old-fields. A soil inoculum experiment was conducted with soil collections from several locations to address spatial variability in biofeedbacks associated with *Microstegium* invasion. *Eupatorium* grown in sterilized soil performed better overall, irrespective of invasion. However, individual sites had remarkable variation in the direction of the soil biofeedback and significance of invasion and sterilization effects. This study documents a high degree of spatial variation in soil biofeedbacks that plants encounter in natural systems. Caution should be taken when interpreting soil-biofeedback studies that involve pooled or single-sample soil collections, until the ubiquity and magnitude of spatial variability in biofeedbacks is known.

Chapter 1.

An Assessment of Soil Recovery within 50 Years of Old-Field Succession

Introduction

Historical land use in the forested ecosystems of eastern North America is extremely complex in the extent, intensity, and duration to which human influence has transformed the landscape (Foster et al. 1998). Many landscapes in this region have experienced the successive changes of forest clearing followed by agricultural abandonment and forest recovery (Whitney 1994; Kirby and Watkins 1998; Flinn et al. 2005). This agricultural disturbance has undoubtedly altered every aspect of the original ecosystem by disrupting biotic and nutrient dynamics along with causing significant shifts in community composition both above and below ground (Foster et al. 1997). As a result, forested areas previously used for agricultural may continue to be influenced by past human land-use (Foster et al. 1998).

Past agricultural practices have the ability to generate landscape heterogeneity and produce a persistent legacy effect on successional plant communities and nutrient availability (Bellemare et al 2002; Dupouey et al. 2002). Successional recovery is dependent on the strength of lasting effects and the interactions between initial site conditions and the nature, duration, and the intensity of agricultural use (Flinn and Marks 2007). The lasting effects of agricultural land use on soils have lead to research into how agriculture generates persistent legacy effects on plant communities. Soil resources in successional communities are distributed heterogeneously and differences in plant community composition likely follow these patchily distributed soil-resources (Inouye et al. 1987; Robertson et al. 1988; Armesto et al. 1991; Collins and Wein 1998; Flinn et al.

2005). After agricultural disturbance, the plant-soil system undergoes recovery, which may function on the scale of years to decades (Peterken and Game 1984; Robertson et al. 1993; Doupouey et al. 2002; Kardol et al. 2007). The resulting landscape is therefore a mosaic of heterogeneity introduced by variation in agricultural history, disturbances and subsequent soil recovery.

Anthropogenic factors such as agriculture degrade soils leading to declines in soil quality, including physical, chemical, and biological processes (Lal 1997). Soil structure is the essential property that affects all soil processes. Agricultural practices have profound effects on soil structure that may generate persistent soil legacies through soil compaction, which can increase bulk density, alter soil pore size distribution, restrict the movement of air and water, and change nutrient availability (Grable and Siemer 1968; Barken et al. 1987; Hoffmann and Jugk 1995). The alteration of these soil characteristics not only has a strong effect on plant growth via mechanical resistance (Bengough and Mullins 1991) but also can alter cation exchange capacity and soil fertility. Soil aeration can have large impacts on soil-ecosystem functions such as the development of mycorrhizal fungi, gas exchange, microbial activity, and nutrient availability (Nadian et al. 1997; Nadian et al. 1998). For these reasons, soil compaction may have profound influences on ecosystem function and plant community structure that persists long after agriculture has ceased.

While the coarser fractions of soils determine the physical structure of soils, the physical and chemical properties are governed by the colloidal and active fractions (Buckman and Brady 1961). The colloidal fraction includes clay and humus that are crucial to nutrient dynamics. Agricultural disturbance alters the physical structure of

soils by rearranging soil particles, which disrupts their nutrient supplying ability. By disrupting physical structure and incorporating subsoil horizons, plowing alters soil chemical properties. These characteristics are further changed through erosion, leaching, and the increased oxidation of organic matter (Kalisz 1986; Anderson 1988).

The aim of this study was to document soil recovery and to quantify the persistent effects of agricultural disturbance. To assess soil recovery, we characterized soils taken from 10 abandoned agricultural fields of known disturbance history that ranged from 41 to 49 years in age, a recently abandoned agricultural field and an old growth forest. This information was used to generate a chronosequence of soil recovery using the newly abandoned field and old growth forest soils as successional endpoints. Variation in soil characteristics among the mid-successional fields was used to assess the potential for vegetation differences to generate variation in soil recovery. Due to the potential for long-term legacy effects of agriculture on soils, we hypothesized that the soils of the mid-successional old-fields would be more similar in properties to those of the recently abandoned agricultural field than the soils of the old growth forest.

Methods

Study Site

This study was conducted at the Hutcheson Memorial Forest Center (HMFC; East Millstone, NJ, USA; 40° 30'N, 74° 34'W). This site consists of a mixed-oak old growth forest surrounded by a series of successional fields and forests and a recently abandoned agricultural field (Figure 1). HMFC is also the site of the Buell-Small Succession Study (BSS), a long-term study of succession in abandoned agricultural fields. The BSS

consists of 10 replicated post-agricultural fields that were abandoned in pairs, every-other year over an eight-year span (1958-1966) and subjected to a variety of pre-abandonment agricultural practices that involved soybeans or a combination of soybeans and sorghum (Pickett 1982). Six of the fields were used to grow row crops (C3, C4, C5, D1, D2, and D3) and four were used for hay production using *Dactylis glomerata* (C6, C7, E1, and E2). The hay fields were utilized as hay fields only a few years pre-abandonment and all fields, including those indicated as hay fields here, were used for row crops for the majority of their agricultural history. Preliminary comparisons of row crop and hay fields showed no effect of pre-abandonment conditions, and subsequently were dropped from further analyses. Each field contains 48, 0.5 × 2.0 m permanently marked plots that are sampled for percent cover and the number of woody stems every year, with sampling switching to alternate years in 1979. Early studies showed that there were no significant differences in nitrate, ammonium, nitrogen mineralization potential, phosphorus, calcium, potassium, soil structure, soil texture, or organic matter between fields of different ages (Robertson and Vitousek, 1981; Robertson, 1982).

HMFC is located in the Piedmont Plain of the Appalachian province that forms a band across the middle of the state with altitudes varying between 3.5 to 76 meters (Kümmel 1940). The bedrock geology of the area is of the Upper Triassic Brunswick formation with the lithologic features of the interfingering Newark group including the Stockton, Lockatong, and the Brunswick formations that underlay the soils of the region (Ugolini 1964). The Brunswick formation is the predominant formation and is represented by soft red shale consisting of siltstone with a high percentage of clay and interbedded sandstone (Kümmel 1940). The soils that have developed are classified

under Penn soils and have been described as being shallow, possessing high clay content, containing shale fragments, droughty, poorly developed, and not very fertile (Ugolini 1964; Pickett et al. 2001).

Approximately 85 % of the study area resides on Penn or similar soils. These Penn channery silt loam soils are characterized by fine-loamy residuum weathered from acid reddish shale, siltstone, and fine-grain sandstone on 2-6 % slopes, being well drained with low water availability (Web Soil Survey, NRCS March 5, 2010). It is worth noting that fields C7, C6, C5, part of C4, E1, and D2 are all on the same Penn channery soil type. Fields C3, D3, and part of C4 are on similar Penn soils. The majority of fields E2 and D1 are also located on these Penn soils as well, with a small portion of the SE corner of field D1 on Lansdowne silt loam that is characterized by 0-2% slopes and a fine-loamy till derived from sandstone and shale over residuum weathered from sandstone and shale, where E2 is bisected by Klinesville channery loam that is characterized by 6-12% slopes, possessing a fine-loamy residuum weathered from shale (Web Soil Survey, NRCS March 5, 2010). The abandoned agricultural field is entirely on Penn silt-loam soils, the same soils that characterize the majority of the study area. The old growth forest has developed on the same Penn silt-loam soils and is bisected by a narrow band of Reaville silt loam that is characterized by 0-2% slopes and derived from interbedded fine-grained fine-loamy residuum weathered from sandstone and siltstone and/or shale that is somewhat poorly drained and has a low water availability (Web Soil Survey, NRCS March 5, 2010). There are only slight variations in soil texture, drainage, and depth across the site (Ugolini 1964)

Sampling Design

Eight soil core samples were taken using a 1.4cm diameter soil probe to a depth of 10cm around 20 individual BSS plots in each field. Any leaf litter was removed prior to soil sampling. The samples were then pooled by plot and stored in sterile bags. The plots were chosen within each field to capture the full spatial extent of each field. Twenty sets of soil samples were also randomly taken from both the old growth forest and the recently abandoned agricultural field, resulting in a total of 240 pooled samples. The old growth forest and the recently abandoned agricultural field were sampled March 11 -13, 2006 and the BSS plots were sampled July 25-27, 2006.

Upon arrival in the laboratory, samples were transferred into aluminum storage tins and allowed to air dry. Samples were placed in an oven over night at 105°C to assure all moisture was removed and weighed and sieved using a 1.4 mm mesh sieve to determine coarse fraction and bulk density. Sieved samples were then used for analysis of pH (1:2 slurry in deionized water), organic matter using the dry-ash method (375°C for 1 hour, 575° for 6 hours) and soil texture analysis using the hydrometer method (see Carter 1993 for full description of methods used). To evaluate spatial variation in soil structure among sampling locations, soil compaction was determined for each BSS plot sampled, the old growth forest, and the agricultural field using a Fieldscout[®] compaction meter (Model SC 900; Spectrum Technologies, Inc., Plainfield, IL) to a depth of 12.5 cm. Compaction measurements were not taken for fields C3, D3, and C7. All values of soil measurements excluding bulk density were natural log transformed to conform to the assumptions of analysis of variance (ANOVA). Soils from each plot were also analyzed for total C and N using an elemental analyzer (Carlo-Erba, Eager 300, ran at the Institute

of Ecosystem Studies, Millbrook, NY). Data were analyzed using a one-way ANOVA followed by Duncan's post hoc tests to evaluate differences among sampling locations.

Field age was correlated with all soil characteristics using Spearman's non-parametric correlation in order to assess soil development over time. In this analysis, the recently abandoned agricultural field was set as the youngest and the old growth forest as the oldest, where the agricultural field was assigned an age of 0 and the old growth forest was set as 300 years old (the approximate age of the oldest trees). As non-parametric tests were used, the actual value assigned for age would not affect the results. The coefficient of variation was also calculated to assess the heterogeneity of each soil characteristic within each field. The coefficient of variance was then used in the same non-parametric analysis to assess temporal changes in spatial heterogeneity.

To determine whether the presence of the old growth forest affected soil recovery, distance from the old growth edge was calculated for each plot and used in a correlation analysis with soil variables. Only those fields with single continuous borders with the old growth forest were used for this analysis. Based on those criteria, fields C4, C5, C6, D1, and D2 were used in correlation analyses with distance from the edge.

Interaction of Soil Characteristics and Vegetation Dynamics

To assess the influence of agricultural legacy effects of the soils on the developing plant community, a principal components analysis (PCA) was employed to condense the soil variables into a few composite axes of variation. Soil compaction data were not used in this analysis as they were not available for all fields. Sample coordinates obtained from the PCA was then used in a multivariate analysis of variance

(MANOVA) to analyze differences among fields. Then, using the most recent species cover data from the BSS, a non-linear multi-dimensional scaling (NMS) ordination was used to quantify vegetation composition among the 10 successional fields. The NMS vegetation coordinates were used in a correlation analysis with the axis scores from the soil PCA to assess the relationship between vegetation composition and soil characteristics.

Results

Soil Characteristics

All soil characteristics showed significant differences among sample locations (Figure 2(3)) with the mid-successional fields consistently intermediate between the old growth and newly abandoned field except for bulk density. Bulk density varied significantly among sampling sites ($F_{11, 228} = 6.225, p = < 0.001, R^2 = 0.241$). While the newly abandoned agricultural field consistently showed the lowest values of bulk density among all sampling sites (1.29 g/cm^3), the old growth forest soils were found to be similar to most of the successional fields (C7, C3, C5, D2 & E1). Field D1 had the highest observed bulk density among all sampling locations (1.46 g/cm^3).

Coarse fraction varied significantly among location and also followed topographic position and relative location to one another (Figure 2; $F_{11, 223} = 9.135, p = < 0.001, R^2 = 0.310$). Fields C3, C5, C6, and C7 all had similar coarse fraction values and were also similar with D1 and D2 that ranged from a minimum average of 34.48 g and maximum of 45.60 g. The recently-abandoned agricultural field had the lowest observed values for coarse fraction (23.37 g) and was similar to the old growth soils (26.58 g). Fields C3 and

E2 had the highest observed values among all fields and were only similar to one another and ranged from an average of 56.69 g to 77.84 g.

pH showed the greatest separation among sampling locations, where the old growth forest consistently had the lowest observed values, with an average of 4.68 and the agricultural field had the highest overall average of 5.88 (Figure 2; $F_{11, 217} = 31.809$, $p = < 0.001$, $R^2 = 0.617$). Only two combinations of adjacent fields (C3/C4 and E1/E2) had similar values in pH.

Organic matter also showed a high level of variation across the site, differing substantially among adjacent sampling locations (Figure 2; $F_{11, 218} = 17.350$, $p = < 0.001$, $R^2 = 0.467$). The highest values of organic matter were found in the old growth soils (10.02 %) with the lowest values observed in field C7 (7.10 %). The agricultural field was similar to fields C3, C6, D1, and D2 ranging from an average of 7.64 % to 7.73 % organic matter. Fields E1 and E2 had proportionately high levels of organic matter (9.15 % and 9.02 % respectively), where observed values in fields E1 reached a percentage of organic matter statistically similar to the old growth forest soils. Adjacent fields often did not have similar values in organic matter. For example, fields C6 and C7 had different levels of organic matter with C6 and D1 being more similar.

Nutrient Analysis

The total carbon content of soils varied significantly among sampling locations (Figure 3; $F_{11, 216} = 33.374$, $p = < 0.001$, $R^2 = 0.630$). The lowest total carbon values were found in the agricultural field, ranging from 1.25 % to a high of 3.76 % in the old growth forest soils, a 2-fold decrease in total soil carbon. Soils taken from the mid-

successional fields were intermediate in carbon content. The carbon content of soils largely differed even among adjacent fields. Nitrogen analysis yielded similar results where fields exhibited significant variation, often among adjacent fields ($F_{11, 216} = 25.396, p = < 0.001, R^2 = 0.564$). Nitrogen levels ranged from a low of 0.13 % in the newly abandoned field to a high of 0.26 % in the old growth forest. Nitrogen levels within mid-successional fields were again higher than those in the recently abandoned agricultural field but were lower than those of the old growth soils (Figure 3). The newly abandoned agricultural field had the lowest C:N ratio while the old growth forest soils possessed the highest. The BSS fields, however, exhibited little variation in C:N ratio with nearly all fields similar at a level that ranged from 11.76 to 12.36 except for field E1 which was slightly higher with a value of 13.34 (Figure 3).

