

FINAL REPORT

Development of Ecological Indicator Guilds for Land Management

SERDP Project RC-1114B

DECEMBER 2005

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SERDP Research Project CS-1114B

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FINAL

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Executive Summary

This research is a SERDP-SEMP funded project “Development of Ecological Indicator Guilds for Land Management” (CS 1114B). Agency land-use must be efficiently and cost-effectively monitored to assess conditions and trends in ecosystem processes and natural resources relevant to mission requirements and legal mandates. Ecological Indicators represent important land management tools for tracking ecological changes and preventing irreversible environmental damage in disturbed landscapes. The overall objective of the research was to develop both individual and integrated sets (i.e., statistically derived guilds) of Ecological Indicators to: quantify habitat conditions and trends, track and monitor ecological changes, provide early-warning or threshold detection, and provide guidance for land managers. The derivation of Ecological Indicators was based on statistical criteria, ecosystem relevance, reliability and robustness, economy and ease of use for land managers, multi-scale performance, and stress-response criteria. The basis for the development of statistically based Ecological Indicators was the identification of ecosystem metrics that analytically tracked a landscape disturbance gradient.

Research was conducted in the Fall-Line Sandhills at Fort Benning Georgia. This area represents the complex physiographic ecotone between the Piedmont and Coastal Plain. In Phase I research, nine sites were selected in adjacent watersheds of upland mixed pine-hardwoods forest with loamy-sand soils; three sites each in High, Medium, and Low disturbance classes, based on current and past U.S. Army mechanized infantry training activities. High sites were currently experiencing active mechanized infantry training. Medium sites were subjected to past military training activities, but current use was primarily by foot infantry, and vehicles were restricted to existing roads and trails. Low sites had neither current nor previous military activities and were exposed to minimal foot traffic.

This research has made important scientific advancements in five areas: 1) the identification of individual and classes (guilds) of Ecological Indicators (EIs) that quantify and characterize landscape disturbance; 2) the use of this information to construct Site Comparison or Site Condition Indices (SCIs); 3) new insights into the relationships among landscape disturbance,

biodiversity patterns, ecosystem processes, and the intermediate disturbance hypothesis; 4) detailed identification of species-habitat/environment and landscape disturbance relationships; and 5) the clarification of complex ecosystem and physiological processes. This report essentially deals with the first two, because these were the primary objective of the proposed research. Numbers three and four are currently being investigated with statistical modeling, especially examining the relationships between biodiversity patterns and landscape disturbance. These analyses are directed primarily at understory (ground cover) and canopy (trees) vegetation and selected invertebrates, especially ant communities. Number five is directly addressed by several publications produced by our team, and additional analyses are continuing.

Eleven Ecological Indicator Systems based on extensive research team experience and literature reviews were selected for evaluation in Phase I with respect to *a priori* selected desirable properties of EIs. These EI systems represented a very broad range of potential physical, chemical, physiological, community, and ecosystem indicators. Eight of the eleven researched EI systems *as a group* were very effective at distinguishing among the three disturbance classes (High, Medium, Low). These successful EI systems were: General Habitat, General Ground Cover, Floristics Ground Cover, Soil Chemistry, Microbial Community Dynamics, Nutrient Leakage, Soil Mineralization Potential, and Ground/Litter Ant Communities. Developmental Instability (DI), Plant Physiology, and Spatial Organization in Plant Communities were unable to reliably distinguish among disturbance classes. DI is the phenotypic asymmetry response to stress in the early embryonic development of an organism. DI was overly sensitive to a wide range of environmental perturbations, including drought, fire (and nutrient pulses), herbivory, and gall parasitism. Additionally, DI poses significant problems in selection of test species, field sampling, statistical interpretation, and resource-intensive laboratory requirements.

Individual EIs and EI guilds were identified and derived with a number of statistical procedures. Multivariate (MANOVA) and univariate (ANOVA) analysis of variance and discriminant analysis (DA) were used to identify indicator metrics and extract suites of variables (guilds) that successfully discriminated among the disturbance classes. Principal components analysis was useful for reducing field or laboratory data sets (e.g., community species composition, DI metrics) with many variables with high multicollinearity into fewer uncorrelated variables (i.e., vectors) for input into subsequent analyses.

All eight successful EI guilds in Phase I, differing widely in tracking ecosystem condition and responses, demonstrated that the Low and Medium disturbance classes were similar to each other, but differed a great deal from the highly disturbed sites. This indicates that the Medium sites may be well on their recovery trajectory from past military training activities. Nevertheless, Low and Medium sites were also successfully differentiated by all eight EI guilds. DA results from these guilds were reliable, consistent, and robust. Therefore, DA consistently provided a quantitative assessment of the relative ecological differences among the three disturbance classes (i.e., the relative locations of the three disturbance classes in discriminant space).

Soil A-horizon depth and soil compaction were the only EI metrics among all habitat parameters that successfully and significantly ($P < 0.001$) distinguished among the three disturbance classes of Phase I. Indeed, these two EIs and soil mineralization potential (consists of two metrics) were the only metrics that *individually* could distinguish the three disturbance classes. These

identified metrics have profound assessment and monitoring implications. Soil is considered the major template for maintaining ecological processes and landscape sustainability. The A-horizon forms at the soil surface by accumulation of humus, and is the layer of highest biodiversity, biological activity, decomposition, and nutrient recycling. Soil compaction has many negative impacts on ecosystem processes including: reduced seed germination and root growth, retarded aeration and water infiltration, increased runoff and erosion, decreased microbial activity and nutrient dynamics, increased difficulty in invertebrate and vertebrate burrowing activities, and discouraging the development of biologically active surface crusts and litter mixing.

It was indeed encouraging to learn that EIs that reflected and mirrored complex ecosystem properties and their dynamics, and community structure and composition were relatively simple; and could economically be monitored by land managers. Research is continuing with the emphasis on multivariate modeling to further weave the tapestry for understanding these complex relationships and interdependencies.

Six of the EI systems successful in Phase I research were validated in a much broader landscape context in Phase II. Soil Mineralization Potential and Nutrient Leakage were not evaluated, because they require a great deal of effort, time, laboratory analyses, and specialized equipment and expertise for monitoring; and are not readily applicable for large sample validation experiments such as our Phase II. However, these Ecological Indicators would be very useful for long-term monitoring of specific fixed sites. Forty sites (including the original nine) were selected throughout Fort Benning, representing relatively pristine to severely degraded military training areas in all available upland vegetation communities and forest types. Site selection was based on eight GIS databases, site criteria and data from other SEMP research teams, and extensive field ground-truthing. The 40 sites were classified into 10 landscape disturbance classes, based on pre-data collection visual assessment of military training damage to vegetation and soils by a single experienced field ecologist (AJK). A-horizon depth, soil compaction, and DF1 (discriminant function 1) of general ground cover characteristics (dominated by bare ground) when plotted on the 10-class disturbance gradient clearly verifying the utility of these EIs. These three EI metrics were much better at characterizing this disturbance gradient than the more traditionally used NDVI (normalized difference vegetation index) derived from satellite imagery.

A Site Comparison (or Condition) Index (SCI) was constructed from seven EI metrics: A-horizon depth, soil compaction, soil organic content (correlates with carbon), litter cover (100-bare ground), canopy cover, basal area, tree density; and the NDVI. These eight variables were statistically selected by the criteria that each metric individually varied highly significantly ($P < 0.001$) along the 10-class disturbance gradient. Furthermore, the seven indicator metrics could be measured in the field by minimally trained field personnel using simple and inexpensive equipment. Statistically, NDVI did not contribute any additional information to the SCI. The EI metrics and NDVI were standardized and weighed by statistical procedures. SCI scores for the 40 sites were plotted against the 10 disturbance classes. The SCI modeled the disturbance gradient monotonically and smoothly. The unbiased analytically derived SCI scores reproduced almost perfectly the ranking assigned by a very experienced observer, thus non-subjective uniformity of ranking was achieved. The histogram of SCI scores for the SCI ranked 40 sites

revealed a sigmoid logistic decay function, analytically demonstrating that relatively few sites were either very high quality or very severely degraded, and suggested a “threshold effect” of rapid decline in SCI values as disturbance increased from “pristine sites” or as severely degraded sites were approached. Discrepancies between SCI and disturbance class rankings revealed interesting ecosystem patterns that are being investigated.

A SCI was similarly calculated for each of the 160 transects at the 40 sites using the seven EI metrics. Each metric was standardized and weighed by statistical criteria to develop the final composite SCI for the 160 individual transects. A histogram of SCI scores versus the SCI ranked transects again displayed a sigmoid logistic decay curve. The continuity of the curve demonstrated that the complete upland disturbance gradient was characterized at Fort Benning. Based on their ranked SCIs, the 160 transects were classified into five disturbance classes: Low, Low-Med, Medium, Med-High, and High. These five disturbance classes represent an analytically derived and unbiased classification with which to assess the behavior of the other four EI systems: General Ground Cover, Floristics Ground Cover, Microbial Community Dynamics, and the Ground/Litter Ant Community.

Discriminant analyses were conducted on each of these EI systems, and discriminant function (DF) scores were plotted for the five new disturbance classes to assess the response of each EI system along the disturbance gradient. The DFs are vectors that represent weighed linear combinations of the original variables (e.g., indicator metrics). DF1 is the optimal combination of the original metrics that best distinguished the five disturbance classes, and we call this vector a “guild”, because it represents a functional group capable of quantifying and characterizing landscape disturbance. DF1 for both the General and Floristics Ground Cover guild reliably portrayed the disturbance gradient, while for the Ant Community it distinguished between lower and higher disturbance classes. DF2 in all three cases represented a pattern that verified the intermediate disturbance hypothesis. This hypothesis predicts that species richness is highest in sites that are subjected to moderate disturbance, as contrasted to lack of disturbance or severe disturbance. The intermediate disturbance hypothesis has important implications for ecological theory, biodiversity conservation, habitat restoration, and land management. DFs 3 and 4 assisted in the separation of disturbance classes that may have been closely associated in lower order discriminant space. The Microbial Dynamics guild was not included in this report, because errors were detected in its database, and these are currently being resolved. Additional analyses and statistical modeling are continuing, particularly in the association of multiple guilds, the relationships of landscape disturbance and biodiversity patterns, and species-habitat modeling.

Our research results in identifying Ecological Indicators and classifying their metrics into guilds in a wide variety of upland vegetation communities in the complex physiographic ecotone and disturbance regimes at Fort Benning are indeed encouraging. Nevertheless, the data were collected at a single location in the Fall-Line Sandhills. Additional data is required from a larger geographic area and an even greater variety of vegetation communities and soil types (especially clayey), both in the Southeast and in other regions of the United States.

Objective

Military land-use must be efficiently and cost-effectively monitored to assess conditions and trends in natural resources relevant to training/testing sustainability, ecosystem maintenance, and the timing and success of restoration efforts. **Ecological Indicators** represent important land management tools for tracking ecological changes and providing early-warning detection of threshold impacts to prevent irreversible environmental damage. The objective of this research was to develop Ecological Indicators and classes of indicators (e.g., guilds and indices) based on statistical criteria, ecosystem relevance, reliability and robustness, economy and ease of use for land managers, multi-scale performance, and stress-response criteria. The purpose of the Ecological Indicators was for monitoring ecological changes directly relevant to biological viability, long-term productivity, and ecological sustainability of military training and testing lands.

The general strategy for this ambitious undertaking will be to identify combinations of indicators or suites of indicators (i.e., statistically derived guilds) that are economical and relatively easy for land managers to monitor. Nevertheless, these simple indicator metrics will be statistically related to important ecological processes and community composition, and hence, possess the capability to assess and monitor ecosystem condition and trends. The basis for the development of statistically based Ecological Indicators was the identification of ecosystem metrics that analytically tracked a landscape disturbance gradient.