Soil Compaction

Compaction at the soil surface was not significantly different across sampling locations (Figure 4; $F_{8, 167} = 1.819, p = 0.077, R^2 = 0.080$). However, as depth increased significant differences in compaction developed across sites. The old growth soils were 54 % and 45 % less compacted at depths of 7.5 cm and 12.5 cm, respectively, when compared with the agricultural field. At the 7.5cm depth ($F_{8, 167} = 5.499, p = < 0.001, R^2 = 0.209$), the agricultural soils showed the greatest amount of compaction and the old growth soils had the lowest observed values. Compaction at the 7.5 cm depth was similar among BSS old fields, with values intermediate between the agricultural field and the old growth forest. At a depth of 12.5 cm, significant differences mainly existed between all former agricultural fields and the old growth forest ($F_{8, 167} = 4.700, p = < 0.001, R^2 =$

0.184).

Soil Texture

Soil texture varied across the site, with differences in sand ($F_{11, 216} = 3.158$, $p = 0.001$, $R^2 = 0.139$), silt ($F_{11, 216} = 2.357$, $p = 0.009$, $R^2 = 0.107$), and clay ($F_{11, 216} = 5.868$, $p = < 0.001$, $R^2 = 0.230$) (Figure 5). Field D1 had the lowest percentage of clay and was similar to the adjacent D2 field along with C3, E1, C5, D2, and the old growth forest. Adjacent fields often varied in clay content. For example, the two E fields were significantly different from one another as were the C fields. The agricultural field had the highest amount of clay while the old growth forest soils had some of the lowest clay content observed in the texture analysis. The lowest percentage of sand was found in field D3 and the highest in fields E1 and D2. The amount of sand among adjacent sampling locations tended to be more consistent than other soil characteristics. The C fields tended to be similar with respect to the percent sand content to each adjacent neighbor (e.g. fields C3 with C4 with C5 etc.). The two topographically lowest fields, D1 and D2, were also similar to each other. Although D3 was significantly different from fields D1 and D2, it had values similar to C3 which occur along the same upland ridge. Proportionately, sand made up a large fraction of the texture content of the agricultural field. The old growth forest soils did not differ from the sand and silt content of most sampling locations.

Influence of Forest Edge on Soil Characteristics

Proximity to the old growth forest had little influence on the soils of the BSS

fields as there were few significant correlations between distance from the forest edge and soil measurements (Table 1). The only significant relationship between distance from the edge and coarse fraction was in field C4 where coarse fraction increased as distance from the forest edge increased. Percent organic matter was related to distance from the edge only in field C5. The other significant correlation observed was that of soil compaction at a depth of 7.5 cm in field C4. Although most correlations were non-significant, large variation in the direction and magnitude of responses of soil variables to the edge existed among adjacent fields.

Soil recovery over time

The age of the soils was relatively unimportant in explaining differences among the collection sites. The correlation of age with the soil characteristics showed that only pH, compaction at 7.5 cm, and percent clay significantly decreased over time (Table 2). The correlation of age with the coefficient of variance showed only a significant increase in the variation in percent clay over time.

Multivariate analyses of soil and vegetation

The PCA analysis of soil variables extracted three significant axes that accounted for approximately 66 % of the total variation in the data. The MANOVA showed significant differences among sampling locations with each significant PCA axis (Overall MANOVA: $F_{11, 221} = 49.30, p = \leq 0.001, R^2 = 0.7209$; Axis 1: $F_{11, 221} = 49.30, p = \leq 0.001, R^2 = 0.7209$; Axis 2: $F_{11, 221} = 3.21, p = \leq 0.001, R^2 = 0.1411$; Axis 3: $F_{11, 221} = 9.39, p = \leq 0.001, R^2 = 0.3296$). Fields were plotted in ordination space for the first two PCA axes (54.16 % of

the cumulative variation) which showed marked variation among sampling locations. As in the univariate analyses, the mid-successional fields were intermediate in characteristics between the agricultural field and the old growth forest. Within ordination space, there appears to be a high degree of variation among the 10 successional fields with some overlap, however, all fields are more similar to the old growth forest soils than the agricultural field soils. To interpret the differences among fields, loadings from the PCA were plotted on the same ordination axis. Based on this analysis, successional development was most strongly associated with PCA axis I and showed a decrease in bulk density and increase in fertility over time. Changes in texture were largely independent of disturbance history.

Contemporary vegetation composition was also associated with soil characteristics. The correlation between vegetation NMS 1 axis and soil PCA 1 axis was significant (Figure 7; $R = -0.07741$, $p = < 0.001$). Fields that had higher C, N, and organic matter (E1, E2, C5, C4, and D3) were associated with liana species such as *Rubus flagellaris*, *Lonicera japonica*, *Rubus allegheniensis* and the tree *Juniperus virginiana*. Fields that had lower C, N, organic matter and pH (Fields C6, C7, and D1) were associated with *Vitis spp.*, *Ranunculus abortivus*, *Circaea lutetiana*, *Junglans nigra*, *Quercus velutina*, and *Microstegium vimineum*.

Discussion

Many landscapes worldwide bear the imprint of historical land use (Hermy and Verheyen 2007). The effects of agricultural land use on ecosystems may vary in persistence and magnitude (Flinn and Marks 2007). For example, Dupouey et al. (2002)

found that soil physical and chemical characteristics remained in an altered state from Roman agriculture, spanning centuries. Other studies have shown that legacies of agriculture within old-field successional systems up to a century or more (Koerner et al. 1997; Wilson et al. 1997; Verheyen et al. 1999; Compton and Boone 2000). While some measures of ecosystem functioning may recover relatively quickly from agricultural disturbance, other characteristics such as levels of organic matter, carbon, phosphorus, and the homogenization of soil properties may remain different from primary forests for much longer periods (Flinn and Marks 2007). Recovery of former agricultural systems in less than 100 years has also been shown to occur (Kalisz 1986; Compton et al. 1998; Dzwonko 2001; Graae et al. 2003). Our data suggests that the fields of the BSS still exhibit evidence of disturbance after 49 years in nearly all soil characteristics. However, the legacy that agriculture left has begun to diminish as soil recovery has occurred to varying degrees across the BSS site.

When compared with the undisturbed old growth forest soils, it is evident that land-use has triggered a series of successional changes that occurs through the varying levels of soil organization through time (Christensen 2001). It is not possible to determine whether the differences documented in soils among BSS fields were related to past land use or were a product of the successional vegetation which has developed on the site. Large variation in vegetation composition has arisen among the BSS fields as a result of different patterns of assembly through time (Myster and Pickett 1994). The soil heterogeneity within the BSS may be the result of differing plant composition that lead to variation in vegetation-mediated soil conditions (Finzi and Canham 1998 a; Finzi et al. 1998b; Verheyen et al. 2003). Robertson and Vitousek (1981) and Robertson (1982)

compared soils from other HMFC fields in the 1st, 4th, and the 19th year since abandonment along with the old growth forest where significant changes were observed in soil physical and chemical properties as succession progressed. In these two studies, there was little variation within and among the successional seres, but significant decreases in Ca, Mg, bulk density and pH and significant increases in organic C, total N and C:N ratios were observed in old growth forest soils when compared with successional fields (Robertson and Vitsousek 1981).

The correlation between the vegetation NMS and the soil PCA suggests a linkage between the structure of the vegetation and soil properties. Because plants have little if any effect on soil physical properties outside of aggregation and aeration, differences in vegetation would only influence total C, total N, organic matter content and soil pH in this study. This can be seen in the association of soil texture to PCA axis II, which was not associated with vegetation composition. Fields E1 and E2 are characterized by extensive *Juniperus virginiana* stands with persistent open canopies that have accumulated more C and N due to decreased decomposition rates in open areas and accumulation of recalcitrant litter. The linkage of C and N accrual with *J. virginiana* is similar to findings made by McKinley and Blair (2008) and may lead to increased net N mineralization and decreased C mineralization in these fields. Fields C4 and C5 also had *J. virginiana* mixed with hardwoods which established early after agriculture had ceased. *Juniperus virginiana* is a pioneer species that is often the first tree to repopulate cleared or damaged land. As the E fields did not share a border with the old growth forest, they were relatively isolated from seed sources for many tree species, leading to a dominance of the bird-dispersed *J. virginiana*. Therefore, variation among fields in vegetation due to

pre-abandonment conditions (Myster and Pickett 1990) or dispersal limitation may have contributed to the differences in soil. It is likely that soil recovery and vegetation dynamics have interacted to jointly influence each other as the plant community developed over time.

Agricultural practices that alter fertility through nutrient transfers (i.e. grazing, tillage, or spread of animal manures) can lead to long-term modifications of soil organic matter (Koerner et al. 1997). Due to the continual removal and transfer of plant biomass, along with repeated tillage, large stores of carbon were removed from this system and these deficiencies remained 50 years post-abandonment. Agricultural practices, such as tillage, have been shown to decrease soil microbial biomass and organic matter by 50 to 75 % (Cambardella and Elliot 1994). In this study, a 30 % reduction in the average amount of organic matter was observed between the old growth forest and agricultural field. Because of organic matter's influence on soil properties and microbial activity, and consequently on nutrient availability, this alteration is critical to soil functioning (Buckman and Brady 1961). Because the BSS fields have not achieved full recovery in soil organic matter to pre-disturbance levels, the deficiencies exhibited by the fields in soil organic matter may have far reaching impacts on nutrient cycling, microbial activity, plant establishment, and other ecological processes.

The accrual of soil carbon is directly dependent upon the inputs provided through organic matter (Hooker and Compton 2003). Accretion of organic matter within the BSS fields has not reached the levels of the old growth forest soils. This may be due, in part, to the historical lack of a developed canopy structure that would favor organic matter accumulation (Kalisz 1986). Because of the proportionately low organic matter

availability within certain old field soils such as fields C3, C6, C7, D1, and D2, organic material might not be available for decomposition due to adsorption on clay minerals or by entrapment in soil structures which cannot be entered by micro-organisms (VanVeen and Kuikman 1990). The latter, would only be magnified by increased soil compaction. Therefore, full system recovery to pre-disturbance functionality may be repressed for many years as these inhibitors become saturated.

Recovery of soil organic matter and nutrient dynamics may encounter a lag phase between plant production and soil C storage, where long-term accumulation may take up to 50 years and as long as 200 years (Hamburg 1984; Burke et al. 1995; Compton and Boone 2000). The 2-fold decrease in total soil carbon observed within the agricultural field provides insight into the initial deficiencies the BSS fields would have encountered post-abandonment and tell us two things. One, the low levels of C exhibited by the agricultural field shows that agriculture has used these soils as a source of carbon, where continual removal of crop yields stripped C away from the system. Two, large spans of time are required for successional systems to replace the carbon lost via agriculture. The mean and variance of organic matter increased over time, litter inputs or other influences from the old growth forest had almost no effect on soils as there were few edge effects detected. This suggests that internal litter inputs and vegetation influences are much more important to soil recovery and that variation in local vegetation may be an important driver of soil recovery.

Our results from the pH analysis follow other studies that show as forests develop on old fields, soil acidity increases (Thorne and Hamburg 1985; Richter et al. 1994; Hooker and Compton 2003). With increases in soil acidity, the exchangeable stores of

Ca and Mg would markedly decrease over time due to accumulation in plant biomass (Robertson and Vitousek 1981; Richter et al. 1994). However, rapid losses of cations should also occur through leaching and can lead to depreciable declines over time. A comparison of pH levels between the agricultural field with the average pH of 5.88 and the old growth forest with an average pH of 4.68 shows a 10 fold decrease. The large variation in pH among BSS fields could have been influenced by differences in plant composition and litter chemistry across the site. For example, through large litter inputs of *Juniperus virginiana*, soils can become more acidic as decomposition occurs. Continual litter inputs of the oak-hickory forest over time also may have played a significant role in the acidification of HMFC soils through large tannin inputs (Thorne and Hamburg 1985).

As in many other studies, this study showed an increase in nitrogen with succession (Robertson and Vitousek 1981; Gleeson and Tillman 1990; Compton and Boone 2000) where old growth forest soils on average exhibited a 50 % greater soil N than the agricultural field. These findings are consistent with the studies performed by Robertson and Vitousek (1981) and Robertson (1982) at HMFC that showed no differences among the successional fields in N mineralization and nitrification rates, with increased N mineralization and nitrate production in the old growth forest soils. While the BSS fields have not reached N levels exhibited by the old growth forest soils, they have shown some recovery of total N. As suggested by Gleason and Tilman (1990), initial low levels of nitrogen within the old-fields may have driven community dynamics within the assembling plant communities. Subsequent variation in nitrogen among

sampling locations could be the result of interspecific differences in litter production and the rate of litter decomposition (Finzi et al. 1998c).

In analyzing the physical changes that agriculture imposed, it was not surprising that soils from the recently abandoned agricultural field had the highest percentage of clay. The high clay content of the abandoned agricultural field is most likely due to constant tillage that would incorporate the substratum into the upper soil layers. This continual mixing of lower horizons could contribute to higher pH values due to the reincorporation of Ca^+ and other cations that have leached down through the soil.

Agricultural practices increase soil compaction, which may reduce root growth, gas exchange, and nutrient uptake (Nadian et al. 1997). Compaction in this system was dependent on position within the soil profile as time since abandonment increased. There were no differences in surface soil compaction among sampling locations. Deeper in the soil profile, soils became less compacted through time. The BSS fields show some soil recovery from compaction as they were less compacted than the abandoned agricultural field, but were more compacted than the old growth forest soils at both 7.5 and 12.5 cm depths. The newly abandoned agricultural field showed consistent compaction at all depths, while the old growth forest soils became significantly less compacted with depth. The greater compaction observed in the agricultural field and the BSS fields could have large impacts on phosphorus uptake decreased via fungal hyphae, mycorrhizal biomass and root surface area (Nadin et al. 1998). Coupled with the inhibitory effects of compaction on root-fungi interactions, plants initially colonizing abandoned fields may have difficulty establishing due to the increased resistance through the soil profile. There appeared to be a threshold depth at 12.5 cm, where there was small variation in

compaction among the BSS fields and the agricultural field, perhaps indicative of tillage effects.

Chapter 2.