Introduction and Background

The motivation to develop reliable and robust ecological indicators was provided by the requirement for long-term sustainable land management under very challenging and potentially contradictory multiple land-use. The Department of Defense manages over 10 million hectares of land in the United States. This land area is comparable in size to the National Park system. Military ecosystems contain a disproportionately large share of federal and state threatened and endangered species (Flather et al. 1994, Shaw et al. 1997), possessing more Federally listed threatened and endangered species (approximately 200) than any other federal or state agency (Boice 1996). The U.S. Army has made a strong commitment to environmental mandates (Pringle 1991) and concerns for biodiversity and endangered species management (e.g., Krzysik 1994, Keystone Center 1996, Leslie et al. 1996, Schreiber et al. 1997, Cassels et al. 2001). This commitment is particularly dedicated in the longleaf pine forests and savannas of the southeastern United States (Kulhavy et al. 1995, Harper et al. 1997).

~~The development of r~~Reliable and integrative ecological indicators that track landscape conditions relevant to ecosystem structure and dynamics ~~would represent are~~ a significant practical tool for land managers. ~~Such indicators should track landscape conditions relevant to ecological structure and dynamics so that managers can to~~ assess current conditions and monitor ecological changes or the trajectory of ecosystem processes. Recent reviews have covered the context, use, and application of ecological indicators (Fisher 1998, NRC 2000, Dale and Beyeler 2001, EPA 2002, Niemi and McDonald 2004). The U.S. Environmental Protection Agency has had a long history and interest in the evaluation and application of environmental indicators (Hunsaker and Carpenter 1990, Fisher et al. 2003). The quality and reliability of scientific

information provided to the public and decision makers on the condition of our environment and the relationship to societal values have benefited from the use of ecological indicators (e.g., Harwell et al. 1999, Heinz Center 2002, EPA 2003).

Land Management Challenges and Ecological Assets

Military installations in the southeastern United States are landscape habitat islands in a sea of urbanization, agriculture, and short-rotation pine plantations. Nowhere is this better demonstrated nationally than in the Fall-Line Sandhills, which extend from extreme eastern Alabama to southeastern North Carolina. The Fall-Line Sandhills are a relatively narrow band of low-relief rolling hills of deep sands adjacent to the Fall-Line, forming a biogeographic transition between the Coastal Plain and the rolling hills of the Piedmont Plateau (Frost 1993, Peet and Allard 1993). The Fall-Line region consists of Coastal Plain strata overlaying crystalline Piedmont basement rocks providing the opportunity for geologic and biotic complexity and heterogeneity. The Sandhills are the result of major and very diverse geomorphic processes: the erosion of inland high mountains of Cretaceous age, ancient fossilized sand dunes, sandy-loamy Cretaceous marine sediments, and historically recent fluvial deposits from the Piedmont after forest clearing and agriculture. Historic losses of Piedmont soils ranged up to 30.5 cm (Orme 2002). Trimble (1974) reported that the average soil loss from agriculture in the Georgia Piedmont was 19 cm. Sand Hill soils are light textured loamy-sands that are friable, low in nutrients, easily eroded, and readily susceptible to deep gully incision. These soils are well to excessively drained, resulting in some of the topography having higher elevations than the adjacent Piedmont (Peet and Allard 1993). Because of the character of Sandhills soils, agriculture was short-lived in the region, and inexpensive degraded lands were readily available to the military throughout the Sandhills during the first-half of the twentieth century. All the major Army installations in the Southeast lie throughout the Sandhills arc from Fort Benning in westcentral Georgia to Fort Gordon (northeastern GA), Fort Jackson (central SC), and Fort Bragg in southeastern North Carolina. Air Force bases are also scattered in this region.

The presettlement range of longleaf pine forests occupied 92 million acres from southeastern Virginia through eastern Texas (Frost 1993). Today, less than 2 percent remains, making it one of the most endangered ecosystems in the United States (Noss and Peters 1995). The longleaf ecosystem is home to two of the most intensely researched high-profile endangered species: Red-cockaded Woodpecker (McFarlane 1992, Kulhavy et al. 1995) and Gopher Tortoise (Auffenberg and Franz 1982, Eubanks et al. 2002). However, what is not appreciated is the high biological diversity of these ecosystems. Peet and Allard (1993) documented 23 different compositional longleaf pine communities within longleaf pine forests and savannas, with several local plant communities representing the highest densities of species richness reported for the entire western Hemisphere. Hardin and White (1989) listed 191 rare plant taxa associated with the longleaf-wiregrass ecosystem, 66 of them were believed to be local endemics. Much of this diversity is probably attributed to fire disturbance, preventing the evolution of dominants (Huston 1994, see discussion and references pgs. 121-122). The vertebrate fauna of the Southeast is also particularly diverse (Echternacht and Harris 1993). The regional amphibian and reptile fauna contains 290 native species, 170 of these are found in the remnant longleaf pine ecosystem, many are endemics, many are endangered (Dodd 1995). The aquatic fauna of the southeastern United States is the most diverse temperate freshwater fauna on the planet and one of the most endangered (Williams et al. 1993, Walsh et al. 1995, Benz and Collins 1997).

The Southeast indeed represents concerns of global significance: biodiversity, endemism, endangered species, and endangered ecosystems. Military landscapes are at the same time training facilities and regional biotic refugia, facing the most diverse and challenging resources management and land-use decisions of all public or private landscapes. They are federally mandated to not only provide for the security of our nation with their military training and testing missions, but also to accommodate an ever-increasing burden of biodiversity and endangered species management, as regional landscapes become degraded and fragmented with urban sprawl and other intensive economy-directed land-use. Additionally, military installations typically have local cooperative agreements for: recreational hunting, timber harvest, grazing, and agriculture.

The Need for Ecological Indicators

Indicator species have been used for at least a century, when Merriam (1898) based his North American life zones on vertebrate indicators. Recommended indicator species have spanned the entire biological realm of organisms: bacteria, fungi, algae, lichen, plants, protozoans, invertebrates, and vertebrates (see references in NAS 1979, Landres et al. 1988, Hunsaker and Carpenter 1990, NRC 2000). Indicator species have been applied to classifications (Hall and Grinnel 1919), environmental and resource management (Kimmins 1990, Jeffrey and Madden 1991, Harig and Bain 1998), wilderness management (Belnap 1998), and the assessment and monitoring of environmental quality (Thomas 1972, NAS 1979, Zonneveld 1983, Bock and Webb 1984, Newman and Schreiber 1984, Müller et al. 2000, NRC 2000). Indicators have particularly been favored in aquatic ecosystems, because water quality is highly relevant to anthropocentric values and established legal mandates (e.g., Phillips 1980, Berkman et al. 1986, Rosenberg and Resh 1993).

Despite their long history of operational value, the use of indicator species has been controversial and their use criticized (Landres 1983, Graul and Miller 1984, Mannan et al. 1984, Morrison 1986, Block et al. 1987, Karr 1987). The fundamental issue is that natural ecosystems possess complex interactions and interdependencies and they are spatially and temporally dynamic. The use of one, several, or even a suite of similar species (e.g., a guild) as an “ecological indicator” and then extrapolating their fate to ecosystem responses or the behavior of other species represents a very tenuous hypothesis. The link between individual species and ecosystem processes has been challenging (Jones and Lawton 1995, Simberloff 1998, Weiher and Keddy 1999), but is progressing (Wardle 2002). Lindenmayer et al. (2000) suggested the use of habitat structural features as biodiversity indicators, because of the difficulties and ambiguities in monitoring individual species. Our 1998 proposal for this specific research also recognized the value of habitat metrics and incorporated them as surrogates of community structure and composition.

There is widespread and growing interest in developing terrestrial metrics or indices that assess landscape condition and are capable of monitoring long-term ecological changes (Belnap 1998, Andreasen et al. 2001, Niemi and McDonald 2004). Terrestrial applications have proven difficult, and lag far behind the over two decades old stream-based IBI (Index of Biological Integrity) developed by Karr and colleagues (Karr and Chu 1999). Broad scale indicator criteria at multiple ecosystem levels have been suggested to monitor biodiversity trends (Noss 1990,

Wickham et al. 1997). Nevertheless, field measurements of responses from a broad range of ecological hierarchies (individuals, populations, communities, and ecosystems) to a landscape disturbance gradient have never been demonstrated. Our ecological indicators research framework was based on the foundations and controversial insights discussed above and our development of desirable criteria and properties of ecological indicators (Table 1). Our research model was that promising ecological indicators could be initially identified by comparing sites in similar vegetation and soils, but exposed to a disjunct land-use disturbance gradient. Identified indicators could then be validated for reliability and robustness in a broader range of disturbance, vegetation communities, and soil types. We measured responses from a broad range of biotic and abiotic ecosystem attributes along a military training disturbance gradient. The complex heterogeneous landscape of the southeastern Fall-Line Sandhills provided an ideal research setting. This general approach has been used for military war-game scenarios in the Mojave Desert (Krzysik 1984) and to visitor use at Arches National Park (Belnap 1998).

The Guild Concept

Root (1967, 1973) originally introduced the guild concept as a group of species that exploit similar resources in a similar way, without regard to taxonomic affiliation. However, MacNally and Doolan (1986) suggested that guilds be defined as closely related species using food resources in similar ways. Guild structure investigations became standard practice for avian ecologists, because birds are easy to observe in their natural habitats relative to what they are feeding on, where and on what substrate they are feeding specifically, and the feeding strategies employed (e.g., Willson 1974, Holmes et al. 1979, Landres and MacMahon 1980, DeGraaf et al. 1985). Guilds based on genera have conveniently been used as classes of similar resource use: food and reproduction for dung beetles (Hanski and Koskela 1979), arthropods feeding on nematodes (Walter and Ikonen 1989), and ecosystem use by amphibians (Krzysik 1998a). Nevertheless, the concept of the guild has been broadened considerably (Landres 1983, Hawkins and MacMahon 1989, Simberloff and Dayan 1991). Guilds have been used to classify forest cover and age classes (DeGraaf and Chadwick 1984), stream habitat (Gorman 1988), and even environmental management, assessment, and monitoring (Järvinen and Väisänen 1979, Block et al. 1987, Hawkins and MacMahon 1989). Krzysik (1985) used Impact Guilds to classify rodent and bird responses to military landscape-scale training activities at the Army's National Training Center in the central Mojave Desert. Guilds in this research simply reflect classes (suites) of species composition and abundance (or percent cover) or chemical/physical metrics all sharing a statistically derived relationship to an *a priori* defined disturbance gradient. Our research model is presented in Figure 1.

Military Landscapes are Ideal for Managing with Ecological Indicator Tools

Military lands are truly multiple-use landscapes where the viability of ecological processes must be cherished and maintained far into the future. Not only is ecological integrity necessary for the maintenance of functional refugia for fragmented regional biota, it is essential for realistic and safe military training operations. Therefore, these lands must be efficiently and cost-effectively monitored to assess conditions and trends in the landscape relevant to ecosystem maintenance, native biodiversity, target species management, conservation of natural resources, the timing and success of restoration efforts, and the sustainability of military training and testing missions. Ecosystem based indicators represent valuable land management tools for tracking ecological

changes and providing early-warning detection of threshold impacts resulting from military missions and other land-use.