Spatial Variation in Soil Biotic and Abiotic Controls on Tree Regeneration in Old Field Succession

Introduction

Central to plant ecology is the interaction between plants and soil, where physical and chemical characteristics of soils determine plant community composition and structure. Biological interactions also play an integral role, where soil biota affects plant biomass production, species diversity, and succession (Reinhart et al. 2003; Callaway et al. 2004a; Mitchell et al. 2006). Plant-soil interactions can be divided into three main categories: physical, biogeochemical, and biotic. Each one of these interactions can individually affect plant communities, yet work together as one intricate, complex whole (Ehrenfeld et al. 2005). These three interactions are organized into a dynamic system that is influenced by geologic history, climate, topography, and local soil biota (Nikiforoff 1941). After a large-scale disturbance, such as agriculture, the plant-soil system undergoes successional recovery which may require years to decades to occur (Kardol et al. 2007). These successional processes may create a plant and soil community similar to the original community or can result in an alternative state (Westoby 1980). The patterns of soil and community development after the disturbance have the potential to vary from the local to landscape scale. Soil resources in successional communities are distributed heterogeneously due to agricultural practices such as plowing and tillage where differences in plant community composition are likely to follow these patchily distributed soil-resources (Inouye et al. 1987; Robertson et al. 1988; Armesto et al. 1991; Collins and Wein 1998; Flinn et al. 2005). The impact of past agricultural uses can create differences among landscape units that may persist for 300 years or more (Peterken and Game 1984;

Robertson et al. 1993; Doupouey et al. 2002). The resulting landscape is therefore a mosaic of heterogeneity introduced by variation in agricultural history, disturbances and subsequent soil recovery.

Plant- Soil Interactions

Soils can be thought of as products of ecosystem engineering where the plant community interacts with the physical, chemical, and biotic components of soils (Van Breemen and Finzi 1998). Plants can directly influence the physical state of soils by altering water dynamics and soil stabilization, which then has the ability to have large impacts on soil processes (Angers and Caron 1998). Plants also have an effect on biogeochemical cycling by providing soil organic matter inputs such as leaf and root biomass, root exudates or by changing nitrification and carbon fluxes (Robertson et al. 1988; Finzi et al. 1998c; Van Breeman and Finzi 1998; Ehrenfeld et al. 2001). These processes can then, in turn, alter the plant community, forming a reciprocal interaction.

Some of the most interesting plant-soil interactions are with the soil biota, which includes the entire community of pathogens, parasites, herbivores, and mutualists that may form a biotic feedback loop (Bever et al. 2001). These feedbacks are common, often negative, and can be generated by a diversity of soil organisms (Bever et al. 1997; Bever 2003). Soil biofeedbacks have the ability to affect the establishment, abundance and species diversity of plant communities (Van der Putten et al. 1993; Molofsky 1994; Mills & Bever 1998; Molofsky et al. 2001; Klironomos 2002; O'Hanlon-Manners & Kotanen 2004; Reinhart et al. 2003; 2005). The composition of soil organisms involved in creating the feedback response (i.e. nematodes, pathogenic bacteria, fungi, etc.) will

determine the net influence of the soil community. For example, VAM fungi, while associated broadly with plant hosts, have host-specific growth responses that can influence the outcome of competitive interactions between plant species (Klironomos et al. 2002).

Many exotic plant species have the ability to alter the chemical and biological properties of soils (Ehrenfeld et al. 2001; Kourtev et al. 2002; Wolfe and Klironomos 2005; Bezemer et al. 2006) which can indirectly modify the plant community through the generation of positive or negative biofeedbacks. Plant-soil feedbacks may influence changes in species assemblages after exotic plant invasion. The magnitude and direction of the net effect of the plant-soil feedbacks have been shown to play a pivotal role in regulating both the population dynamics and impacts of non-native plant species (Callaway et al. 2004a; Wolfe and Klironomos 2005). The accumulating body of evidence has expanded the conceptual framework of invasion to include indirect effects of soil biofeedbacks (Kourtev et al. 2002; Reinhart et al. 2003; 2005; Callaway et al. 2004b; Reinhart and Callaway 2005; Levine et al. 2006; Mitchel et al 2006; Reinhart and Callaway 2006; Eppstein and Molofsky 2007).

Spatial Variation & Legacy Effects

As soil properties and biotic communities are likely to vary over small spatial scales (Bever 1994; Molofsky 1994; Bever et al. 1997), it is likely that the net direction and magnitude of biofeedbacks will also vary dramatically. Spatial variation in biofeedbacks may be indicative of changes in the relative abundances and species composition of the soil biota. Furthermore, the soil biota may vary temporally; the

successional development of an ecosystem may be depend on the plant-soil interactions at any moment in time due to changes in the abundance and species composition of the soil community (Kardol et al. 2006). Changes in the presence and abundance of the organisms that generate a feedback may lead to dramatic alteration of the soil-plant interaction across a heterogeneous landscape.

Successional community development may be influenced by both soil legacies from previous land usage and soil biofeedbacks. As both of these influences may vary spatially across a landscape, their net influence on plant community dynamics may also vary. This is particularly important if key successional transitions are affected.

Successional dynamics are dependent on the strength of lasting effects and the complex interactions between initial site conditions and the persistent legacy generated by the nature, duration, and the intensity of past agricultural use (Flinn and Marks 2007). With the large influence of non-native plant invasions on community structure above and below ground, it is crucial to assess the impacts of invasion upon key transitions within dynamic plant communities. The transition from a shrub-grass dominated system to a forested system results in large shifts in species composition and numerous soil processes. Alterations in physical, biological, and chemical soil process during the transition can occur, such as nutrient cycling and increases in root mass and soil penetration that result in modifications to the water use regime, and changes in the soil community composition. Following this transition, continued tree regeneration is vital to the maintenance of a forested system.

In order to study the invasion of *Microstegium vimineum* (Trin.) and its effects on *Acer rubrum* (L.) regeneration across a successional system through the alteration of the

soil community, and to address the theory of spatial partitioning of soil biofeedback mechanisms, a soil inoculum experiment was conducted. To specifically investigate the spatial aspect of soil biofeedbacks associated with *Microstegium*, replicate soil samples from several locations to quantify the role of soils in regulating tree regeneration was investigated within a single landscape mosaic. The results from the inocula experiment were compared with natural patterns of *A. rubrum* regeneration across the site.

Methods

Study Site and Species

The invasive species examined in this study was *Microstegium vimineum*, (Japanese stiltgrass, hereafter referred to as *Microstegium*) an exotic grass introduced into the United States from Asia. *Microstegium* was first identified in the United States in Knoxville, Tennessee in 1919 and was recorded in New Jersey by 1959 (Fairbrothers & Gray 1972). *Microstegium* can rapidly invade and dominate areas disturbed by natural and anthropogenic processes (Cole & Weltzin 2005) and can form extensive monocultures that reduce the abundance of native herbs and alter ecosystem properties (Hunt and Zaremba 1992; Gibson et al. 2002). Although this plant is a C4 grass, it is shade-tolerant, allowing it to invade forest understories. This species exhibits high morphological plasticity to light conditions and soil nutrients contributing to its regional success (Claridge and Franklin 2002; Cheplick 2005; Cole and Weltzin 2005; Leicht et al. 2005). *Microstegium* also alters nitrogen cycling inducing a change in the composition and/or activity of the microbial community, making this species a good candidate to investigate for biofeedbacks (Ehrenfeld et al. 2001).

This study was conducted at the Hutcheson Memorial Forest Center (HMFC; East Millstone, NJ, USA; 40° 30'N, 74° 34'W). This site consists of a mixed-oak old growth forest surrounded by a series of successional fields and forests (Figure 1). Within this site, *Microstegium* had become widespread, initially establishing within the old growth forest. HMFC is also the site of the Buell-Small Succession Study (BSS), a long-term study of succession in abandoned agricultural fields (Pickett 1982). The BSS consists of 10 replicated post-agricultural fields that were abandoned over several years (1958-1966) and was subjected to a variety of pre-abandonment agricultural practices (Pickett 1982).

Six of the fields were used to grow row crops (C3, C4, C5, D1, D2, and D3) and four were used for hay (C6, C7, E1, and E2). Each field contained 48 0.5 × 2.0 m permanently marked plots that were sampled for percent cover and the number of woody stems every year, with sampling occurring in alternate years since 1979. By 2005, *Microstegium* had spread from the old-growth forest into the adjacent BSS fields, dominating large areas. Since the invasion of the BSS fields, *Microstegium* has exponentially increased in both cover and frequency.

In the Eastern U.S., *Acer rubrum* is often the first hardwood tree species to colonize successional fields and riparian areas and is increasing in dominance throughout northeastern deciduous forests (Rankin and Pickett 1989). The establishment of *A. rubrum* is therefore extremely important within successional systems, especially the HMFC. *Acer rubrum* is the dominant successional hardwood in the BSS, occupying approximately 25% of all plots. This tree species initially invaded the fields of the BSS within the first year of abandonment and continued to establish itself within the vegetation 12 years later (Buell et al. 1971; Rankin and Pickett 1989). While *A. rubrum*

is a canopy dominant, there is little regeneration occurring, with few plants making it past the seedling stage.

Experimental design

To assess the importance of soil biofeedbacks and landscape variation regulating the establishment of *A. rubrum*, soils were collected from each of the BSS fields and the old growth forest. Collecting samples this way allowed for detection of variation within the soil in replicated communities across the same landscape. In each sampling location, eight soil cores were randomly collected using a 7cm diameter soil auger to a depth of 10 cm. Half of the samples were taken from areas invaded by *Microstegium* and the other half from areas with no apparent sign of invasion. Samples were collected on March 21, 2006, placed in sterile collection bags, and kept on ice during transport to ensure all components of the soil community remained dormant (Reinhart et al. 2005). In the laboratory, soil samples were kept refrigerated until processed. Samples were sieved using a 1.4 mm mesh sieve and pooled within collection location (each individual field) and treatment (invaded/uninvaded) to ensure even soil inocula.

Half of each pooled sample was autoclaved (25 minutes, 252° F, at 17 psi) to remove the influence of the microbial community. Sterilized soil cone-tainers (Stuewe & Sons, Inc., Corvallis, OR) were filled approximately two-thirds full with sterile potting mix (Sunshine®, SunGro Horticulture, Ltd, Canada), inoculated with 10 mL of soil inocula, which was incorporated into the sterile mix, and capped off with an additional 5 cm of sterile potting mix. A small amount of inoculum (10 mL) and a potting mix with added nutrients was utilized to minimize the influence of any variation among collection

locations in nutrient levels and to minimize any pulse of nutrients released from autoclaving inocula. Three seeds of *A. rubrum* were planted within each inoculated container. After germination, the largest seedling was selected and all other seedlings removed. Containers with no germinated seedlings were replanted after 1 week. At the end of a 90-day growing period, plants were harvested to determine above ground biomass. Leaf area (LA) was determined using a Li Cor LA2000 (Li-Cor Inc, Lincoln, NE). Leaves were dried at 45.6° C, to determine dry leaf mass (LM). Using LA and LM, specific leaf area (SLA) was calculated as cm^2/g .

As many of the response variables were correlated, MANOVA was used to assess the treatment effects of collection location, soil sterilization, and *Microstegium* invasion for all response variables. As a follow-up, individual ANOVAs were used to assess treatment effects on each response separately.

Regeneration patterns

Data from the BSS were used to assess the pattern of the distribution of red maple across the site in the most recent sampling year (2006). ANOVA followed by a Duncan's post-hoc test was used to test for variation among fields in *Acer rubrum* cover, stem density, and *Microstegium* cover. A correlation analysis was used to test for an association between *Acer rubrum* and *Microstegium* cover. Using the number of stems per plot and the cover of *Microstegium*, a correlation analysis was used to examine the association between the establishment of red maple and the *Microstegium* invasion.

Results

Variation among locations

The multivariate analysis showed no influence of the biotic components of invasion and sterilization with the only significant treatment effect being sampling location (Table 3). Despite the correlation among the response variables, they showed different individual responses to the experimental treatments than those observed in the MANOVA (Figure 8). Like the MANOVA, univariate analyses indicated a consistent effect of sampling location. Measures of plant performance had large variation across sampling locations, even when locations were spatially close (Figure 9; a, b, & c). For example, soil from field C5 consistently produced seedlings with lower biomass, LA, and LM, where field C6, directly adjacent, consistently produced larger plants and bigger, heavier leaves (Figure 8). Field E2 usually had the highest observed values of all fields for the four response variables. Despite the large variation among sampling locations observed, the general trend in response variables was similar across sampling locations. Total biomass, leaf area, and leaf mass were all positively correlated with one another, while SLA was negatively correlated with total above ground biomass and LM (*all p* < 0.01). Furthermore, soils taken from the old growth forest consistently did not differ from most fields in all measures of plant performance.

Multivariate analysis showed a non-significant interaction of the invasion and sterilization treatments (*p* = 0.053), but when broken down into individual responses, total biomass and LA had significant interaction (Table 3; Fig. 10). Sterilization of invaded soils led to a decrease in seedling biomass while sterilization of uninvaded soil increased biomass (Figure 10). The increased biomass in the sterilized uninvaded soils

shows an antagonistic background effect of the uninvaded soil community upon *A. rubrum*. SLA did not show a similar response. The individual univariate analyses also revealed that among different measures of plant performance, small, subtle variation in plant allocation occurs. Biomass and LA showed a significant interaction of the invasion \times sterilization treatment ($p = 0.026$ & $p = 0.003$; Figure 10). LM and SLA did not have a significant invasion \times sterilization interaction; however LM did follow the same general trends of biomass and LA.

Seedling response to the invasion of *Microstegium* varied from one field to the next. LM was the only variable that responded significantly to the sampling location \times *Microstegium* invasion and the sampling location \times soil sterilization treatments ($p = 0.036$ & 0.035 , Table 3; Figure 11). However, most fields had no effect of invasion on LM. Variation between invasion treatments only occurred in four fields, with LM of seedlings grown in fields C6 and D2 larger in the uninvaded treatment while in fields C3 and D3 grown in invaded treatments were larger (Figure 11). The differences in these four fields are responsible for the significant interaction. Leaf mass had no general trend in response to the sterilization treatment across the site. Some plants had larger leaves in sterilized soils (C3, C4, C6, and D2), while plants from C7, D1, D3, E1 and the old growth had larger leaves in unsterilized soils (Figure 11). The significant field \times invasion \times sterilization interaction ($p = 0.005$) for SLA was generated by large variation in the effect of *Microstegium* invasion and sterilization among sampling locations that was observed in no other measure of plant performance. The results of the three-way interaction indicate spatial partitioning of soil communities across the site; where negative soil background effects and the facilitative effect of *Microstegium* were

responsible for small, subtle allocational shifts in tree seedlings that occurred locally among sampling locations.

Regeneration patterns

There was significant variation ($F_{9, 470} = 21.509, p = < 0.001$) in the cover of red maple across the HMFC, with the highest amount of cover in field C3 and all D fields (Figure 7a). In these four fields, there was no correlation between the percent cover of *A. rubrum* and the cover of *Microstegium* (C3: $r = -0.011, p = 0.943$; D1: $r = -0.106, p = 0.474$; D2: $r = 0.039, p = 0.791$; D3: $r = -0.283, p = 0.791$; Figure 9a & 9b). The other fields did not have an adequate amount of red maple cover to test for an association.

There was significant variation ($F_{9, 470} = 14.212, p = < 0.001$; Figure 9c) among fields in the number of stems of *A. rubrum* (Figure 9c). Those fields that had the most stems per field were different from fields with the highest cover of *A. rubrum* (indicating red maple recruitment). Three of the four sampling locations (C4, D2, and E1) that had the most stems were positively correlated with *Microstegium* cover (C4: $r = 0.259, D2: r = 0.259, E1: r = 0.268; all p < 0.05$) indicating that the areas of highest seedling densities occur in areas with higher densities of *Microstegium*.

Discussion

Undoubtedly, soil biofeedbacks can play an important role in regulating plant communities both directly and indirectly. Theoretical models and empirical studies have shown that with the invasion of non-native species, these biofeedbacks are often negative (Bever 1994; Van der Putten et al. 1993; Bever et al. 1997; Ehrenfeld et al. 2001;

Klironomos 2002; Callaway et al. 2004a; Bonanomi et al. 2005; Wolfe and Klironomos 2005; Levine et al. 2006). However, in this study, *Microstegium* had little negative influence on the success of *A. rubrum* in most sites and overall had a net facilitative effect on performance that varied in magnitude across the site. It appears that *Microstegium* had small, local influences on seedling establishment that resulted in subtle alterations of allocation patterns. Abiotic differences among sampling sites appeared to have larger influences for *A. rubrum* seedlings.

The results from this study show the possible role of soils in regulating establishment of tree seedlings within this system. Differences among sampling locations in all measures of plant performance indicate that strong abiotic soil differences existed among the fields and the old growth forest despite attempts to minimize this variation. Some soils seemed better for seedling establishment, while others tended to hinder or at least produce slower initial growth. Field data showed that *A. rubrum* seedling densities were positively correlated with cover of *Microstegium*, indicating an enhancement of red maple establishment. This effect appears transient due to the lack of older seedlings of *A. rubrum* suggesting post-establishment mortality is great from competition with *Microstegium* and other plants.

Although several samples were sampled within each of the soil collection sites, a large spatial variation was distinguished across the site. The variation among locations observed in this experiment cannot be attributed to the overall abundance of *Microstegium* or *A. rubrum* within the site. As stem densities were positively correlated with areas invaded by *Microstegium*, and because measures of plant performance did not show strong negative effects when grown in soils taken from areas invaded by

Microstegium, biotic effects appear not to exhibit strong influence on *Acer rubrum* regeneration. With the consistent and strong influence of sampling location and weaker biotic effects (Figure 8), the variation observed is largely driven by abiotic differences across the site.

Interactions between the soil and plant community have been shown to be influenced by soil history and may account for some of the variation among fields. For example, Kulmatiski et al. (2006) showed that exotic plant distribution was best explained by soil history when comparing abandoned agricultural land with undisturbed land. However, the landscape variation in soil influences observed in this study showed no effect of soil history, as there were no consistent differences between fields once used for agriculture and the old growth forest. The old growth forest could be expected to be different for several reasons. First, the forest has been preserved since European settlement (Ugolini 1964), thus leaving it unaffected by past agricultural practices, where the surrounding landscape that includes the BSS fields and the agricultural field had been under heavy agriculture during the same span. Second, the invasion of *Microstegium* originated in the old growth forest and has developed a near monoculture of *Microstegium*. Because of the substantial invasion of *Microstegium* in the old growth forest, the soils may have been conditioned by the species and would have experienced successive generations to develop the soil biofeedback. The BSS fields are still being invaded by *Microstegium* and, therefore, the soil biofeedback would be weaker.

While there do not appear to be strong effects of past agricultural use (row vs. hay crops) in this study, more subtle abiotic differences among fields may have generated the variation among adjacent agricultural fields (i.e. differences in bulk density, coarse

fraction, pH, texture, etc.). Some measures of ecosystem functioning may recover relatively quickly from agricultural disturbance such as soil physical properties and chemical processes associated with pH, while other characteristics, such as levels of organic matter, carbon, and phosphorus may take longer (Flinn and Marks 2007). The time frame that a particular system requires to fully recover from agricultural disturbance is governed by a multitude of variables, including the magnitude and duration of the disturbance, environmental conditions, along with the successional plant community itself and has been shown to take as little as 100 years up to 2,500 years (Kalisz 1986; Compton et al. 1998; Dzwonko 2001; Dupouey et al. 2002; Graae et al. 2003).

In a study by Reinhart and Callaway (2005) that contrasted plant-soil community interactions for two *Acer* species in native and non-native ranges, a general negative effect of the background soil community in native regions on seedling growth was observed. In this experiment, an inhibitory effect of background soil was revealed on red maple seedlings, where plants grown in unsterilized, uninvaded soils tended to be smaller than plants grown in unsterilized, invaded soils. The observation that most fields with the highest percent cover of red maple were different from fields that had the highest stem density per plot may be indicative of density dependent interactions of *A. rubrum* through the soil community. Those fields that have established red maple trees may be creating a more hostile soil community toward recruits of the same species, thus implementing density-dependent control of seedlings at local scales. However, the experimental data shows a conflicting pattern with regards to density dependent control regulation of red maple seedlings. Two of the fields with the highest stem density per field, C4 and E1, show a density-dependent control pattern, where seedlings performed worse in these

soils. The other two fields with the highest stem densities, C3 and D2, occur in fields that have high *Acer rubrum* cover and produced relatively large seedlings. When relating seedling biomass to *Acer* cover as a better predictor of density dependent interaction, no strong pattern emerges while field effects show large variation in seedling biomass and no apparent pattern with red maple cover. Although these results do not show a strong pattern of antagonistic effects by established *A. rubrum* on conspecifics, density dependent control on seedling regeneration may provide further insight into controls on red maple establishment.

Although site differences were not largely biologically driven, the field effects involving the interactions of field \times *Microstegium* and field \times sterilization may be a response to diverse soil communities over the contiguous landscape. Soil communities would have the ability to affect plant communities, especially if the different soil biota also influences abiotic processes that indirectly affect plants. Variation among sampling locations may be indicative not only of changes in the relative abundances of soil biota, but may also reflect changes in the composition of the species pool.

It appears that invasion by *Microstegium* lead to a reduction in the antagonistic interactions that *A. rubrum* had with the background soil biota. *Microstegium*'s invasion may have resulted in soil communities with decreased abundances of pathogens specific to *A. rubrum*. Indirect facilitative interactions have been shown to arise between native communities and invading species which disrupt species-specific pathogens (Keesing et al. 2006). When a pathogen-resistant non-native species invades a community, a dilution effect may lead to a decreased transmission efficiency of the pathogen that leads to an increase in the performance of native residents (Kopp and Jokela 2007; Telfer et al.

2005). The positive correlation between red maple stem density and *Microstegium* cover seen in the field data could be indicative of indirect facilitation of *A. rubrum* establishment.

In this experiment, plants grown in sterilized soil should have performed the same regardless of invasion because the sterilization process should have eliminated the effect of the biotic community and chemical differences in soils should have been minimized by the use of a small volume of inoculum. However, when soils were sterilized the two sterilized treatments were not equal. Plants grown in sterilized invaded soils performed worse than those soils in sterilized uninvaded soils, possibly indicating a nutrient release in uninvaded soils. The ability of *Microstegium* to alter soil chemistry (Ehrenfeld et al. 2001) may have been responsible for these observed differences in sterilized soils, where the sterilization process may have released compounds into the soil that adversely affected plant growth. Also, sterilized soils would not remain sterile for the duration of the experiment and re-colonizing microbial immigrants may have different effects in sterilized vs. unsterilized soils where the existing community of unsterilized soils may selectively filter colonization events (Reinhart and Callaway 2006).

While within-site variation in the strength of biotic control on seedling performance was expected, these were relatively small and largely generated changes in seedling allocation patterns. The subtle shifts in allocation patterns observed was due to variation that occurred among a few fields while other fields had no discernable difference. Soil sterilization reduced seedling performance in invaded soils, indicating the presence of beneficial soil organisms that outweighed the benefits of any nutrient pulse resulting from sterilization. The large variation in seedling allocation patterns

among locations and with invasion indicates that some areas may be more suitable for *Acer* regeneration, and that more suitable sites will be due to a combination of abiotic differences and *Microstegium* invasion. *Microstegium*'s role in *Acer* regeneration appears to be an overall facilitative one that aids in the initial establishment of seedlings. However, *Microstegium* will likely out-compete these *Acer* seedlings, reducing regeneration within the community. The subtle differences in plant performance in association with invasion measures observed in this study may alter the competitive ability of seedlings that do survive.

Further assessment is needed on the abiotic differences that might exist among fields to quantify any long-term effects on seedling establishment. Nutrient analyses would assist in determining fine scale differences throughout the site and provide insight into understanding nutrient heterogeneity and the factors that affect the differences among sampling locations that appear to be controlling community-level processes such as tree regeneration (Robertson et al. 1993). Research on the influence *Microstegium* invasion on tree regeneration should take three approaches. One approach should be directed toward the relationship of *Microstegium* as a facilitator for seedling establishment. The second approach should focus on the interaction between tree seedlings and *Microstegium* at later stages of development to analyze the competitive effects of *Microstegium* invasion on seedling establishment. Finally, the idea of conspecifics enacting density dependant control through negative soil feedback on *Acer* seedlings should be evaluated. Because of the large variation among fields observed, focus is needed at finer scales to fully examine the heterogeneity of both the biotic and abiotic effects of soils on tree regeneration. The results of the experiment do not allow

for the complete dismissal of indirect effects of the soil biota, and analysis at smaller scales may allow for a better assessment of soil biofeedbacks on seedling establishment and development.

Chapter 3.

Landscape Scale Variation in Plant-Soil Biofeedbacks: A Cautionary Tale

Introduction

A number of mechanisms have been proposed to explain changes in species assemblages after exotic species invasion, as invaders often reduce diversity or otherwise impact community structure. Much of this research has focused on the importance of the alteration of resource levels, particularly light and soil nutrients, in determining the outcome of competitive interactions. Accumulating evidence has also identified soil biota as having a potentially important role in invasion biology and has expanded the conceptual framework of invasion to include indirect effects (Bever 1994; et al. 1997; et al. 2001; 2003; Reinhart et al. 2003; 2004; and Reinhart and Callaway 2005; Bonanomi et al. 2005; Bezemer et al. 2006; Kardol et al. 2006; Levine et al. 2006). Many exotic plant species have the ability to alter the physical, chemical, and biological properties of soils (Ehrenfeld et al. 2001; Kourtev et al. 2002; Wolfe and Klironomos 2005; Mitchel et al. 2006) which can indirectly modify the plant community through the generation of positive or negative biofeedbacks.

Soil feedbacks have the ability to affect establishment, influence plant abundance and alter species diversity (Van der Putten et al. 1993; Molofsky 1994; Mills & Bever 1998; Molofsky et al. 2001; Klironomos 2002; O'Hanlon-Manners & Kotanen 2004; Reinhart et al. 2003; 2005). These feedbacks appear to be common, often negative, and can be generated directly or indirectly by a variety of soil organisms (Bever et al 1997; Bever et al. 2001; 2003). The specific composition of soil organisms involved in creating the feedback response (i.e. nematodes, pathogenic bacteria, fungi, etc.) will determine the

net influence of the soil community on plant assemblages. For example, VAM fungi, while associated broadly with plant hosts, have host-specific growth responses that can influence the outcome of competitive interactions between plant species (Klironomos 2002). The magnitude and direction of the net effect of all plant-soil biota interactions would then determine the feedback's potential in regulating both the population dynamics of the invader and its impacts within the community.

Contrasting results of soil biofeedback experiments may be due, in part, to naturally occurring temporal and spatial variation in plant-soil biota interactions over the focal species range (Reinhart and Callaway 2006). However, most studies have not examined spatial variation within soil biofeedbacks, but have focused on the influence of pooled soil communities. As soil properties and biotic communities are likely to vary over small spatial scales (Bever 1994; Molofsky 1994; Bever et al. 1997), it is likely that the net direction and magnitude of biofeedbacks will also vary dramatically. If these localized soil communities are pooled, creating one homogenous sample, the spatial variation that generates fine-scale heterogeneity in feedback processes would be lost. Therefore, the results from one single pooled soil community may yield markedly different effects on population dynamics than those occurring in response to localized soil communities.

To assess the role of soil biofeedbacks in explaining the decreases in abundance of a commonly associated native forest herb, *Eupatorium rugosum* (L.), in response to the invasive understory grass *Microstegium vimineum* (Trin.) a soil inoculum experiment was conducted. More specifically, to quantify the importance of spatial heterogeneity in soil biofeedbacks associated in regulating plant invasions, soil was collected with replicate

soil samples from several locations within a single landscape mosaic from areas that have been invaded by *Microstegium* and areas those areas that have not and compared.

Methods

Study Site & Species

The exotic grass *Microstegium vimineum* (hereafter referred to as *Microstegium*) is an aggressive colonizer that can form extensive monocultures that reduce the abundance of native herbs and alter ecosystem properties (Hunt and Zaremba 1992; Gibson et al. 2002; Cheplick 2005). Although this plant is a C4 grass, it is shade-tolerant and exhibits a high degree of plasticity in response to light and nutrient availability. These physiological attributes allow it to invade forest understory communities and expand into open habitats (Cheplick 2005; Cole & Weltzin 2005; Leicht et al. 2005; Claridge & Franklin 2002). While *Microstegium* is usually slow to invade undisturbed areas, it can rapidly increase in areas that have been disturbed by natural and anthropogenic processes (Cole & Weltzin 2005). This study was conducted at the Hutcheson Memorial Forest Center (HMFC; East Millstone, NJ, USA 40° 30'N, 74° 34'W). This site consists of a mixed-oak old growth forest surrounded by a series of successional fields and forests (Fig. 1).

Within this site, *Microstegium* has become widespread, initially establishing itself within the old growth forest. HMFC is also the site of the Buell-Small Succession Study (BSS), a long-term study of succession in abandoned agricultural fields. The BSS consists of 10 replicated post-agricultural fields that were abandoned over several years (1958-1966) and subjected to a variety of pre-abandonment agricultural practices (Pickett 1982). Six of the fields were used to grow row crops (C3, C4, C5, D1, D2, and D3) and

four were used for hay (C6, C7, E1, and E2). By 2005, *Microstegium* had spread from the old-growth forest into the adjacent BSS fields, dominating large areas. Within the BSS data, the diversity and cover of understory herbaceous species have decreased with the invasion of *Microstegium* (S.J. Meiners, unpublished data). Since the invasion of the BSS fields, *Microstegium* has exponentially increased in both cover and frequency (Fig. 10). To test the importance of soil biofeedbacks in facilitating the spread of *Microstegium* and its impact on the herbaceous community, the influence of *Microstegium* on *Eupatorium rugosum* (hereafter referred to as *Eupatorium*) was examined. This perennial herb is common within the forest understory at HMFC and was expanding within the BSS fields prior to the *Microstegium* invasion (Fig. 12).