The Landscape Disturbance Gradient

Fort Benning represents the ideal research opportunity, because it provides a very broad range of habitat conditions, consisting of landscape mosaics that vary from highly disturbed patches to areas that show little or no disturbance. Direct habitat disturbance occurs primarily as a result of mechanized infantry training activities and their associated tactical elements. Fort Benning is the U.S. Army's primary infantry training facility, and also hosts a mechanized brigade. The training mission is to teach and practice mechanized, airborne, and on-foot tactics. The vehicles best identified with mechanized infantry maneuvers are the 4-WD Humvee (HMMWV - High-Mobility Multipurpose Wheeled Vehicle), 3900 kg; and three tracked vehicles: the M2 Bradley Fighting Vehicle, 29,900 kg; the M1A1 Abrams tank, 57,000 – 63,000 kg; and the M109 Paladin 155-mm self-propelled Howitzer (the large artillery piece that appears to be a "tank with an unusually large gun"). Additionally, a broad assortment of logistical and supply vehicles are also deployed. The Humvee gets a lot of field time, because it is a light-weight and exceptionally maneuverable vehicle that was designed to deploy an extreme diversity of weapon platforms, including light and heavy machine guns, TOW missiles, and Stinger surface-air missiles. TOW (Tube-launched-Optically-tracked-Wire-guided) missile capabilities are commonly deployed and trained, making the Humvee a very effective "tank-killer". All military training activities disturb vegetation and soils to varying degrees, but tactical tracked and wheeled vehicles cause specific severe changes to soil structure in a wide variety of soils (Webb and Wilshire 1983, Prose 1985, Milchunas et al. 1999, Garten et al. 2003). Soil damage includes compaction, shearing forces, and disruption and mixing of soil horizons. Although tracked tactical vehicles are very heavy, the actual pressure they exert on the ground is actually less than that of a cow or full size pick-up truck, because of their high surface area contact with the ground; 0.7 kg/cm² for an M2 Bradley and 1 kg/cm² for the M1A1 Abrams tank (Laur and Llanso 1995). Nevertheless, tracked vehicles present high shear forces to soils several centimeters below the surface, where compaction can increase soil density by over 30 percent over that caused by static forces alone (Bodman and Rubin 1948, Taylor and Vandenberg 1966). In addition to military training activities, regular prescribed burning, and timber harvest activities have impacted Fort Benning soils, vegetation composition and physiognomy, and ecosystem processes (Hatchell et al., 1970).

Tactical vehicles along with foot personnel and bivouacking create a great deal of damage to vegetation and soils. Nevertheless, the Fort Benning landscape is not completely degraded, because training scenarios and tactical engagements are relatively specific and consistent with well-planned operations. Tactical vehicles travel along established roads and trails when moving around in the landscape to their engagement positions. Major roads/trails are along ridgelines, providing convenient boundaries for watersheds and training compartments. Both trails and vehicles only enter streams and riparian zones at established "hardened" crossings. Actual engagements only occur in relatively small landscape patches, on the order of 20 to 40 hectares. These areas are highly fragmented with extensive and complex habitat mosaics of bare ground, fields of early succession herbaceous vegetation, dense trail networks, and relatively undisturbed habitat patches.

Many large areas of the installation consist of relatively pristine tracts of forest or savanna, because they are protected for their conservation or compliance values or they represent the large area of an long-range artillery-fire safety fan. Conservation and compliance areas include protection and management of threatened/endangered species (e.g., Red-Cockaded Woodpecker, Gopher Tortoise, listed plant species); and unique, valuable, or rare habitats and plant communities (i.e., Fort Benning's 15 "Unique Ecological Areas"). An important factor in the maintenance of these relatively pristine ecosystems is that public access to the installation's training ranges is limited, regulated, and carefully controlled.

Research Approach

Ecosystems consist of soil, bacteria and fungi (decomposers and nutrient cyclers), primary producers (plants), consumers (animals), and the abiotic environment in a given physiographic-climatic continental setting. Based on our research team's experience and an extensive literature review, we selected eleven Ecological Indicator Systems (EISs) that represented major ecosystem structure, function, and processes components:

- 1) General Habitat Metrics,
- 2) General Ground Cover,
- 3) Floristics Ground Cover,
- 4) Soil Chemistry,
- 5) Nutrient Leakage,
- 6) Soil Mineralization Potential,
- 7) Microbial Community Dynamics,
- 8) Ground/Litter Ant Communities,
- 9) Developmental Instability (DI) of selected plants, invertebrates, and fish;
- 10) Plant Physiology Metrics,
- 11) Spatial Organization of Plant Communities.

Our working hypothesis was to statistically characterize the response of these EISs along a military training disturbance gradient. Suites of environmental variables within EISs that analytically portrayed the disturbance gradient were called Ecological Indicator Guilds. We hypothesized that Ecological Indicators could be identified that not only successfully tracked a broad landscape disturbance gradient, but were also easy and economical for land managers to monitor. Our strategy was that we would through statistical modeling analytically associate these successful simple and fundamental indicator metrics (e.g., General Habitat, General Ground Cover, Soil Chemistry) with the more complex, less tractable, but more relevant ecosystem condition and processes Ecological Indicators such as nutrient dynamics, microbial function and dynamics, and ant community structure. In Phase I research, we would identify promising EISs from among the eleven above, at sites that possessed similar historical land-use, upland vegetation, and soils; with sites differing only in military training intensity and history. In Phase II research, we would evaluate the validity and robustness of the Phase I selected Ecological Indicators at sites representing the fullest range of disturbance (including the most pristine areas), upland plant communities, and soil types available at the installation.

Methods and Materials

Research Location

The research was conducted at Fort Benning in westcentral Georgia. The installation is in the Fall-Line Sandhills, the physiographic ecotone between the Coastal Plain and Piedmont in the Southeast, characterized by a gentle rolling topography and geological and ecological heterogeneity. There is also the intrusion of Loamy Hills physiography from Alabama to further complicate the landscape. This biogeographical transition zone is characterized by high landscape and species richness, the occurrence of ecotonal taxa, and fire dependent taxa and plant communities. The Fall-Line Sandhills consists of deep porous sands deposited by the advance and retreat of early seas, with added soils and clays from erosion of the Piedmont. Erosion has resulted in a landscape of rolling hills. The soils formed in two types of parent material: marine sediments that have undergone considerable *in situ* weathering, and water-deposited material on stream terraces and floodplains (Mason 2002). Paleudults are found on slopes where the upper sandy strata are thick, while Hapludults are found on thinner sand deposits underlain by more clayey materials. The upland flora is typically xerophytic.

Mean summer temperature at Fort Benning is 27°C, while mean winter temperature is 9 °C; annual rainfall is 130 cm, with 53% falling from April through October (Mason 2002). The mild humid climate favors the growth of bacteria and fungi, increases the rate of chemical reactions in the soil, results in rapid decomposition of organic matter, and facilitates the formation of soils low in organic matter and nitrogen and poor water holding capacity. The high precipitation leaches large amounts of nutrients and soluble bases and moves fine particles deep into the soil, resulting in acidic sandy soils low in fertility. Continuous leaching of the soil, along with the uptake of nearly all nitrogen results in low total nitrogen and an extremely high carbon/nitrogen (C/N) ratio (Barry 1980).

The regional presettlement landscape consisted of longleaf-shortleaf-loblolly pines and hardwoods forest communities, and was transitional to the longleaf pine – wiregrass or bluestem pyroclimax community further down the coastal plain where fire was more frequent and important in structuring communities (Frost 1993). Although all our research sites and the surrounding landscape were subjected to agricultural activities for at least four decades prior to 1943 (Kane and Keeton 1998), some of the current forest patches at Fort Benning reasonably reflect presettlement tree community composition. However, loblolly pine typically dominates its congeners, because of post-agriculture silvaculture practices. Currently, Fort Benning has an active three-year burn cycle, specifically addressing Red-Cockaded Woodpecker and longleaf pine management priorities. Fort Benning maintains the largest population of Red-Cockaded Woodpeckers in existence (Fort Benning, Integrated Natural Resources Management Plan). Ground cover is diverse consisting primarily of woody vegetation (tree seedlings, shrubs, and vines) along with perennial forbs and some grasses. Annual forbs are uncommon both in species and numbers. The shrub layer is poorly developed because of the frequent burn-cycle.

Phase I Research Sites (2000-2001-2002)

Before the initiation of field research, desirable Ecological Indicator criteria were developed (Table 1). The selection of ecosystem metrics for field and laboratory evaluation to assess Ecological Indicator performance was strongly guided by these criteria.

Phase I research was conducted in two adjacent third-order watersheds, Bonham Creek and Sally Branch, within the installation's Delta training compartments. Nine research sites were established in similar upland mixed pine-hardwoods forest with similar soils, mainly Troup Loamy Sand (NRCS 1997). Elevations ranged between 100-150 m. The nine sites consisted of three each in three disjunct disturbance classes based on current and past military training land-use (Figure 2). High disturbance sites are currently used for landscape-scale mechanized infantry training maneuvers. Medium disturbance sites had experienced similar activities in the past, but current use is light and consists of foot traffic. Vehicles are confined to existing roads and trails. Low disturbance sites had no evidence of current or prior military training activities, and foot traffic is light. They are currently protected for their conservation and endangered species compliance values, or represent landscape safety fans for long-range artillery practice. Sites numbered 1 or 2 were in Bonham Creek, and those numbered 3 were in Sally Branch.

High sites were characterized by extensive habitat fragmentation and vehicle trails, reduced canopy and ground cover, extensive sandy loose soils with reduced A-horizons, large patches of bare ground, deep gullies, rock pedestals, active erosional head-cutting, and widespread evidence of construction engineering to retard erosion (e.g., rip-rap, water channeling). There was abundant evidence of recent military training (tracked and wheeled vehicle tracks, concertina wire, spent ordnance, empty MRE [meals ready to eat] packages, etc.). Medium sites possessed high ground cover and an intact forest canopy. Nevertheless, deep gullies were present and there was evidence of surface soil losses. There was less evidence of vehicle and infantry use (e.g., concertina wire, small arms casings). Low sites had minimal disturbance to soils, high ground cover, a well-developed woody ground cover and shrub layer, and the highest tree densities. Vehicle tracks and other signs of military presence were absent. Although occasional gullies were present, they were historical (pre-military installation) with well-developed vegetation, including the presence of pines exceeding 50 years of age.

Phase II Research Sites (2003)

Ecological Indicators derived in Phase I that were effective in statistically separating the three disjunct disturbance classes were validated in the broader disturbance gradient, plant communities, and soil types of Phase II research. Forty research sites were selected throughout the installation in April-May 2003, including the original nine, to represent the full range of upland habitats at Fort Benning: most pristine to most degraded from military training habitat disturbance, upland forest community types, and Fort Benning's "Unique Ecological Areas". The selection of these sites was based on eight GIS databases, site criteria and data from other SEMP research teams, and extensive field ground-truthing (Appendix A). These 40 sites were classified into 10 landscape disturbance classes, based on the visual assessment of military training damage to vegetation and soils. Relatively pristine sites were classified as Disturbance Class 1 (DC1), and correspondingly, the most severely degraded sites were classified as DC10. The classification was conducted before any field data were collected by a researcher (AJK) with over 20 years of field experience with military training habitat disturbance.

Statistical Design and Analyses

An unusual attention to statistical rigor and analytical detail was used in our statistical design to identify Ecological Indicators. Statistical rigor was particularly stressed in three areas (Krzysik 1998b):

- 1) unbiased systematic-random sampling designs
(i.e., adequate spatial coverage and representation, concurrent with sample independence and an accurate estimation of P-values);
- 2) the minimization of Type I error
(i.e., rejection of a true null hypothesis), the finding of significance when there is none, using conservative statistical inference when possible;
- 3) analyses with high statistical power, minimizing Type II error, (i.e., failure to reject a false null hypothesis); requires high sample sizes.