Experimental design

To assess the importance of spatial variation in soil biofeedbacks, soil was taken from each of the BSS fields and the old growth forest. Collecting samples this way allowed for the detection of variation within the soil community in replicated communities across the same landscape. At each sampling location, eight soil cores were randomly collected using a 7cm diameter soil auger to a depth of 10 cm. Half of the samples were taken from areas invaded by *Microstegium* and the other half from areas with no apparent sign of invasion. Samples were collected on March 21, 2006, placed in sterile collection bags, and kept on ice during transport to ensure all components of the soil community remained dormant (Reinhart et al. 2005). In the laboratory, soil samples were kept refrigerated until processed. Samples were sieved using a 1.4 mm mesh sieve

and pooled within treatment (invaded/uninvaded) from each collection location to ensure even soil inocula.

One half of each pooled sample was autoclaved (25 minutes, 252° F, at 17 psi) to remove the influence of the microbial community. Sterilized soil cone-tainers (Stuewe & Sons, Inc., Corvallis) were filled approximately two-thirds full with sterile potting mix (Sunshine®, SunGro Horticulture, Ltd, Canada), inoculated with 10 mL of soil inocula, and capped off with an additional 3 cm of sterile potting mix. This yielded 10 replications per treatment per field for a total of 40 replicates for each field and the old growth forest. We used a small amount of inoculum (10 mL) and a potting mix with added nutrients to minimize the influence of any variation among collection locations in nutrient levels and to minimize the influence of any nutrients released from autoclaved inocula. Seeds of *E. rugosum* were germinated in the same sterile potting mix and transplanted into each inoculated cone-tainer. Seedlings that died within the first week after transplant were replaced.

To assess *E. rugosum* performance, plant height was measured every ten days after transplant for a total of 60 days. At the end of the growing period, plants were harvested to determine above ground biomass. Any plants that had flowered or produced flowering buds were also recorded. ANOVA was used to analyze treatment effects of collection location, soil sterilization, and *Microstegium* invasion in a full factorial design on the total above ground biomass and final height of *Eupatorium*. This analysis was followed by individual ANOVAs conducted separately for each sampling location to assess the treatment effects within each sampling location. A repeated measures ANOVA was also used to conduct a similar analysis on the periodic height

measurements. Logistic regression was used to assess the influence of field, soil sterilization, invasion and plant biomass on the probability of flowering.

Results

Biomass of *Eupatorium* varied dramatically across fields and with inoculum sterilization, with no consistent influence of *Microstegium* invasion (Table 4). Plants grown in sterilized soil overall performed better irrespective of *Microstegium* invasion across the entire site. The significant three-way interaction of location \times invasion \times sterilization indicated the influence of invasion and sterilization varied spatially among soil collection locations (Table 5). Repeated measures analysis of height and ANOVA of final height yielded qualitatively the same results as the ANOVA performed on biomass and are not reported here.

To understand the three-way interaction, it was necessary to examine the results of each sampling location individually. Within each location, local feedback effects often existed, where the invasion of *Microstegium* played a significant role in determining plant performance. The analysis also revealed remarkable variation in the direction and significance of the main effects and interactions among collection sites (Table 5; Fig. 13). Soil inocula from several locations had no impact on *Eupatorium* performance (Fields C3, D2, E1 and E2). Some of the individual locations followed the overall (all locations pooled) effect of only soil sterilization (Fields C5 and old growth), where others had an invasion \times sterilization interaction (Fields C4, C6, C7, D1). Furthermore, those fields differed in the direction of the influence of *Microstegium* invasion on *Eupatorium* growth. In two fields (C4 and D1), invasion resulted in an increase in growth for

unsterilized soils relative to uninvaded soils. In contrast, there was a strong reduction in growth of *Eupatorium* in unsterilized invaded soils of C7. In field C6, sterilization of invaded soils resulted in a marked depression of *Eupatorium* growth.

Reproductive success was not directly related to any of the experimental treatments, (*all* $P > 0.05$) but was strongly influenced by plant mass. Larger plants were more likely to flower irrespective of invasion or sterilization, which lead to an inconsistent relationship with experimental treatments (*Wald* $\chi^2 = 11.478$; $P < 0.001$; *Exp* (β) = 8.643).

Discussion

Undoubtedly, soil biofeedbacks play an important role in regulating plant communities both directly and indirectly. Theoretical models and empirical studies have shown that with the invasion of non-native species, these biofeedbacks are often negative (Bever et al. 1997; van der Putten and Peters 1997; Ehrenfeld et al. 2001; Klironomos 2002; Bonanomi et al. 2005; Kardol et al. 2006; Levine et al. 2006). However, in this study, *Microstegium* had little negative influence on the success of *Eupatorium* in most sites and in a few instances had a net facilitative effect on this dominant understory species.

In non-sterilized soils, plants grown in uninvaded soil often performed worse than plants grown in invaded soil, suggesting a disruption in the antagonistic effect of the background soil community on *Eupatorium*. Indirect facilitative interactions have been shown to arise between native communities and invading species mediated by species-specific pathogens (Keesing et al. 2006). When a pathogen-resistant non-native species

invades a community, a dilution effect may occur caused by a decreased transmission efficiency of the pathogen that can lead to a net increase in the performance of the native residents (Kopp and Jokela 2007; Telfer et al. 2005). Within the data presented here, it appears that invasion by *Microstegium* lead to a reduction in antagonistic interactions with soil biota, possibly because *Microstegium* invasion has resulted in soil with decreased abundances of pathogens specific to *Eupatorium*.

In most of the sampling locations, plants grown in sterilized soils grew better than plants in live soils. This indicates an overall antagonistic interaction between the native soil communities and *Eupatorium*. Since nutrient differences among locations and sterilization were minimized by using small inocula in combination with fertile potting mix, this indicates a biotic agent as the primary factor that reduced plant performance. This study confers no speculation on the identity or diversity of soil organisms responsible for the observed soil biota effects since only net influences on *Eupatorium* were observed.

Other interactions between the soil and plant community have been shown to be influenced by soil history and may account for some of the variation seen among fields and the old growth forest. For example, Kulmatiski et al. (2006) showed that exotic plant distribution was best explained by soil history when comparing abandoned agricultural land with undisturbed land. However, the landscape variation in feedbacks observed in this study showed no effect of soil history, as there were no consistent differences between fields once used for agriculture and the old growth forest. While there do not appear to be legacy effects of past agricultural use in the study, more subtle historical differences among fields may have generated the variation among adjacent fields.

Flowering was strongly correlated with biomass, where larger plants showed a higher probability of flowering regardless of experimental treatment. These findings seem to be incongruent with observed declines of *Eupatorium* within the old growth forest throughout the *Microstegium* invasion. If *Microstegium* is not substantially influencing, or in some cases facilitating *Eupatorium* growth, then one would expect *Eupatorium* to increase as *Microstegium* spreads. However, this is not the case, suggesting the dominance of direct competitive interactions over soil biofeedbacks in regulating invasion impacts in this system.

Spatial Variation in biofeedbacks

Soil samples are often pooled in soil feedback studies creating one homogenous sample and eliminating natural variation within soil communities. Within these plant-soil biota studies, experimental consideration of spatial variation is usually lacking but may be acknowledged to occur in theory. To complicate the issue further, spatial variation may be indicative not only of changes in the relative abundances within soil biota, but may also reflect changes in the actual composition of the species pool. These changes in the presence and abundance of the multitude of organisms that generate a net feedback may lead to dramatic alteration of the soil-plant interaction.

The most important result of this experiment is the extreme variation in soil feedback despite the close proximity and similarity in soil type of all soil collection sites within the landscape of HMFC. Sample sites located adjacent to one another showed different effects of sterilization and, in some cases, *Microstegium* invasion. For example, site D1 showed sterilization by *Microstegium* effects while field D2, directly adjacent,

showed no treatment effects. Although samples were pooled within each sampling location, large differences were distinguished among locations. Further studies utilizing sampling within each location would provide insight into fine scale soil feedback processes, which may be even more variable than the results reported here.

Spatial variation in soil biofeedbacks must be addressed because the effects yielded by pooled samples across a large habitat may not reflect the local impacts experienced by individual plants (Tilman et al. 1997; but see Reinhart and Callaway 2005). If all of the samples had been pooled, it is possible that only the overall effect of sterilization would have been observed, and none of the individual effects seen within fields (shown by the statistical pooling of data). However, it is likely that pooled samples may bias studies towards negative effects generated by pathogens. The soil pooling process would spread rare or localized pathogens throughout the inocula. Given the rapid life cycle of most pathogens, even those occurring in low densities may spread rapidly leading to their dominance within the experimental soil community and their influence on target species. Therefore, the net feedback observed for experiments using pooled samples may be much more negative than the biofeedbacks that most plants experience *in situ*. The consequence of this bias is the potential to make erroneous conclusions about the magnitude of soil biofeedback effects and generalizations about the interactions structuring the plant community.

More and more studies are yielding insight into the importance plant-soil biota interactions within plant community ecology. The findings presented here suggest that although it is important to gauge the potential for soil biofeedbacks to regulate plant communities, assessment is needed at smaller spatial scales to adequately understand the

prevalence of these feedbacks within landscapes. The data presented shows that the common usage of one homogeneous soil community in biofeedback studies is limiting to our understanding of soil biofeedbacks. With the multitude of soil organisms shown to affect resident plant populations and invading populations, it is short sighted to believe that a single assessment of soil biofeedback can be widely applied to explain the diversity of community interactions across a landscape, and possibly even within a site. Ecologists must now begin the essential task of incorporating spatial variability into plant-soil biota studies to fully understand the role of soil interactions as a determinant of plant community dynamics (see Eppstein and Molofsky 2007). Further research is needed to explore the spatial aspect of soil biofeedbacks that can develop across an otherwise homogeneous environment and to determine how common spatial variation is in these systems. Once soil biofeedbacks are shown to exist, the next step must be to identify the variation in these biofeedbacks at both fine and coarse scales.

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Tables and Figures.

Table 1. Correlation coefficients of soil characteristics and compaction variables corrected for multiple comparisons with distance from the edge from the old growth forest.

FIELDS	C4	C5	C6	D1	D2
ln Coarse Fraction	0.472*	0.334	-0.014	0.348	-0.200
ln pH	-0.349	0.156	0.133	0.172	0.075
ln Organic matter	-0.199	0.707*	0.107	0.040	0.274
ln of compaction at 0 in	-0.039	-0.411	-0.122	0.083	-0.155
ln of compaction at 3 in	-0.548*	0.345	0.076	0.201	-0.153
ln of compaction at 5 in	0.053	0.185	-0.213	-0.082	0.043

** indicates significance at the 0.05 level*

Table 2. Correlation coefficients of all soil characteristics and the coefficient of variance with age.

Soil Characteristics	Mean Value	Correlation Coefficients
ln Coarse Fraction	0.0208	-0.296
ln pH	-0.794**	0.159
ln Organic matter	0.269	0.434
Bulk Density	1.39	-0.363
ln of compaction at 0 cm	-0.050	0.092
ln of compaction at 7cm	-0.815**	0.370
ln of compaction at 12.5	-0.261	0.387
% Sand	0.113	0.289
% Clay	-0.586*	0.790**
% Silt	0.459	0.025

*indicates significance at the 0.05 level

**indicates significance at the 0.01 level

Table 3. MANOVA and follow-up ANOVAs for each measure of plant performance. Pillai's Trace test statistic is reported for multivariate analysis. Full model ANOVA (Biomass) $R^2=0.268$; Full model ANOVA (Leaf Mass) $R^2=0.268$; Full model ANOVA (Leaf Area) $R^2=0.229$; Full model ANOVA (Specific Leaf Area) $R^2=0.211$. Significant terms are indicated in bold at the 0.05 level.

Source	MANOVA		ANOVA							
	Pillai's Trace		Biomass		Leaf Area		SLA		Leaf Mass	
	F	P	F	P	F	P	F	P	F	P
Field	2.445	<0.001	5.409	<0.001	3.808	<0.001	2.292	0.013	4.750	<0.001
Invasion	283	0.421	1.919	0.167	0.689	0.407	0.191	0.662	2.700	1.01
Sterilization	283	0.530	.952	0.330	1.674	0.197	0.668	0.414	1.037	0.309
Field × Invasion	1144	0.157	1.574	0.114	1.633	0.096	0.802	0.627	1.977	0.036
Field × Sterilization	1144	0.284	1.793	0.062	1.334	0.211	1.304	0.228	1.987	0.035
Invasion × Sterilization	283	0.053	4.985	0.026	8.767	0.003	0.144	0.705	3.458	0.064
Field × Invasion × Sterilization	1144	0.113	0.864	0.568	0.933	0.503	2.627	0.005	0.832	5.98

Table 4. ANOVA of the influence of invasion and soil sterilization on *Eupatorium rugosum* biomass across the study site. Significant terms indicated in bold at the 0.05 level. Full model ANOVA $F = P = R^2 = 0.31$.

Source	df	MS	F	P
Sampling location	10	0.163	3.243	< 0.001
Invasion	1	0.002	0.039	0.843
Sterilization	1	1.098	21.883	< 0.001
Location Invasion	10	0.113	2.245	0.015
Location Sterilization	10	0.256	5.093	< 0.001
Invasion × Sterilization	1	0.015	0.293	0.589
Location × Invasion × Sterilization	10	0.239	4.769	< 0.001
Error	392			

Table 5. ANOVA of the influence of sterilization and invasion of *Microstegium* on biomass of *Eupatorium rugosum* for all data pooled (overall) and for each individual field of the BSS and the bordering old growth forest. Values presented under treatment are F-values with their corresponding significance. Field names (e.g. C4) represent grid locations of each field within the BSS. Significant terms indicated in bold.

Field	N	Sterilization	Invasion	Sterilization×Invasion	R-square
Overall	792	17.609***	0.4	0.595	0.04
C3	78	0.377	3.861	2.77	0.16
C4	80	3.762	0.034	6.534*	0.22
C5	80	12.236**	2.888	0.073	0.3
C6	80	5.753*	5.113*	4.861*	0.37
C7	78	3.349	1.693	15.610***	0.37
D1	78	1.054	2.366	10.261**	0.29
D2	80	0.217	0.255	0.004	0.01
D3	80	42.462***	5.898*	3.056	0.59
E1	80	2.218	0.392	0.784	0.09
E2	80	6.963	1.085	0.389	0.19
Old Growth	78	7.906**	0.316	0.003	0.19

* $P < .05$, ** $P < .01$, *** $P < .001$

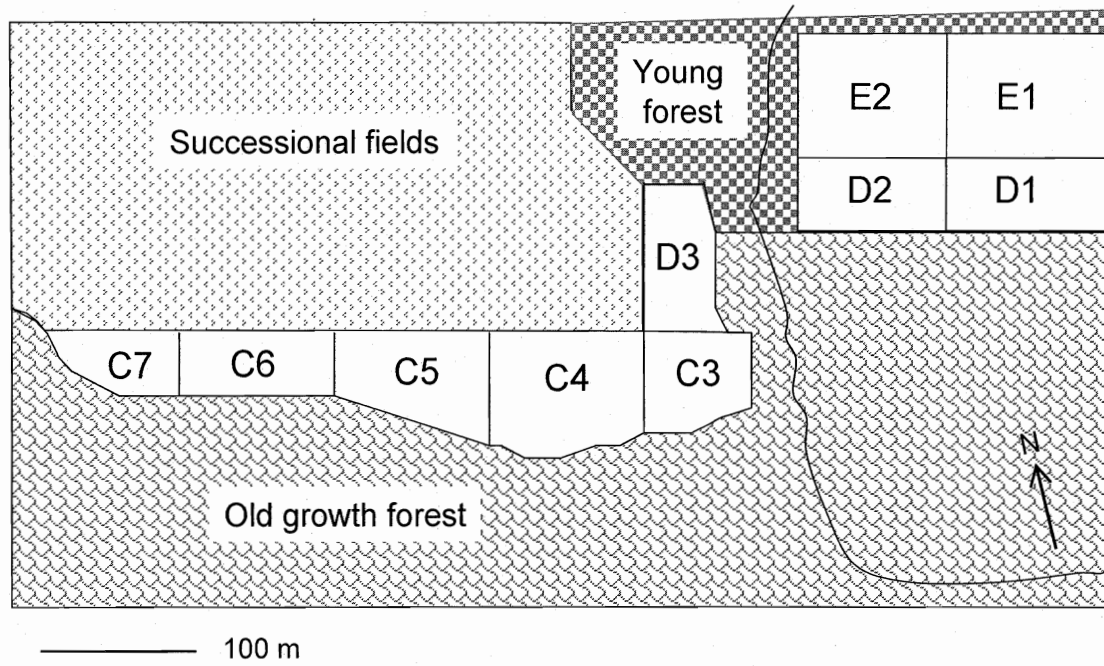


Figure 1. Site map of HMFC, the BSS fields, and the old growth forest. The newly abandoned agricultural field, which is located due east, is not located on this map.

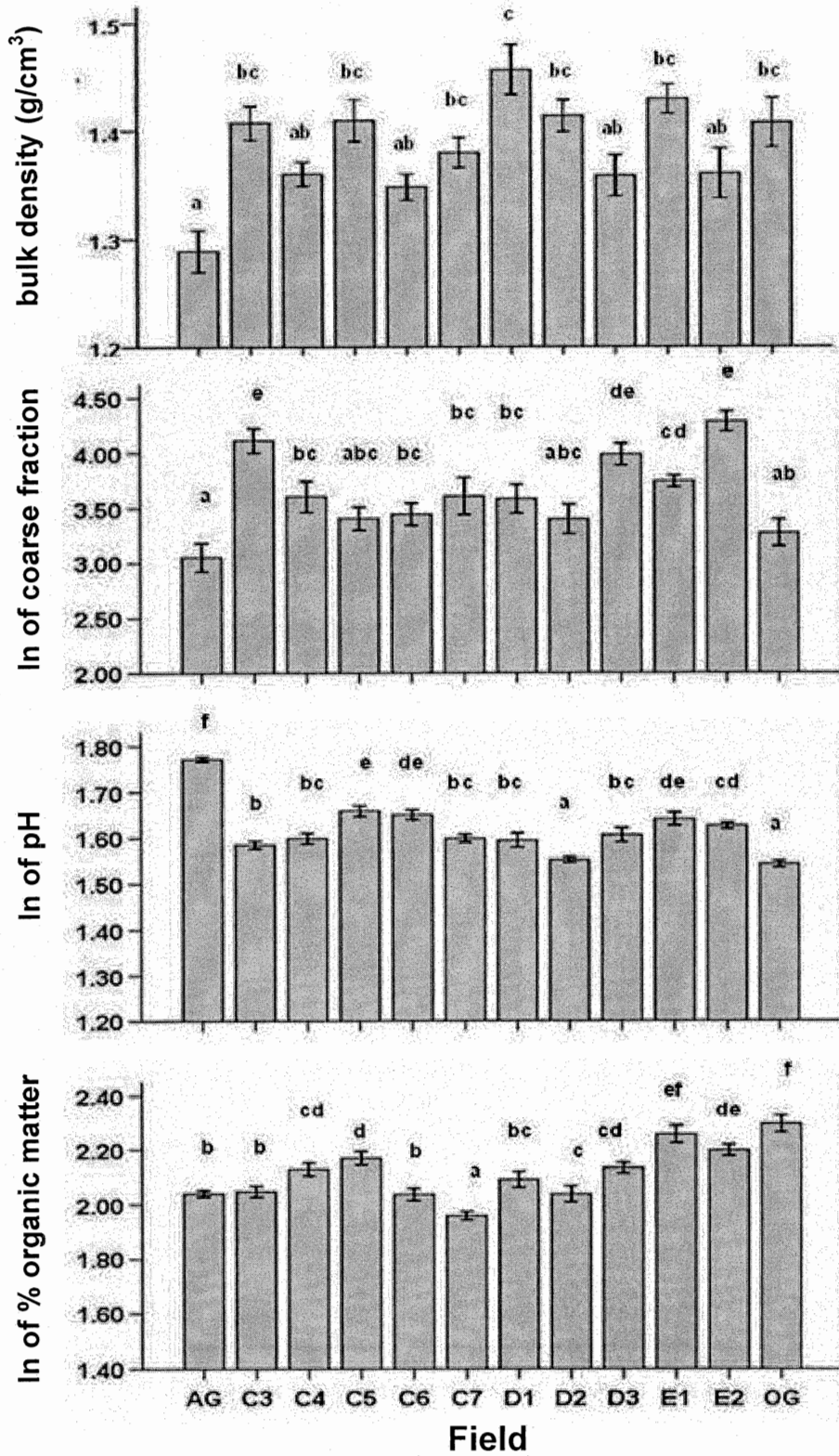


Figure 2. Variation in soil characteristics among BSS fields, the old growth forest, and the recently abandoned agricultural field. Bars represent mean + 1- SE, letters indicate post hoc tests.

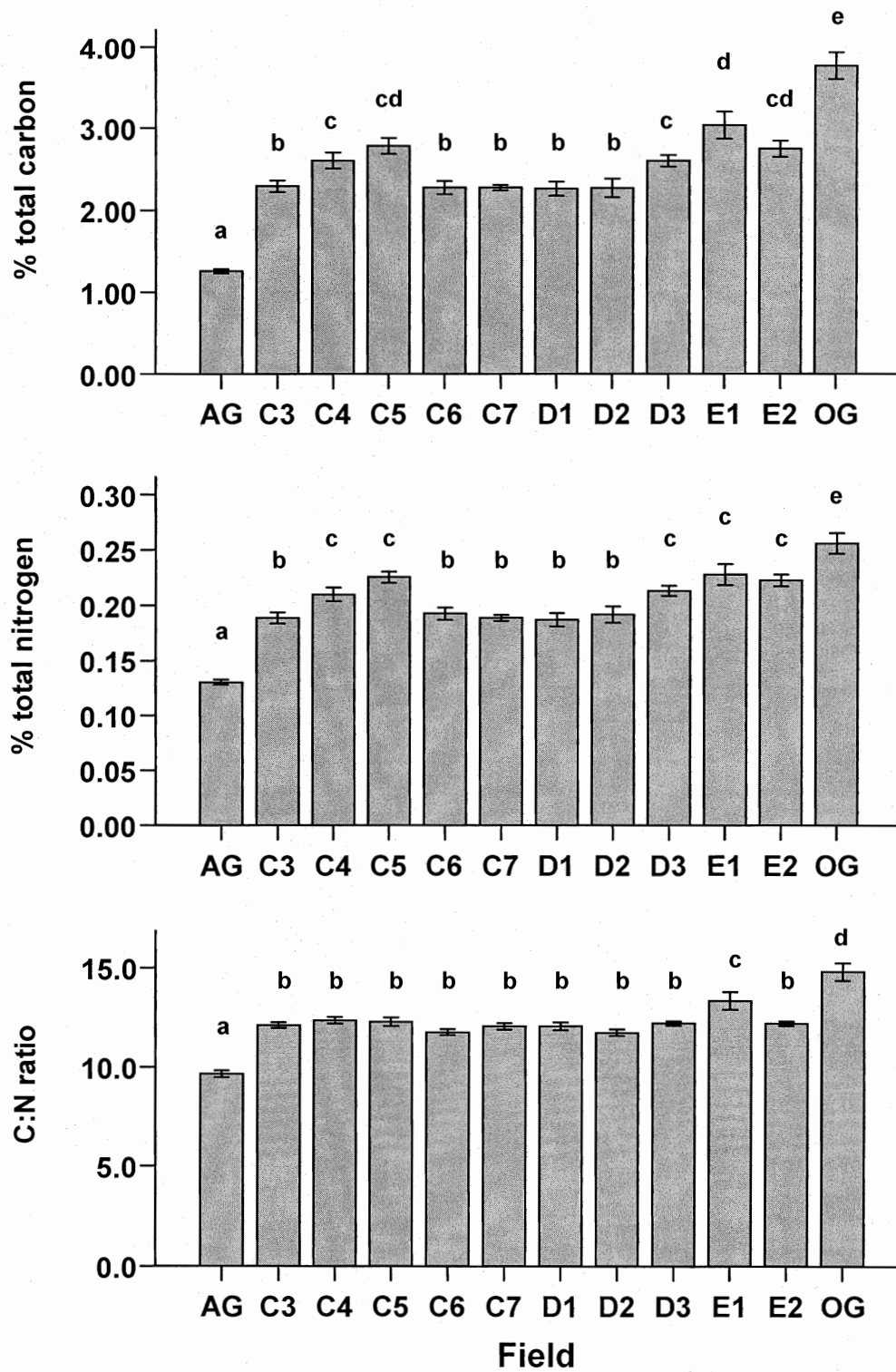


Figure 3. Variation in the % C, % N, and the C:N ratio among all sample locations.

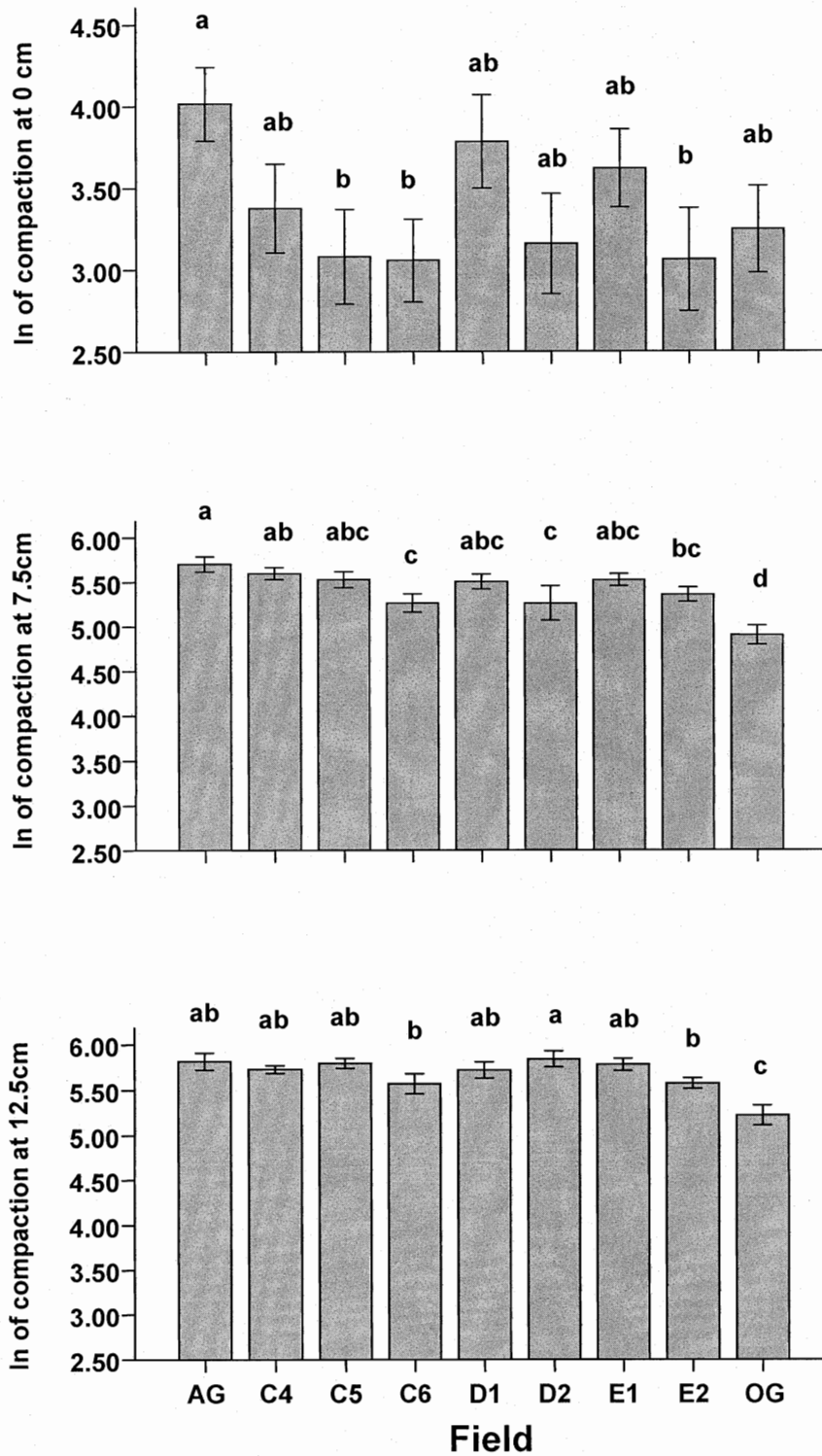


Figure 4. Variation in soil compaction across 7 of the BSS fields, the agricultural field, and the old growth forest.