Analysis of Variance – ANOVA and MANOVA

Initial exploratory analysis to assess variable (e.g., indicator) differences among factors and disturbance classes were carried out with multivariate analysis of variance (MANOVA) when there were multiple dependent variables, or univariate factorial analysis of variance (ANOVA) when a single dependent variable was used. Levene's test was always used for testing homogeneity of variances (Levene 1960). MANOVA and ANOVA *post-hoc* comparisons were always assessed with Tamhane's T2 multiple comparison test. This test is not only very conservative (minimizes Type I error), but is the recommended procedure when variances are heterogeneous, typical of ecological data (Tamhane 1979). This test was used even when variances were homogeneous. We felt it very important to use conservative inference throughout our analyses to minimize Type I error. In other words, when variables were found to be statistically significant among disturbance classes, we wanted to be certain in their assignment as "indicators", even at the possibility of "missing potential indicators" (Type II error). This was the statistical criteria used for determining significant differences among disturbance classes in Phase I research. Variables input into analyses were used both untransformed and appropriately transformed (Sokal and Rohlf 1995). Typically, variable transformations did not change analyses results, indicating robustness. Variables were transformed for analysis of variance, but untransformed variables were used for discriminant analysis.

Statistical significance of factors in Multivariate Analysis of Variance (MANOVA) was assessed with four metrics: Wilk's Lambda, Hotelling's Trace, Pillai's Trace, and Roy's Largest Root. The first three are conservative inference metrics, gave comparable results, and were used for inference. The latter was occasionally very liberal, increasing the potential for Type I error, the rejection of a *true* null hypothesis. In other words, finding significance when there really was none.

Discriminant Analysis

Discriminant analysis (DA) was selected to identify and quantify the importance of variables (i.e., indicators) possessing high discrimination to separate disturbance classes. Discriminant analysis is a powerful multivariate procedure to estimate the relative importance of predictor variables in characterizing (i.e., separating) pre-established groups or classes (Tabachnick and Fidell 2001). Recall, that DA "does not know" any ranking or ordinal nature of the groups. It

only knows that there are N-groups that need to be distinguished by a variable set common to all groups. DA has important extensions and relationships with multiple regression analysis and MANOVA (McGarigal et al. 2000). DA has a long history of successful practical applications in community and habitat classifications (Norris and Barkham 1970, Goldstein and Grigal 1972, Matthews 1979, Gerdol et al. 1985, McCune and Allen 1985, Krzysik 1987). DA has been equally successful at elucidating species-habitat relationships with a broad range of species: birds (Anderson and Shugart 1974, Able and Noon 1976, Rice et al. 1981), forest management and birds (Conner and Adkisson 1976, Crawford et al. 1981), salamanders (Welsh and Lind 1995), turtles (Reese and Welsh 1998), but especially small mammals (M'Closkey 1976, Holbrook 1978, Dueser and Shugart 1979, Cavallaro et al. 1981, Jorgensen et al. 1998). See McGarigal et al. (2000) for numerous applications in wildlife management. Like any other multivariate method, DA is subjected to a long list of assumptions, which of course, may be tenuous with real-world ecological field data (Williams 1983, Tabachnick and Fidell 2001). Williams (1981) review was particularly pessimistic. Nevertheless, there is a great deal of evidence that DA is reasonably robust to these assumptions, particularly when large and relatively equal sample sizes are used (Harris 1975, Williams 1983, Legendre and Legendre 1998).

DA was performed on all Ecological Indicator Systems to extract Ecological Indicator Guilds. DA weights the predictor variables (e.g., habitat variables) such that their linear combination maximally distinguishes (discriminates) among two or more predetermined groups or classes - three disturbance classes in Phase I research. The well known F-ratio tests the criterion for measuring class differences, sums of squares between groups versus sums of squares within groups: $F = SS_B / SS_W$. By rewriting sums of squares terms in the form of vectors of weighed linear combinations of predictor variables, the matrix form reduces to:

$$v'Bv / v'Wv = \lambda$$

where λ represents the discriminating criterion. The discriminant problem reduces to extracting the set of weights, or coefficients, that maximizes λ . In other words, covariance structure among groups is maximized, while minimizing within-groups covariance. Discriminant Function 1 (DF1) maximally distinguishes the groups, DF2 represents the second best discriminant function that is orthogonal (i.e., uncorrelated) to the first, and so on. The number of derived discriminant functions is equal to the number of predictor variables or one less than the number of groups in the analysis, whichever is less. Therefore, in Phase I Research to identify variables separating the three disturbance classes only two DFs were derived. DA has several desirable properties. When sample sites lie along a disjunct landscape disturbance gradient, and appropriate habitat metrics are collected, DA identifies which variables, and their relative importance, best define or characterize the gradient. Because the method is sensitive to data matrix singularity, variables that possess high collinearity with other variables, a common situation with environmental parameters, are *a priori* rejected from analysis (Tabachnick and Fidell 2001). This is known as the "Tolerance Test". Both direct (all variables entered simultaneously) and step-wise methods were used in DA to extract reduced subsets of discriminating variables. Caution has been suggested in the use of step-wise methods (Green 1979, Stevens 1996), but their use in conjunction with direct analysis assists in variable selection and assessing the robustness of analyses. The step-wise approach provides additional information to the DA. It provides the Wilks' Lambda statistic, which assesses the addition of each step-wise input variable to its contribution to group centroid separation, concurrent with group cohesiveness. Additionally, the F-to-remove statistic assists in interpreting the relative contribution of a specific variable to

group discrimination. The variable with the largest value contributes the most to overall discrimination, independent of the contributions made by the other variables (McGarigal et al. 2000). The results of direct analyses were used as the final analysis to derive the discriminant function scores that were plotted in the figures. Similar results were obtained with both transformed and untransformed variables. Therefore, variables were not transformed for final analyses.

Principal Components Analysis

A very specific strategy was essential for the principal components analysis in the Ground Cover Floristics Guild for reducing plant taxa with many correlated variables into fewer independent principal components. The first step was to remove rare species, and therefore rare occurrences, from the analysis. The taxa variables were not transformed, the covariance matrix was used as input, the solution was Varimax rotated, and N=6 (Phase I), N=10 (Phase II) PCs were selected for extraction.

In Phase I, 126 species or morpho-species were identified in the Floristics EIS. This was reduced to 67 taxa by deleting 59 rare taxa with <5 occurrences. Six PCs were extracted.

In Phase II 262 species or morpho-species were identified in the Floristics EIS. This was reduced to 239 by deleting 23 rare species with single occurrences. The 239 taxa were further reduced to 10 orthogonal (independent) variables with a principal components analysis using covariance matrix input and interpreting the factor loadings of the varimax-rotated solution. Varimax rotation enhanced the interpretation of higher order principal components. Discriminant function (DF) scores were plotted for the five new disturbance classes to assess the response of each EIS along the disturbance gradient. The DFs are vectors that represent weighed linear combinations of the original analysis variables. This vector is the optimal combination of the original metrics that best distinguished the five disturbance classes, and we call this vector a guild, because it represents a functional group capable of quantifying and characterizing landscape disturbance.

Classification of 40 Research Sites into 10 Upland Forest Communities

Forty-three tree species (plus Pine Snags and Deciduous Snags) representing 7031 individuals were identified at the 40 sites, ranging from 1433 Loblolly Pines to four species represented by only a single individual. Twenty-six species and snags (N=6903, 98.2 percent of all individuals) were used to develop an Upland Forest Classification for the 40 sites. Trees with less than 18 sampled individuals were not included in this analysis. Six species of trees were represented by 17 to 12 individuals, but their inclusion in the tree database for classification analysis was not considered to be important.

Tree species/snags were represented by their **Basal Areas** for forest classification. The tree data based on basal area was analyzed by Hierarchical Agglomerative Cluster Analysis using Ward's criterion (Ward 1963) with squared Euclidean distance as the similarity metric. Ward's method is a minimum variance clustering procedure that seeks to form N clusters under the criteria that the trace of matrix W is minimized, where W is the matrix obtained by summing within-cluster sums of squares and products matrices (i.e., variance-covariance matrices) over all N clusters. A large number of clustering algorithms were experimented with in these analyses, including Average Linkage, Single Linkage (Nearest Neighbor), Complete Linkage (Farthest Neighbor), Centroid, and Median methods. Ward's method gave very similar results to the Average

Linkage (Unweighted Pair-Group Averages) method. The Average Linkage method is the most commonly used technique (Romesburg 1984), has desirable properties (Sneath and Sokal 1973), and was very effective with field data simulations (Krzysik 1987). However, Ward's method was most successful at developing a Tree Community classification that made the most ecological sense in tree species compositions. Both Ward's and Average Linkage methods are procedurally recommended (Romesburg 1984). Ten upland forest communities were identified with cluster analysis.

Independently, the tree community data based on basal areas was ordinated with Nonmetric Multidimensional Scaling (NMS) (McCune and Mefford 1999). The author has a great deal of experience with this method, and finds that it is robust and informative at uncovering underlying community patterns. The NMS ordination of the 40 sites readily identified seven forest community types that were extracted with cluster analysis. The first NMS axis represents a long gradient in basal area, clearly separating the **highly disturbed** sites with low basal areas from the mature stands of **Longleaf Pine Forests** on opposite ends of this gradient. The second axis represents a landscape moisture gradient, ranging from the **Oak-Hickory** Deciduous Mesic Forest to **Xeric Scrub Oak – Pine Savannas**. Three pine-hardwood mixed forests were closely clustered in NMS space: Loblolly/Shortleaf – Hardwoods, Mixed Pine – Oak – Hickory (Loblolly Dominant), and Mixed Pine – Southern Red Oak (one pine dominant, the other hardwood dominant).

Soil Texture Classification of 160 Transects at the 40 Research Sites

Soil samples were collected to a depth of 10 cm with a 2 cm corer. Samples were taken systematic-random along the entire length of each transect: six random samples were obtained near the beginning, middle, and end of each transect. These 18 samples were composited for a single soil texture determination for each transect using particle size analysis by the hydrometer method (Tan 1996). Soil Texture classes were calculated from the percentages of sand and clay in the sample using the American System Pedosphere.com soil texture and bulk density online calculator.

http://www.pedosphere.com/resources/bulkdensity/worktable_us.cfm

Deriving the Site Comparison (Condition) Index (SCI)

The Ecological Indicator Systems of General Habitat and Soil Chemistry were identified in Phase I as potential Ecological Indicators. They were evaluated and quantified in Phase II by the following statistical procedure. A linear regression for each General Habitat and Soil Chemistry metric was conducted with the 10 disturbance classes as the dependent variable. Only metrics with $P < 0.001$ were selected for incorporation into a composite SCI. Each selected variable was standardized by giving the specific variable a score of 100 at the site where this variable had its highest value, and then proportionately adjusting the values of that variable at each of the remaining 39 sites. Next, three statistical metrics were used to derive standardized weighed coefficients:

- 1) F-values from all the individual simple linear regressions,
- 2) t-values from a single multiple linear regression, and
- 3) Spearman's rho nonparametric correlations from each bivariate comparison.

Multiple regression only selects variables that add statistical relevance, because variables that add no unique information to the regression (i.e., exhibit high multicollinearity) are excluded

from the final multiple regression equation. A standardized composite SCI was derived for each of these methods by deriving proportional weighing coefficients for each of these methods, and the final SCI was calculated as the average of these three.

Most analyses were conducted with SPSS 13.0 (SPSS 2004).