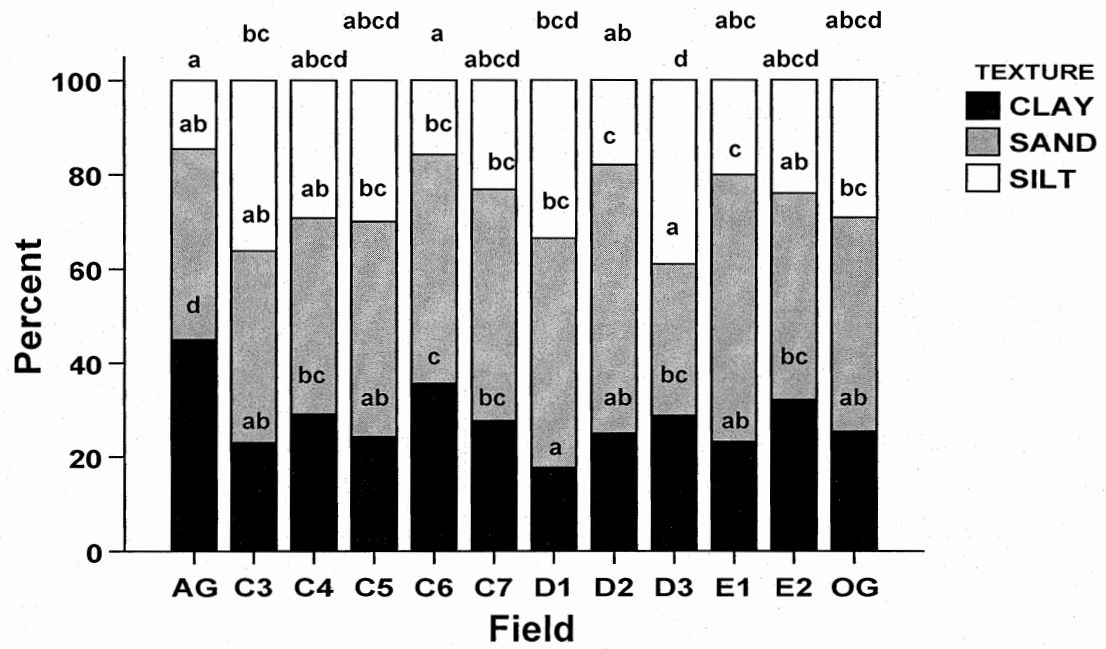


Figure 5. Soil texture for all sampling sites. Bars represent mean relative percentage of sand, silt, and clay for each sampling location. Letters directly above each separation indicate significant differences among texture class.

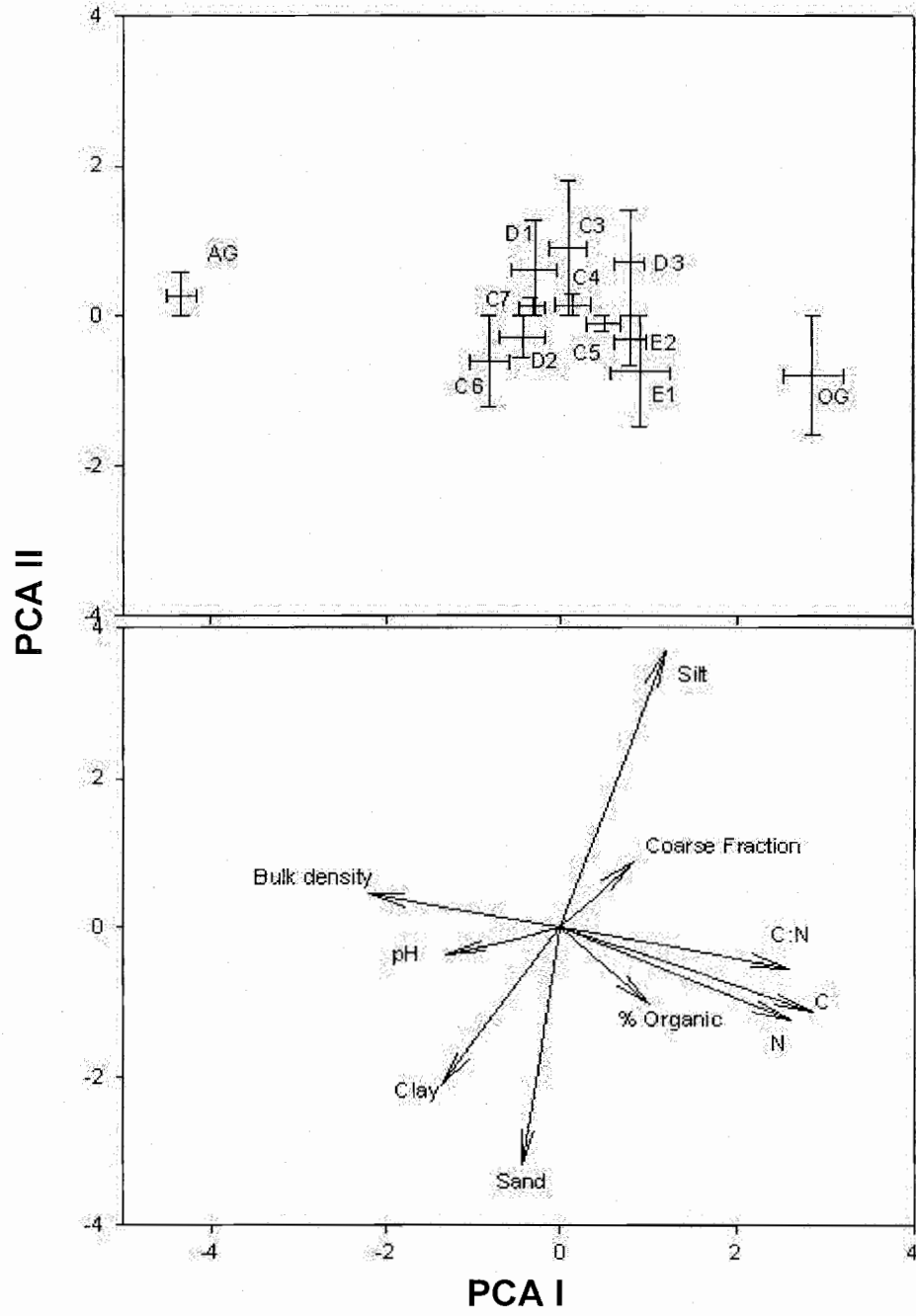


Figure 6. Principal components analysis of physical and chemical soil properties. The mean \pm one SE is plotted for each sampling location. In the lower panel, soil variables are plotted in the ordination space as vectors

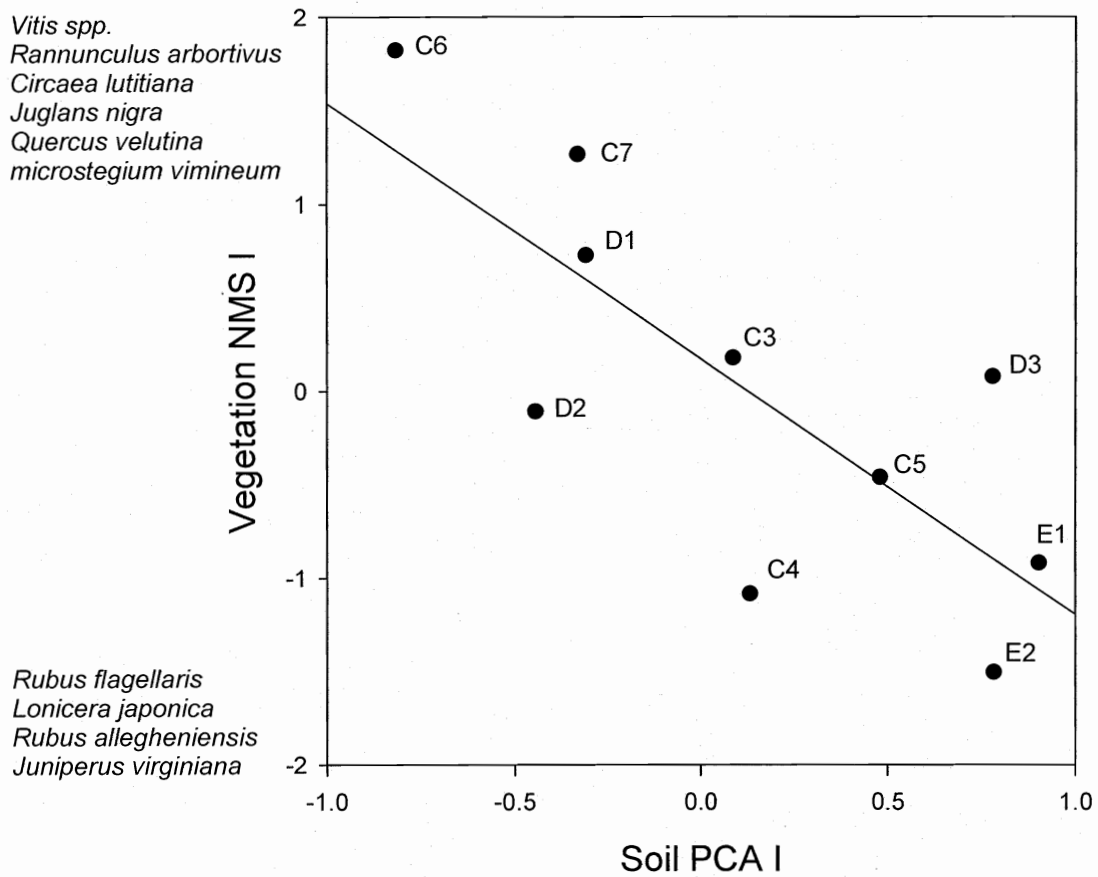


Figure 7. Correlation between the vegetation NMS axis I and soil PCA axis 1. The vegetation NMS axis shows significant species loadings along the axis. Loadings for the PCA axis are shown in figure 6.

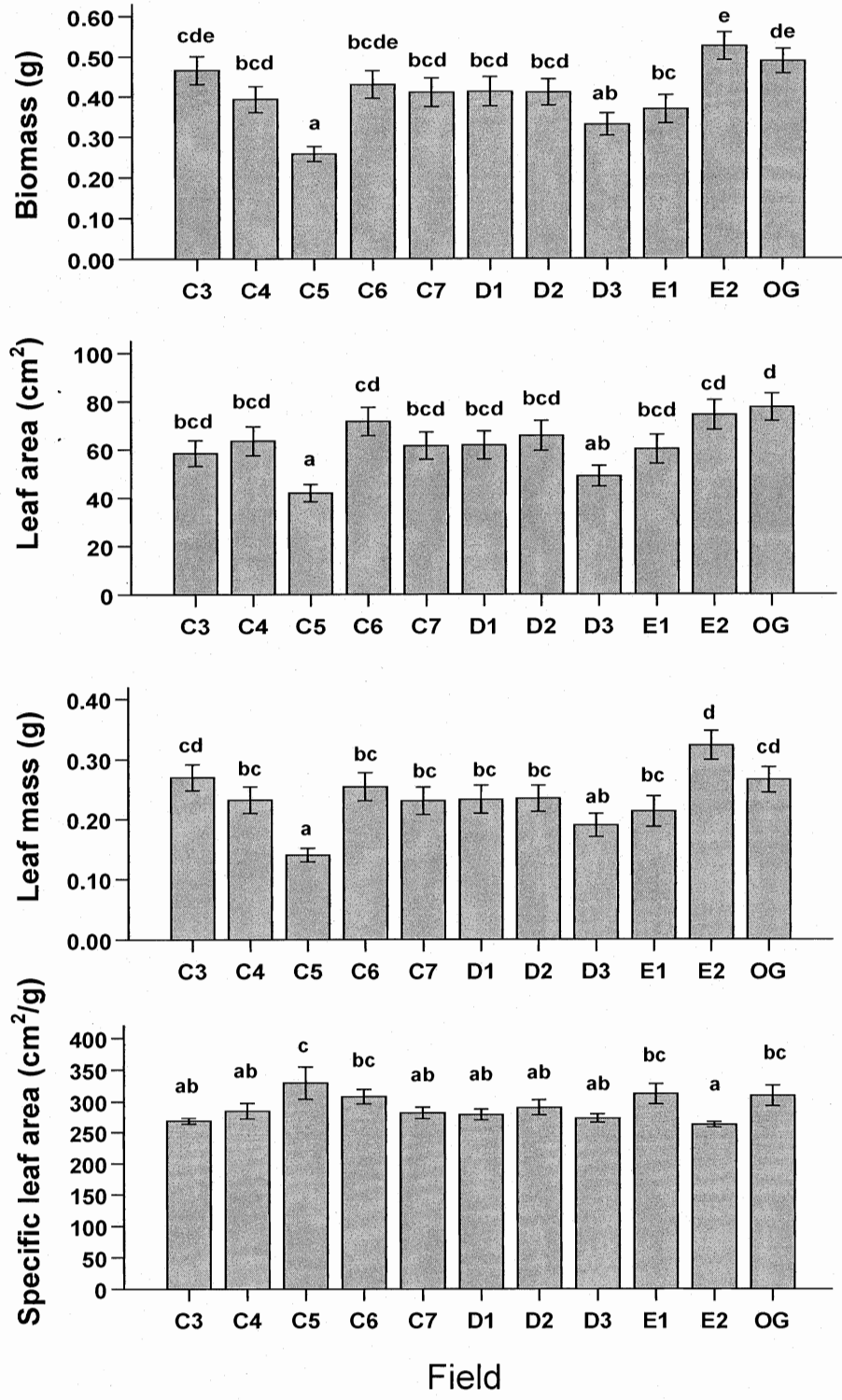
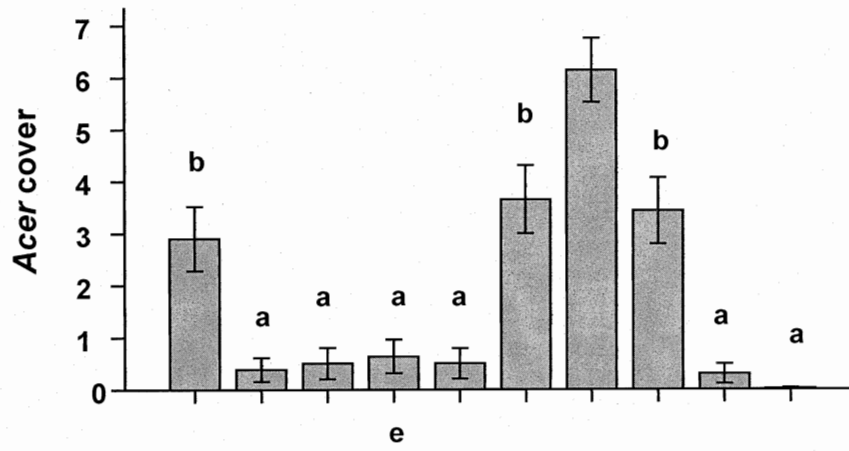
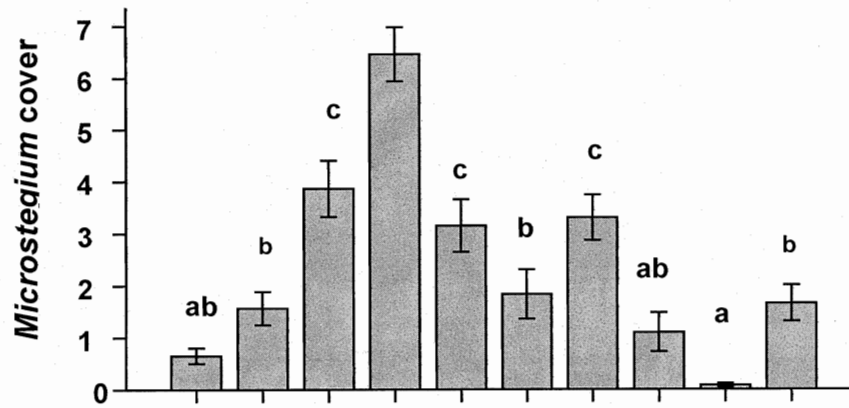


Figure 8. Variation in the measures of seedling performance across the 11 sampling locations. Mean \pm 1 S.E.

a)



b)



c)

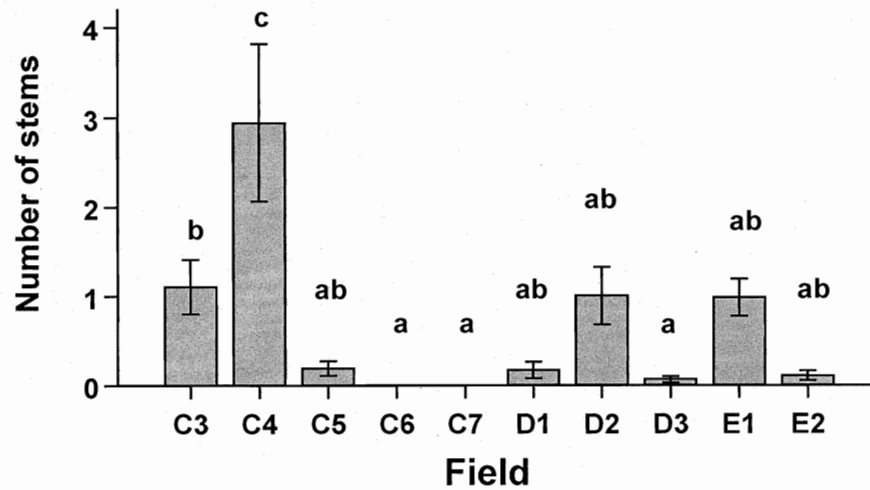


Figure 9. a) Spatial distribution of *Acer rubrum* canopy cover, b) *Microstegium* invasion, and c) seedling density across the 10 BSS fields. Mean \pm 1 S.E.