Field Methods

Phase I Field Methods

The research sites were approximately 10 hectares in size. An **upland base transect** was established in the center of each site that was perpendicular to the slope of the gentle topography. A lowland base transect was established in the lowland habitat located down-slope of the uplands. All additional transects, plots, and site sampling were based on this upland base transect. Lysimeters, three each, were placed along both upland and lowland base transects. Most parameters were collected based on the upland base transect. Nutrient leakage and soil chemistry samples were the only data collected at both upland and lowland habitats. Random sample locations were determined using a pair of dice by a method developed by AJK.

General Habitat

General habitat variables (A-horizon depth, soil compaction, canopy cover, basal area) were collected by systematic-random protocols along four perpendicular 30 m transects centered on the upland base transect.

A-horizon depth: A garden trowel and a 15 cm stainless steel metric ruler were used to take two random samples at five locations in 2001, intersection of the four perpendicular 30 m transects, and at their 30 m endpoints (N=10/site). In 2002, samples were systematic-random obtained every 5 m along each transect from 5 m to 30 m (N=24/site). Because of the difficulty and subjectivity involved in locating the base of the A-horizon, estimates were always made by the same individual (DAK) to 0.5 cm.

Soil compaction: Soil Compaction data (2001-2002) were systematic-random obtained every 1 m along each transect from 1 m to 30 m (N=120/site), data in Lang Units, using a Lang Penetrometer, Forestry Suppliers.

Soil Shear Stress: Soil Shear Stress data (2002) were systematic-random obtained every 3 m along each transect from 3 m to 30 m (N=40/site), data in kg/cm², using a Vane Shear Tester, Model E-285, Forestry Suppliers.

Canopy cover: Canopy cover samples (2001-2002) were taken at five locations, intersection of the 30 m transects and at their endpoints using a Concave Spherical Densimeter, Model C (Forestry Suppliers). At each sampling point, 96 “canopy hits/misses” were determined at each of four 90-degree apart sighting positions. Therefore, there were 384 “canopy hits/misses” per sampling point or 1920/site (N=5/site).

Basal area: Basal area (2001-2002) was determined at the identical locations as canopy cover using a Cruz-All Basal Area Factor Gauge (Stock No. 59795, Forestry Suppliers). At each

sampling point, BAFs of 40, 20, 10, and 5 were determined. In the database, the data were converted to m²/ha, and the largest value of Basal Area from the four readings was used as the final point Basal Area estimate (N=5/site).

Tree Community: Tree: density, species richness, mean DBH, number/species were collected on four 0.36 ha circular plots centered on the intersection of the 30 m transects in 2001 and 2002. Each tree in the plot was identified to species and its DBH (Diameter Breast High) was measured with a 5 m fiberglass DBH Tape (Forestry Suppliers Inc., No. 59571). DBH was recorded to 0.1 cm, and only individuals with a DBH $\geq$ 5 cm were tallied. Pine snags and deciduous snags were also measured and counted.

General Ground Cover

General ground cover variables were collected along six 65 m transects using 13 systematic-random 0.58 m² round quadrats on each transect in 2001 and 2002. Two transects coincided with the base transect, and the other four were located 45 degrees from the base transect. The percent cover of vegetation < 2 m in height was visually estimated: total forbs (including legumes), total legumes, total grass (includes sedge-like nongrass), woody vegetation, ferns, *Yucca*, *Opuntia*, pine seedlings, and total pine. Additionally, the percent cover of bare ground, pine litter, deciduous litter, and forb/grass litter was estimated.

Floristics Ground Cover

Ground cover floristics variables were collected in the identical quadrats described above. The percent cover of each species or morpho-species (plants that were recognizable, but could not be identified to species) was estimated in each quadrat. Although data were collected in 2001 and 2002, only 2002 data was used in the analysis because of greater confidence in correct species identifications.

Microbial Community Dynamics

Six composite soil samples were taken along each base transect in both upland and lowland habitats, two samples in the vicinity of each lysimeter. Each sample consisting of a composite of six individual soil samples taken with a garden trowel to a depth of 15 cm. Data were collected in the spring (May) and fall (October or November) of each year. Soil samples were refrigerated and processed in the laboratory using two established standardized procedures: BioLog for bacteria (Zak et al. 1994) and FungiLog for Fungi (Sobek and Zak 2003). For each procedure, two different microbial responses, *Total Activity (Amount) of Substrate Utilization* and *Functional Richness (Diversity) of Substrate Use*, were assessed for growth on 95 nutritional substrates. Functional richness equals the number of substrates from the total of 95 that were utilized. Laboratory trials determined that the 95 nutritional substrates could be effectively classified into seven **substrate guilds**: simple carbohydrates, complex carbohydrates, amines/amides, amino acids, carboxylic acids, polymers, and nucleotides. Therefore, a total of 28 variables comprised the microbial dynamics guild, with the variables coded as follows: bacteria (B), fungi (F), total activity (A), functional richness (R), and substrate guild (7 substrate codes).

Two Phase I separate data sets were developed for the microbial guild:

Bonham Creek – 6 sites (Numbers 1 & 2), data collected in 2000, 2001, 2002

9 Sites – Bonham Creek and Sally Branch (Numbers 1, 2, & 3), data collected in 2002.

Bacteria and fungi were each analyzed separately for each data set using identical statistical procedures. First of all, MANOVA (with Tamhane's T2 multiple comparisons test) was used to assess the significance of factors (treatments) and factor interaction terms. Second, because many of these were highly significant, separate univariate analysis of variance (ANOVA) (with Tamhane's T2 multiple comparisons test) were carried out for each: year, habitat, and dependent variable (microbial variable). Finally, discriminant analysis (DA) was conducted combining years and seasons, but **not** habitats. This resulted in a statistically very conservative approach to DA. Because it was reasoned that if DA could effectively separate the three disturbance classes with the confounding effects of years and seasons, it would indeed be a very robust result, successfully identifying landscape disturbance independent of weather and seasonal environmental effects. Other multivariate and ordination methods will also be applied to this rich data set, and importantly, in combination with our other Ecological Indicator Guilds.

Soil Chemistry

Soil chemistry analyses were conducted in the identical soil samples used for the microbial dynamics guild analysis described above, and were analyzed in the laboratory with standard analytical methods. The soil variables measures were: organic content, microbial biomass carbon, ammonium (NH_4^+), nitrate (NO_3^-), pH, and moisture.

Nutrient Leakage

Nutrient Leakage was determined from soil solution concentrations collected by six lysimeters at each of the nine sites based on the methods of Kovacic et al. (1990a, 1990b). Three lysimeters were located along the upland base transect, and three were located down-slope in a lowland habitat. Lysimeters were located from 15 to 25 meters adjacent to one another. Lysimeter samples were collected over the entire year, but primarily in the spring and fall. Data could not be obtained for all lysimeters during a given sampling period because of regional drought periods and occasional damage to lysimeters by feral hogs, other wildlife, and prescribed burns. Lysimeter samples were immediately frozen when brought in from the field and subsequently analyzed in the laboratory with a Dionex Ion Chromatograph. Lysimeters were sampled in 2000, 2001, and 2002.

Soil Mineralization Potential

Soil and forest floor litter samples were collected at all nine sites in an identical fashion in the winter (February 2002), a period of low nutrient uptake when soil nutrients are immobilized in the microbial pool. Sandhills forest soils exhibit high spatial heterogeneity. Therefore, the systematic-random sampling protocol described here was designed to provide incubation samples that were not only representative of the sites being sampled, but minimized site specific spatial variance. Two, 40 m transects perpendicular to the site slope were established 10 m upslope and 10 m downslope from the exact center of each base line transect. Flags were placed at the beginning (0 m) middle (20 m) and end (40 m) of each transect. At each of the six flags, six soil subsamples to a depth of 10 cm were randomly taken with a 2 cm soil corer. Each subsample was approximately 2 m from each flag. These six subsamples were composited. Therefore, each site initially had six soil samples. Again, these six samples were composited a

second time resulting in a single sample for each of the nine sites. Therefore, each sample for site incubation analysis consisted of 36 double composited field samples.

A single litter sample was randomly taken at approximately 2 meters from each of the six flags. A 15 x 15 cm plywood template was placed over each sample location. A knife was used to sever all leafy material outside of the sample point and all forest floor within the 15 x 15 cm template was removed, providing an accurate spatial areal measure of the forest floor. As in the case of soil samples, the six litter samples were composited, resulting in a single sample for each site.

Ground and Litter Ant Communities

Ground and litter ant communities were sampled with pitfall traps. Each trap consisted of a plastic 9 oz Solo® plastic cup buried flush with the soil. Approximately 2 cm of propylene glycol (Sierra Antifreeze) was used to trap and preservative the ants. Traps were left out overnight over a total duration of 24 hours. Pitfall traps were only used when there was no precipitation. Four (2000), five (2001), and six (2002) clusters of five traps at each cluster were used at each of the nine sites in 2000 and 2002. Only six sites were sampled in 2001, Bonham Creek number 2 sites were not sampled. The five traps were combined, making a cluster the sampling unit. All years were combined for discriminant analysis. Therefore, there were 120 samples for the 9 sites. The collected ants were preserved in 80 percent ethanol, and counted and identified in the laboratory using available ant keys and extensive consulting with ant taxonomy experts.

Ants were also sampled by two other additional methods. Arboreal ants were sampled in all disturbance classes by using an aspirator to “suck up” ants on tree boles. Pine samples and oak samples were treated as different strata. This method collected many individuals of *Crematogaster ashmeadi* and *C. atkinsoni*, arboreal species with the former being a documented important prey for Red-Cockaded Woodpeckers. Although much higher numbers of arboreal ant species were collected compared to the pitfall traps, no new species were found. Ants were also picked up in sweep-net samples of ground and shrub cover at all nine research sites. These methods collected relatively few ants compared to the pitfall traps, and their data analyses were not particularly informative. The sweep samples added only two additional species to the collection of pitfall ants.

Other invertebrates were collected in the pitfall traps and sweep-nets, especially spiders. The identification of spiders and their distribution in the nine study sites were subjected to detailed analysis. Spiders were virtually impossible to identify to species, because of the poor status of their taxonomy.

Carabid beetles are a major European indicator species collected in pitfall traps (Czechowsk 1982, Niemela et al. 2000). However, they were not particularly abundant in the Sandhills.

Orthopterans (grasshoppers, katydids, crickets) were collected by sweep-nets, as well as, individual time-constrained searches. Some were also collected in pitfall traps.

Developmental Instability and Plant Physiology

Developmental Instability (DI) was assessed in the perennial forbs bullnettle (*Cnidoscolus stimulosus*) and bigroot morningglory (*Ipomoea pandurata*), a woody vine muscadine grape (*Vitis rotundifolia*), a shrub winged (or dwarf or shiny) sumac (*Rhus copallinum*), loblolly pine (*Pinus taeda*), grasshoppers (Orthoptera), and fish (scale patterns). The DI metric used for plants was fluctuating asymmetry (FA) of leaf morphology in individual leaves or leaflets, and leaflet patterns with compound leaves. All plants were collected in the area centrally defined by the upland base transect. The size of the area sampled was approximately 10 hectares.

A significant effort was concentrated on *Cnidoscolus* and its measurements will serve as an example. *Cnidoscolus* has a simple three-lobed leaf with coarse teeth on all leaf margins. FA was calculated as the deviation in bilateral symmetry standardized by leaf size using the following formula $FA = |\log(R) - \log(L)|$, where R and L refer to specific morphological right and left symmetry leaf measures (Graham et al. 1998). These values were then Box-Cox transformed (Sokal and Rohlf 1995). Nine measures of FA were used: upper tooth numbers of lateral lobes, lower tooth numbers of lateral lobes, middle lobe teeth, lower widths of lateral lobes, upper widths of lateral lobes, main vein lengths of lateral lobes, middle lobe widths from main vein, ratio of lower to upper width FA of lateral lobes, and angles between the three lobe veins. Plants were collected over five hectares surrounding the base transect at each of the nine sites. Approximately 45 plants were collected at each site in 2000 (approximately 40 in 2002), and three (two in 2002) leaves were randomly selected from each plant for measurement. Each leaf was used as a sample. There were 1242 leaflets in the sample size for 2000, and 704 for 2002. Plants were pressed in the field and measured in the laboratory with specialized computer scanning software.

A similar protocol was used to measure FA in *Rhus*. This species has compound leaves and 12 FA measures were derived from plants collected over four years, 1999-2002.

Variable fluorescence, transpiration, and stomatal conductance was measured with a CI-510 photosynthesis machine (CID Inc., Vancouver, WA).

Water stress was measured using a pressure bomb (PMS, Corvallis, OR), following the methods of Freeman and McArthur (1982).

Spatial Organization in Plant Communities

Detrended fluctuation analysis (DFA) has been used to assess ecosystem stress by measuring changes in structural complexity of community structure (Alados et al. 2003). Using DFA, increasing disturbance levels (i.e., disruption of community organization) should produce more random community organization patterns. DFA provides a measure of spatial autocorrelation and, thus, the structural organization reflecting dynamic ecosystem properties. In areas experiencing habitat disturbance we should expect organization to be disrupted and, correspondingly, exhibit less autocorrelation. Here we measured spatial autocorrelation in the woody ground cover and ferns (< 1 m in height) that intersected 100 m fiberglass measuring tapes along 300 m transects in six (2001) and nine (2002) sites based on disturbance classes. We recorded the presence (+1) or absence (-1) values from contact points along the transect line for each species. Because we measured all instances of intersection, there were cases where overlap

occurred among different species along the same area of transect, and the same individual plant intersected a transect in more than one place. We scored each centimeter in the 100 m sub-transect as either +1 or -1 for each species. These data subsequently were transformed to scores of intercept or lack of intercept per disjoint segment of 20 cm (for analysis of *Rubus* sp.) or 30 cm (all other species). These larger basic units of intercept were chosen to be at least as large as the estimated crown width of an individual plant of the species of interest. This was done to avoid bias in the DFA analyses.

The application of DFA the basic units of 20cm or 30cm each are strung together into a series of segments composed of one basic unit each, alternatively as a series of non-overlapping segments of two adjacent basic units each, then as a series of four, and so on. Consider now a single segment of length 4 basic units, with intercepts of -1, -1, +1, -1. The cumulative score for the first basic unit in that segment is -1, the cumulative score over the first two basic units is -1 - 1 = -2, over the first three -1 - 1 + 1 = -1, and over the first four -1 - 1 + 1 - 1 = -2. We first regress these scores on the corresponding basic unit number to find the average trend in score over the segment in question. The error variance around this sequence, averaged over all segments of size 4, is denoted $F(4)$. In similar fashion, we calculate the average variance over segments of length 2, 4, 8, etc. Finally, the log of these average variances, $\log(F(L))$, is regressed on the logs of the corresponding segment lengths, $\log(L)$. DFA is based on the concept of a random walk which, as its name implies, describes a purely random sequence of “steps” (of size +1 or -1 per basic unit). There will be a net positive or negative average trend in score over these steps, and a variance (and standard deviation) in cumulative score about that trend. If the steps are random (i.e., if the direction of one step is wholly independent of the direction of preceding steps) then this standard deviation rises with the square root of L , the length of the segment over which it is measured:

$$S_L \propto L^{0.5}, \text{ or}$$

$$\ln(S_L) = c + (0.5)\ln(L).$$

where c is a constant. On the other hand, structural order implies something other than randomness. Therefore, the more general expression is

$$S_L \propto L^{\alpha}, \text{ or}$$

$$\ln(S_L) = c + \alpha\ln(L).$$

If the pattern is non-random, α (alpha) is something other than 0.5. In other words, DFA analysis derives a metric, “alpha”, to assess random versus non-random community structural patterns. Random patterns produce an $\alpha = 0.5$.

We presume that interactions, both with other ramets of the target species and with other species, lead to the development of a dispersion pattern that is non-random, and that is characteristic of the community in which the target species finds itself. However, if these normal patterns of interaction are disrupted due to habitat disturbance, there should follow a breakdown in that dispersion pattern and, accordingly, a degeneration toward randomness. Disturbed areas should exhibit alpha values closer to 0.5 than less disturbed areas. To avoid biases due to sparse information, we looked only at species and sites for which there were at least ten intercepts along a 300m transect, a total intercept length of at least 3m (an arbitrary choice of 1% of the total transect). All corresponding species and sites exhibited an adjusted R^2 for the log-log regression of at least 0.90.

Phase II Field Methods

At the center of each of the 40 sites, four perpendicular 100 m transects were established from a randomly determined coordinate between 0-359 degrees. The random coordinate was identified using a pair of dice. Plot size was 4-hectares. Field data were collected in a systematic-random design along each transect. All field data collected by all research teams were referenced to these four transects. The site center point was identified with two fluorescent pink flags. Each transect was identified with four fluorescent pink flags, placed at intervals of 25, 50, 75, and 100 m. Each flag was marked with its respective bearing and distance from site center. GPS center locations, transect bearings, and maps of all sites were provided to all research teams.

Ecological Indicator guild data were collected by the same methods described in Phase I research. The sampling locations were as follows:

A-horizon depth: systematic-random 10 samples from 10-100 m along each transect (N=40/site).

Soil compaction: systematic-random 50 samples from 2-100 m along each transect (N=200/site).

Ground cover: systematic-random 10 quadrats from 10-100, along each transect (N=40/site).

Canopy cover: two points along each transect, 33 and 67 m from origin (N=8/site, 3072 “hits”).

Basal Area: three points along each transect, 30, 60, 90 m from origin (N=12/site).

Trees: Four perpendicular 100 m x 10 m strip-transects that coincided with the four transects.

Microbial, Soil Chemistry, and Ant Communities:

Sample location was at the 50 m point along each transect (N=4/site).

Results and Accomplishments

Phase I Research (2000-2001-2002)

The purpose of Phase I research was to test all selected Ecological Indicator (EI) Systems and to identify which indicators, either as stand-alones or in groups (guilds), were capable of analytically characterizing a landscape disturbance gradient. An example of an EI guild or an indicator index would be a weighed linear combination of variables that separated disturbance classes. The analysis of each EI system is summarized below.

General Habitat

Variable: **A-Horizon Depth**

Year & Sample Size: 2001 (N=90), 2002 (N=216), Total (N=306)

Tamhane's T2 Multiple Comparison

Statistical Significance (P)

Low > Med	<0.001
Low > High	<0.001
Med > High	<0.001

Variable: Soil Compaction

Year & Sample Size: 2001 (N=1078), 2002 (N=1080), Total (N=2158)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
High > Med	<0.001
High > Low	<0.001
Med > Low	<0.001

Variable: Soil Shear Stress

Year & Sample Size: 2002 (N=360)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.33
Med = High	0.54
Low > High	0.021

Variable: Canopy Cover

Year & Sample Size: 2001 (N=45), 2002 (N=45), Total (N=90)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.51
Low > High	<0.001
Med > High	<0.001

Variable: Basal Area

Year & Sample Size: 2001 (N=45), 2002 (N=45), Total (N=90)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.42
Low > High	<0.001
Med > High	<0.001

Variable: Tree Density

Year & Sample Size: 2001 (N=36), 2002 (N=36), Total (N=72)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low > Med	<0.001
Low > High	<0.001
Med = High	0.065

Variable: Tree DBH

Year & Sample Size: 2001 (N=840), 2002 (N=936), Total (N=1776)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = High	0.34
Med > Low	<0.001
Med > High	<0.001

Variable: Tree Species Richness

Year & Sample Size: 2001 (N=840), 2002 (N=936), Total (N=1776)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low > Med	0.008
Low > High	<0.001
Med = High	0.24

Variable: Number of Trees / Species

Year & Sample Size: 2001 (N=840), 2002 (N=936), Total (N=1776)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.16
Low > High	0.001
Med = High	0.086

Discriminant Analysis (DA) of General Habitat Guild

DA of transect data: Figure 3

Correlation of Variable and Discriminant Functions

<u>Variable</u>	<u>DF1 (99.5%)</u>
canopy cover	0.79
basal area	0.58
A-horizon depth	0.55
soil compaction	-0.47

DA of plot data (Trees): Figure 4

Correlation of Variable and Discriminant Functions

<u>Variable</u>	<u>DF1 (77%)</u>	<u>DF2 (23%)</u>
tree density	0.79	-0.59
species richness	0.68	-0.40
number/species	0.41	0.040
DBH (mean)	0.18	0.91

General Ground Cover

Variable: Bare Ground

Year & Sample Size: 2001 (N=54), 2002 (N=54), Total (N=108), each N has 13 quadrats

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.28
High > Low	<0.001
High > Med	<0.001

Variable: Litter Cover

Year & Sample Size: 2001 (N=54), 2002 (N=54), Total (N=108), each N has 13 quadrats

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.27
Low > High	<0.001
Med > High	<0.001

Variable: Forb Cover

Year & Sample Size: 2001 (N=54), 2002 (N=54), Total (N=108), each N has 13 quadrats

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.91
Low = High	0.15
Med = High	0.37

Variable: Grass Cover

Year & Sample Size: 2001 (N=54), 2002 (N=54), Total (N=108), each N has 13 quadrats

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.42
Low = High	0.74
Med = High	0.95

Variable: Woody Ground/Shrub Cover

Year & Sample Size: 2001 (N=54), 2002 (N=54), Total (N=108), each N has 13 quadrats

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = Med	0.054 (borderline)
Low > High	<0.001
Med = High	0.085

Discriminant Analysis (DA) of General Ground Cover Guild

DA of transect data: Figure 5

Correlation of Variable and Discriminant Functions

<u>Variable</u>	<u>DF1 (94%)</u>	<u>DF2 (6%)</u>
litter	0.85	-0.008
bare	-0.84	0.016
forbs	0.22	0.13
woody	0.47	0.63
grass	-0.045	-0.44

Floristics Ground Cover

Year & Sample Size: 2002 (N=54), each N has 13 quadrats

Originally 126 taxa were identified in the samples at the 9 sites in 2002,
systematic-random sampling design,

6 transects/site, 13 quadrats/transect (0.58 m² quadrats), N = 702 quadrats

59 rare taxa with <5 occurrences were removed from analysis

Principal Components Analysis of 67 Ground Cover Taxa

A most carefully planned PCA

(PCA: untransformed data, covariance matrix as input, varimax rotation, 6 PCs extracted)

Associated ranked taxa based on Pearson Correlation (two-tailed)

PC1 is directly associated with the Disturbance Gradient

<i>Rhus radicans</i>	Poison Ivy
<i>Crataegus flava</i>	Yellow Hawthorne
<i>Diospyros virginiana</i>	Persimmon
<i>Galium</i> sp.	Bedstraw
<i>Coreopsis major</i>	Giant Coreopsis
<i>Prunus angustifolia</i>	Chickasaw Plum
<i>Hieracium venosum</i>	Rattlesnake Weed
<i>Quercus incana</i>	Bluejack Oak

PC2 is associated with LOW Disturbance Sites

<i>Vaccinium stamineum</i>	Deerberry (a blueberry)
<i>Cnidioscolus stimulosus</i>	Bullnettle
<i>Rhus radicans</i>	Poison Ivy
<i>Coreopsis lanceolata</i>	Lance-leaved Coreopsis
<i>Sassafras albidum</i>	Sassafras