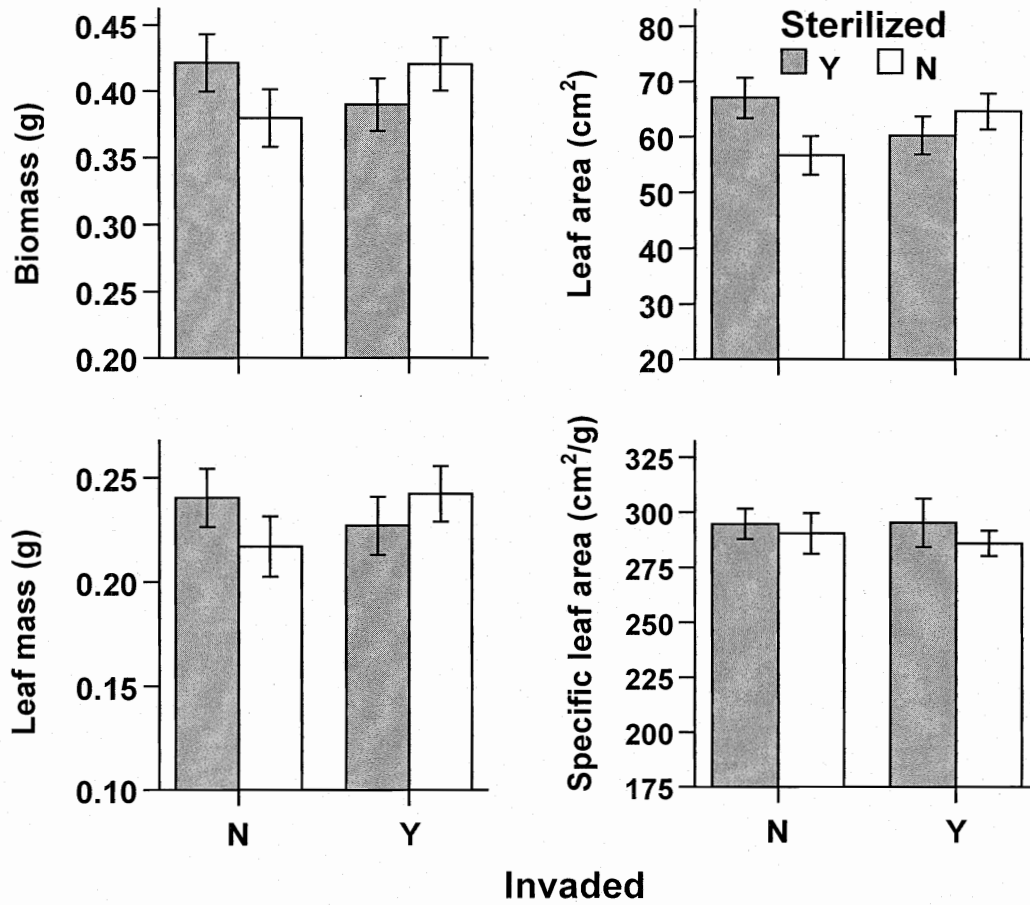


Figure 10. The interaction between sterilization and *Microstegium* for individual performance metrics. Y = soil taken within *Microstegium*. N = soils not taken within *Microstegium*. Mean \pm 1 S.E.

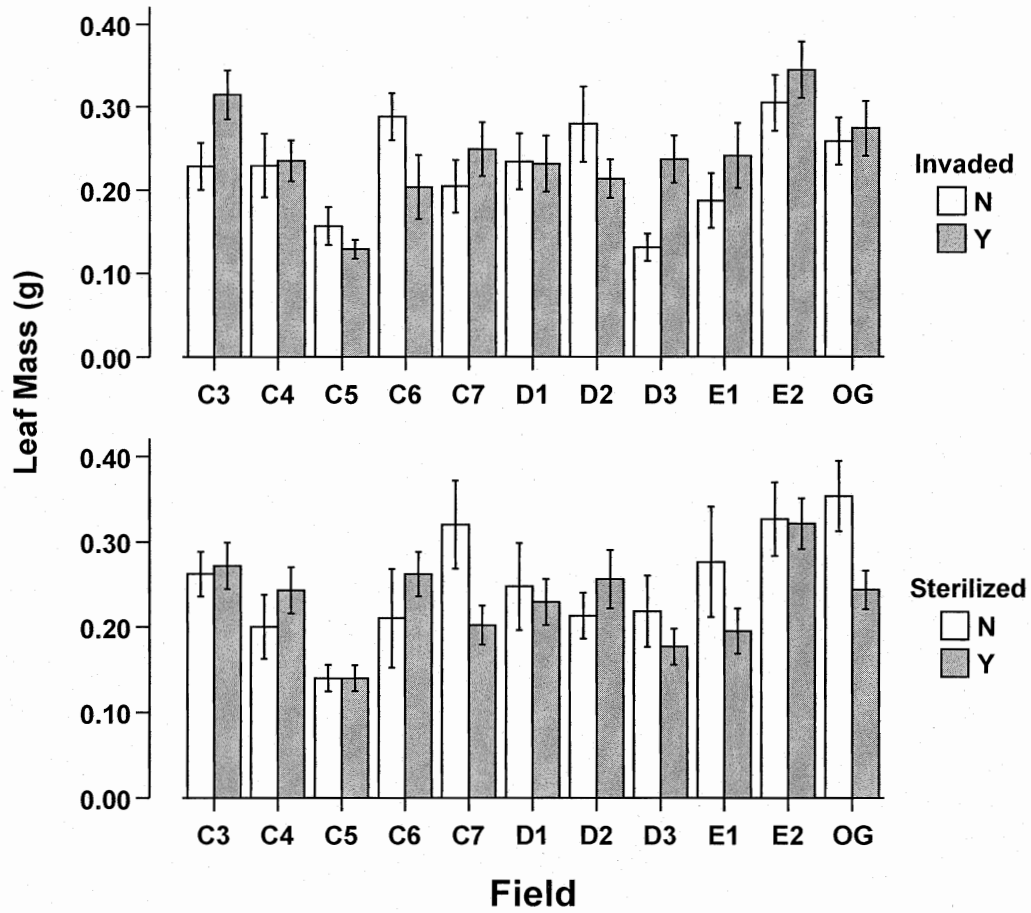


Figure 11. The interaction between *Microstegium* and sterilization for leaf mass across all sampling locations. Mean \pm 1 S.E.

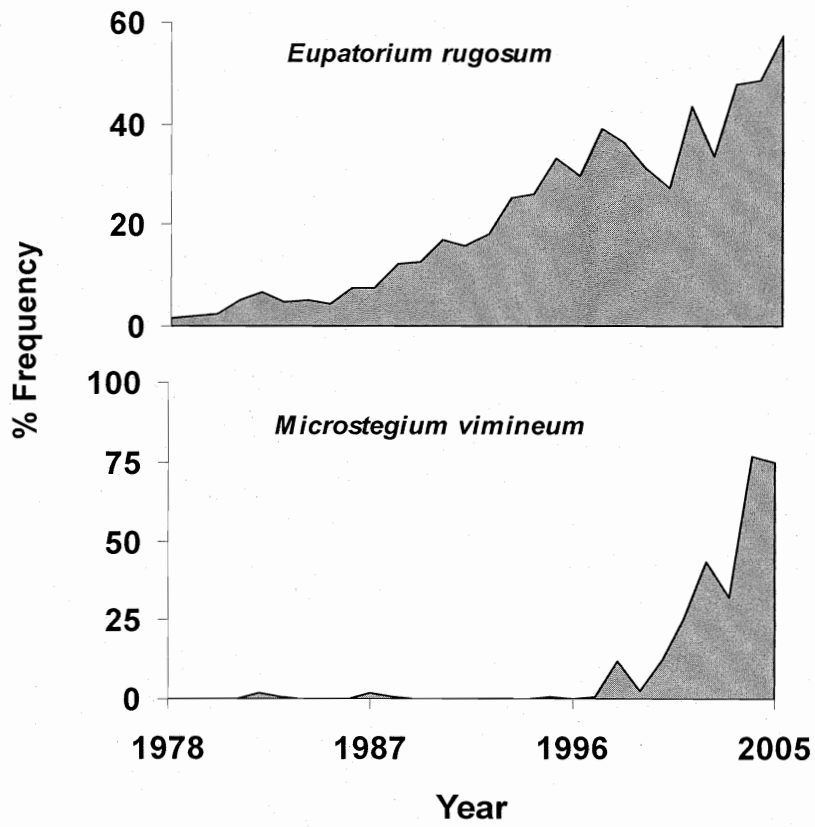


Figure 12. The percent frequency of *Microstegium vimineum* and *Eupatorium rugosum* over time within the BSS fields from the first year it was observed on site to present.

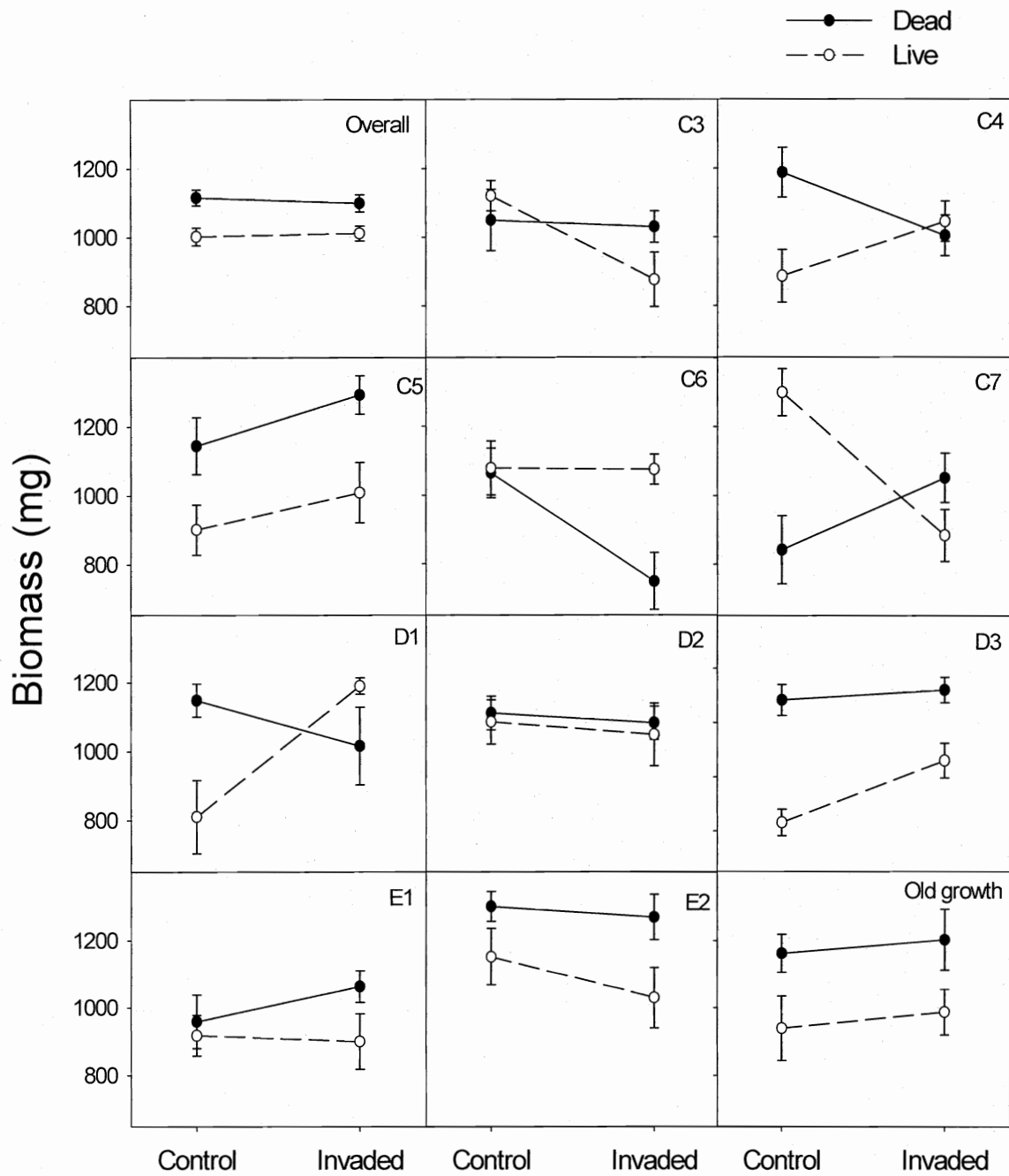


Figure 13. Soil biofeedbacks associated with *Microstegium vimineum* across the BSS site on *Eupatorium rugosum* biomass. Depicts overall univariate interaction (top left panel), followed by 11 individual analyses of each field.