PC3 is associated with less disturbed sites (LOW-MED) versus higher disturbed sites (HIGH)

<i>Carya tomentosa</i>	Mockernut Hickory
<i>Pteridium aquilinum</i>	Brackenfern
<i>Rhus aromatica</i>	Fragrant Sumac
<i>Clitoria mariana</i>	Butterfly Pea

PC4 is associated with HIGH Disturbance Sites

<i>Rubus cuneifolius</i>	Sand Blackberry
<i>Quercus margaretta</i>	Sand Post Oak
<i>Erigeron strigosus</i>	Rough Fleabane
Unknown Forb	Unknown Forb
<i>Conyza Canadensis</i>	Horseweed

PC5 is associated with less disturbed sites (LOW-MED) versus higher disturbed sites (HIGH)

<i>Quercus falcate</i>	Southern Red Oak
<i>Ambrosia artemisiifolir</i>	Common Ragweed

PC6 is associated with MEDIUM Disturbance Sites

<i>Vaccinium arboretum</i>	Sparkleberry (a blueberry)
<i>Pedicularis Canadensis</i>	Wood Betony, Lousewort

Discriminant Analysis (DA) of Floristics Ground Cover Guild

DA of transect data: Figure 6

Correlation of Variable and Discriminant Functions

<u>Variable</u>	<u>DF1 (77%)</u>	<u>DF2 (23%)</u>
PC1	-0.37	-0.26
PC2	0.35	-0.19
PC3	0.17	0.16
PC4	0.13	0.67
PC5	0.40	-0.50
PC6	0.14	0.18

Microbial Community Dynamics

The microbial data consists of two data sets: Bonham Creek (6 sites), 2000-2001-2002; and Bonham Creek and Sally Branch (9 sites), 2002. Microbial activity and dynamics were complex and strongly dependent on soil moisture (precipitation) and temperature. Therefore, bacteria and fungi measured responses were highly variable among years and season of field data collection. The years of data collection, 2000 through 2003 and their corresponding seasons (May or October/November field data) were significantly different, because precipitation patterns varied from severe 25-year drought to flooding conditions from heavy precipitation from severe thunderstorms. The important and complex details of how weather, physical environment, and habitat disturbance interact will be addressed in peer-reviewed professional publications currently in active preparation. The purpose of this Final Report was to identify individual and groups (i.e., guilds) of Ecological Indicators, and their response to anthropogenic landscape disturbance gradients independent of natural disturbance regimes such as weather. See Methods for additional details.

Multivariate Analysis of Variance (MANOVA)

MANOVA significance tests for major factors (treatment responses) were based on four multivariate metrics (see Methods). The Bonham Creek watershed analyses (6 sites, 2000-2001-2002) showed that year, season (May – October/November), habitat (upland – lowland), and disturbance class (Low – Medium – High) for both bacteria and fungi variables were highly significant ($P < 0.001$), with the single exception of season for fungi ($P = 0.56$), see Table 2. Factor interaction terms were highly significant for bacteria: years x season, years x habitat, years x disturbance; and for fungi: years x disturbance, habitat x disturbance (Table 2). These data closely, but not exactly, paralleled the analysis results for the combined Bonham Creek and Sally Branch watersheds (9 sites) in 2002 (Table 2).

Because there were such highly significant differences in factors and factor interactions in the MANOVA results, exploratory univariate analysis of variance (ANOVA) were conducted for separate factors for each *separate* dependent variable (microbial variable). This consisted of **224 separate** ANOVA analyses:

Bonham Creek: 14 microbial variables x 3 years x 2 habitats x
2 microbes (bacteria, fungi) +
9 sites: 14 microbial variables x 2 habitats x 2 microbes
= 224 ANOVA analyses

These univariate exploratory analyses reasonably paralleled and substantiated the multivariate results discussed about.

Tamhane's T2 multiple comparison tests based on MANOVA are presented in Table 3. Tamhane's T2 224 separate univariate analyses based on ANOVA also closely paralleled MANOVA results. Over the three years at Bonham Creek (6 sites), bacteria parameters were better EIs of disturbance class than fungi, and strongly separated High sites from Low and Medium sites. Low and Medium sites were not statistically different. However, fungi parameters were more effective EIs than bacteria at both watersheds (9 sites) in 2002. Interestingly in both analyses, fungi not only readily separated High sites from Low and Medium (as in the case of bacteria), but also was able to statistically separate Low from Medium sites. Although the relative importance between bacteria and fungi was "switched" between these two analyses, interpretation was similar. Bacteria clearly separated the High sites from Low and Medium. Fungi were effective at separating all three disturbance classes – High sites were also clearly separated from Low and Medium sites, while Low and Medium were less effectively separated.

Discriminant Analysis (DA)

The MANOVA results were important and motivated another multivariate analysis, discriminant analysis, to more specifically identify the relative importance of the 14 microbial variables to discriminate among the three disturbance classes for both bacteria and fungi. Two data sets were used: Bonham Creek (6 sites, 2000-2001-2002) and All Sites (9 sites, 2002). Discriminant analyses were conducted separately for bacteria and fungi, and for uplands and lowlands. In each of the eight DA the three disturbance classes were effectively and completely separated by one (N=4) or two (N=4) discriminant functions.

<u>Figure Number</u>	<u>Data Set</u>	<u>Microbial Variables</u>	<u>Habitat</u>
7	Bonham Creek*	Bacteria	Upland
8	Bonham Creek*	Bacteria	Lowland
9	Bonham Creek*	Fungi	Upland
10	Bonham Creek*	Fungi	Lowland
11	9 Sites, 2002	Bacteria	Upland
12	9 Sites, 2002	Bacteria	Lowland
13	9 Sites, 2002	Fungi	Upland
14	9 Sites, 2002	Fungi	Lowland
	* 2000, 2001, 2002		

The following steps were conducted for these eight DA.

Step 1: Using the pooled within-groups correlations between all 14 microbial variables and their respective two standardized canonical discriminant functions, the most important microbial variables contributing to the successful discrimination were selected for each of the two discriminant functions (DF1 and DF2), for each of the eight DA.

Step 2: The important bacteria and fungi variables selected in Step 1 were now additionally selected with the criteria that they were in **common with both data sets** for their **respective** habitats and DFs.

Step 3: The 14 microbial variables were associated with their respective microbe (bacteria or fungi), habitat (upland or lowland), and DF (DF1 or DF2) class derived in Step 2. These results are presented in Table 4.

The pattern in Table 4 is particularly illuminating, and if robust represents a significant technical and innovative advancement for the use of microbial Ecological Indicators to assess landscape disturbance independent of weather perturbations. Note that all 14 microbial metrics and all possible combinations of bacteria/fungi, upland/lowland, and DF1/DF2 are not only *completely* populated in the matrix, but also the population is relatively *sparse and well dispersed*. In relation to the 14 rows in the matrix (microbial variables) and 8 columns (characterizing parameters), ten rows populate only a single column, while four rows populate two columns. Amines/amides (lowlands) and carboxylic acids (uplands) were used exclusively by bacteria, while only polymers were used exclusively by fungi and only in lowlands. Both bacteria and fungi used simple and complex carbohydrates, amino acids, and nucleotides (uplands only). “Used” in these contexts refers to the differential use of microbial variables (substrate guilds) in landscape disturbance patterns, and therefore, providing the ability to discriminate among the three disturbance classes.

Soil Chemistry

Six variables of Soil Chemistry were evaluated from the identical soil samples that provided the Microbial Variables: nitrate (NO_3^-), ammonium (NH_4^+), soil organics (a measure of carbon), microbial biomass carbon, moisture, and pH. Therefore, as in the microbial data, the soil chemistry data consists of two data sets: Bonham Creek (6 sites), 2000-2001-2002; and Bonham Creek and Sally Branch (9sites), 2002. Soil chemistry was strongly dependent on year, season, habitat, and disturbance class; and most interaction terms were also highly significant (Table 5). As with the other Ecological Indicator guilds, this was closely related to very variable weather conditions over the time frame of data collection, from drought to flooding conditions. The details of the differences among the three disturbance classes are provided in Table 6. The less disturbed soils are characterized by higher organic content, lower pH, and lower nitrate. Active decomposition of vegetation produces humic acids which lower pH. Lower nitrate may be related to its active uptake by microorganisms in symbiosis with vegetation. Surprising, microbial biomass carbon does not seem to be related to habitat disturbance.

Discriminant analysis of the two data sets clearly separated the three disturbance classes with two discriminant functions. Figure 15 shows the six upland Bonham Creek sites with all years combined, 2000-2001-2002. DF1 separates High from Low and Medium sites based on soil organics and moisture versus pH. DF2 separated Low from Medium sites based on pH and nitrate. Figure 16 shows the six lowland Bonham Creek sites with all years combined, 2000-2001-2002. DF1 separates the High from the Low and Medium sites primarily with pH. DF2 separated Low from Medium sites based on the contrast between nitrate and soil organics. Figure 17 shows the nine upland Bonham Creek and Sally Branch sites in 2002. Note that figures 17 and 15 are very similar. As in Figure 15, DF1 separates High from Low and Medium

sites based on soil organics and moisture versus pH. DF2 separated Low from Medium sites based on nitrate and ammonium. The loadings were therefore, a little different in this data set. Figure 18 shows the nine lowland Bonham Creek and Sally Branch sites in 2002. DF1 separates the High from the Low and Medium sites primarily with pH, as in Figure 16 (Bonham Creek only). DF2 separated Low from Medium sites based on the contrast between ammonium and moisture-nitrate. This was different than DF2 in Figure 16, as could be expected because the DF2 patterns are different in discriminant space.

DF1 possesses the most discriminating power (73-89% of the variance in these four analyses) and it is refreshing that in both data sets the loadings are very similar for both upland and lowland habitats, effectively separating the High disturbance classes from Low and Medium. However, DF2, which is responsible for separating Low from Medium sites varies with the two data sets for both upland and lowland sites. It was unexpected that the disturbance classes in the lowland habitats were so successfully separated, because the lowlands are not impacted and contain a great deal of vegetation cover at all nine sites. The Soil Chemistry DA analyses also substantiates the results of many other Ecological Indicator guilds by again demonstrating that Low and Medium sites are relatively similar to one other, and dramatically different from High sites.

Nutrient Leakage

Nutrient leakage was assessed by the relative concentrations of 5 anions, 6 cations, and pH in lysimeter solutions. MANOVA results are presented in Table 7. There were highly significant differences in years, habitats, and disturbance classes ($P < 0.001$). This was expected because weather varied from drought to flooding over the three years of the study, and more leached water is innately available in lowlands contrasted to uplands. The interest was in the relative leaching among the three disturbance classes. The statistical significance of ion leaching is shown in Table 8 for both upland and lowland habitats. Upland and lowland habitats were analyzed separately. Upland High disturbance sites lost more nitrate than Low or Medium sites. This is important because Sand Hills soils are deficient in nitrogen (Kovacic et al. 2006). The leaching of nitrate further retards vegetation development in highly disturbed sites. Fluorine was better retained at Low disturbance sites, but more chlorine was leached at the Low sites compared to High disturbance sites. The lowland habitats had a consistent trend where sodium, potassium, magnesium, and sulfate possessed larger lysimeter solution concentrations in both Low and High disturbance sites relative to Medium disturbance classes. This could be related to the higher vegetation productivity at the Low sites, but the poorer retention of nutrients at the High sites. More detailed analyses are being conducted on nutrient leakage incorporating precipitation patterns, fire, and time of year. All three of these variables appear to be very important in nutrient and ion dynamics and their availability in lysimeter solutions.

Separate discriminant analysis (DA) were conducted on upland and lowland lysimeter data with all years (2000-2001-2002) combined. The motivation was to assess if the leaching patterns of the 11 anions and cations and pH were capable of separating the three disturbance classes. The upland DA showed the correct trend in the disturbance gradient with DF1 (Figure 19). DF2 strongly separated Medium sites from Low and High. The DA at the lowland habitats separated Medium sites from Low and High with DF1, and DF2 separated Low and High sites (Figure 20).

It was of exploratory value to plot the actual sites in discriminant space, because a great deal of variability was present in the nutrient leakage data (Figures 21 and 22). Although the general patterns and trends of Figures 19 and 20 are retained, these figures demonstrate that discriminant scores at sites L3, M3, H3, and H2 can vary a great deal from their disturbance class mean. This occurred to a greater frequency in lowland habitats, and apparently at Sally Branch. Because of their location in the landscape, lowland habitats have not been impacted to the same extent as their upland counterparts. Figures 21 and 22 represent a good example to demonstrate intra-disturbance class variability.

The most important **upland** variables for DF1 (ranked) were: sulfate, chlorine, fluorine, and ammonium. The variables for DF2 (ranked) were: nitrate, calcium, magnesium, and potassium. The most important **lowland** variables for DF1 (ranked) were: sulfate, potassium, sodium, and magnesium. The variables for DF2 (ranked) were: pH, calcium, nitrate, ammonium, phosphate, and chlorine.

Soil Mineralization Potential

Variable: Soil Ammonium (NH₄) Concentration Change After 4-Day Incubation

Year & Sample Size: 2002 (N=45)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low > Med	<0.001
Low > High	<0.001
Med > High	<0.001

Variable: Soil Nitrate (NO₃) Concentration Change After 4-Day Incubation

Year & Sample Size: 2002 (N=45)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
High > Med	<0.001
High > Low	<0.001
Med > Low	0.039

Variable: Litter Ammonium (NH₄) Concentration Change After 4-Day Incubation

Year & Sample Size: 2002 (N=45)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = High	0.33

Med > Low	0.005
Med > High	0.023

Variable: Litter Nitrate (NO₃) Concentration Change After 4-Day Incubation

Year & Sample Size: 2002 (N=45)

<u>Tamhane's T2 Multiple Comparison</u>	<u>Statistical Significance (P)</u>
Low = High	1.00
Low > Med	0.001
High > Med	0.006

The soil mineralization potential experimental results are shown in Figure 23. After laboratory incubation of soil samples for 4-days: ammonium (NH₄⁺) concentration significantly decreases with disturbance, while nitrate (NO₃⁻) concentration significantly increases with disturbance.

Ground and Litter Ant Communities

Forty species of ants were identified and 103,251 individuals were counted in the pitfall traps collected at nine sites over the three-year portion of the study. Twelve rare species were represented by 2 to 11 individuals (N=48), and were excluded from discriminant analysis (DA). Therefore, 28 species (N = 103,203) were available for analysis (Table 9). Figure 24 presents the DA of this species composition. DF1 clearly separated the High disturbance sites from the Low and Medium sites. DF2 effectively separated the Low from Medium sites. From this analysis, 13 species were selected that provided the highest loadings on the two discriminant functions. The results of this analysis are shown in Figure 25. The pooled within-groups (disturbance classes) correlations between the 13 ant species and standardized canonical DFs are provided in Table 9. The results were very similar (with a single exception) to the 28 species analysis and the three disturbance classes were easily separated. However, in this analysis, site L3 was intermediate in location between Low and Medium sites for DF2. Several other Ecological Indicators gave L3 lower than expected scores, and we found evidence of historical agriculture in the area.

In these first two DA it appeared that *Dorymyrmex smithi* was dominating the analyses. This species possessed respectively 87 or 96 percent of all individuals, and is a species significantly associated with bare ground and open canopy, and therefore the High disturbance classes. This species was strongly negatively correlated with DF1, and was apparently responsible for the strong separation of the High sites on DF1. This species was removed and DA was repeated with 12 species (Figure 26, Table 9). Interestingly, note that the pattern of both DF1 and DF2 has not been affected by removing a very dominant species. The sole effect being the dramatic decrease in standard error at the High sites for DF1, because the abundance patterns of *Dorymyrmex smithi* are extremely variable among pitfall traps. Note that in the absence of *Dorymyrmex smithi*, species characteristic of less disturbed sites increased in importance to contributing positive values to DF1 (Table 9). It was of interest to test if fewer species of ants could still separate the three disturbance classes. Another DA was conducted with the 6 most

discriminating species of ants (Figure 27, Table 9). Compare Figures 25 (13 species) and 27 (6 species). DF1 has not changed appreciably. Although DF2 appears to be quite different it is not. The main pattern in DF2 is that the positive/negative signs of site means reversed. This is a common phenomenon in DA, and is not significant. The critical feature of DA is its ability to separate groups. Nevertheless, reducing the ant community to 6 species had the effect of producing similar discriminant scores for L3 and M2/M3, and therefore, blurring the distinction between the Low and Medium sites. This is an important result, because the analysis could be telling us that in reality there is not that much difference between at least some Low and Medium sites. A result supported by many of our Ecological Indicators. The final DA was conducted with 5 species, *Dorymyrmex smithi* again deleted (Figure 28, Table 9). The 5 and 6 species DA were essentially very similar. Compare the other two analyses without *Dorymyrmex smithi*. The results with 12 species (Figure 26) and 5 species (Figure 28) are very similar with two exceptions; community reduction to 5 species increases the similarity of L3 with M2 and M3 (especially the former), but decreases the similarity of L3 with M1.

Other Invertebrates

The analyses of other invertebrate taxa were not as informative as the ground/litter ants. Spiders (Araneae) are the dominant predators of the arthropod community. Unfortunately, they are far too diverse and taxonomically difficult to be useful for routine studies. We tabulated more than 200 morphospecies of spiders. Apparently their taxonomy is poorly known and described. Preliminary analysis indicated substantial differences in community composition of spiders among disturbance regimes.

Grasshoppers and katydids are the most conspicuous herbivores in the arthropod community. In grasslands, they are typically the dominant herbivores. Taxonomically, they are easier to identify than either the ants or spiders. Moreover, low species richness (13 species of grasshoppers and 3 species of katydids) makes them easy to work with. We identified and counted 621 grasshoppers (Acrididae) and katydids (Tettigonidae), sampled in 2000-2001-2002. Although there were no differences in either species richness or relative density of grasshoppers and katydids in the disturbance gradient, there were minor differences in community composition. In particular, *Trimerotropis maritima* was restricted to highly disturbed sites. This species is found primarily in coastal strand habitats, and other sandy habitats (Friauf 1953, Squitier and Capinera 2002). *Orphulella pelidna*, *Schistocerca americana*, and *Conocephalus fasciatus* were also more common in High disturbance sites.

The “nutrition condition” (maximum width standardized by total length) of the two most widely distributed species, *Pardalophora phoenicoptera* and *Melanoplus femurrubrum*, was less in the High disturbance sites. After correcting for total length, both *Pardalophora phoenicoptera* and *Melanoplus femurrubrum* had a smaller maximum width in the High disturbance sites (Analysis of Covariance: $P < 0.05$).

Developmental Instability (DI)

Developmental instability (DI) is the deviation from bilateral or radial symmetry of early developing embryonic tissue in response to a biological, chemical, or physical stress or insult. Therefore, in the adult organism these phenotypic asymmetrical deviations can be measured and

quantified, and in theory associated with the environmental perturbation relative to a control (no perturbation). Because there is rarely a “right” or “left” handed preference for the asymmetry, DI is commonly measured by fluctuating asymmetry (FA). FA in simple leaves is measured in a number of ways: leaf lobes, teeth on leaf margins or lobes, and leaf or lobe veins. For example, in the simple three-lobed leaf of the perennial forb *Cnidoscolus stimulosus*, nine metrics of FA can be measured on an individual leaf (see Methods). Compound leaves can not only use the metrics found on individual leaflets, but can also take advantage of any asymmetry arising in the pairing of opposite leaflets. Therefore, giving rise to potentially a very large number of FA metrics. DI is a very time and labor intensive methodology in the laboratory, even when computer imaging and measuring techniques were employed, as in this research, where over 200,000 measurements were recorded.

DI has successfully been applied to a wide variety of environmental stressors in terrestrial, freshwater, and marine ecosystems. See references cited in Duda et al. (2003) and Freeman et al. (2005). A wide variety of organisms have also been used for DI assessment: lower plants (algae), higher plants (angiosperms and gymnosperms), invertebrates, and vertebrates. In this research, a number of different organisms were used as well, see Methods section.

DI was found to be extremely responsive to environmental perturbations in this research. DI in the plants we used appeared to be sensitive to: drought, fire (including nutrient pulses), herbivory, gall parasitism, and possibly disease. DI results were highly variable (i.e., displayed high variance components in analyses) because of this background “noise”, and it was impossible to assess with certainty any additional effects due to habitat disturbance. In the search for Ecological Indicators one gains as much knowledge from experiments that did not work compared to those that did work. At this point in time, we do not recommend the use of plant DI as an Ecological Indicator on the basis of high cost, difficulty and ambiguity in the interpretation of results, severe statistical sampling issues, and probably its inadequacy in tracking habitat disturbance. Nevertheless, a few interesting results were obtained with bullnettle and analyses are still continuing in the plant DI component of this research. The DI work with grasshoppers and fish is still in progress.

The use of plant DI for quantifying habitat disturbance and its use as an indicator of site condition has many problems associated with it. The most immediate and serious problem is that the species used must be present throughout the disturbance gradient. This infers three important life history possibilities:

- 1) either the species is extremely phenotypically and physiologically plastic so that it has evolved to successfully and without stress exploit the full range of environments encountered;
- 2) the species exhibits genetic polymorphism, where specific genotypes are adapted to and occupy different portions of the disturbance gradient;
- 3) or individuals are only germinating or successfully surviving in similar microhabitats through the disturbance gradient.

To assess the relative importance of these three alternatives would require growing plants in the laboratory under highly controlled conditions in a variety of environmental regimes, and genetic analysis. Additionally, it may be desirable to conduct reciprocal transplant field experiments. This would take a great deal of time and at substantial expense. Number 3 is a distinct possibility, because our High disturbance sites were very patchy in soil and vegetation disturbance, and undisturbed micro-sites were certainly present. Additionally, our Low disturbance sites had a great deal of natural disturbance in them, including canopy gaps from tree falls and digging by feral hogs. The prescribed burning also caused a great deal of ground cover disturbance at all disturbance classes, but the High sites were burned less frequently.

Another problem is related to field sampling, which is also related to Number 3 above. In order to obtain the leaves for DI measurement a plant must first be found, and this presents spatial bias in sample collection. The sampling is biased because the plant germinated and survived at a specific micro-site, and this micro-site may be quite different than other micro-sites randomly distributed in the given habitat.

Another problem relates to the statistical interpretation of DI analyses. Because for a given species a large number of DI metrics can be obtained and tested for significance concurrent with no *a priori* knowledge of which metrics are environmentally meaningful or interpretable, there exists the danger of “statistical mining” to extract significant metrics. This problem is compounded by the addition of more species to analyze or more years of data collection, because it increases the probability for finding spurious statistical significance. The confounding effects of weather, fire, herbivory, parasitism, disease, and possibly military chemicals add to the uncertainty of interpretation.

Developmental Instability in Grasshoppers

There was no difference in FA (a measure of DI) of two leg traits and four wing traits among *P. phoenicoptera* and *M. femurrubrum* with respect to the three disturbance classes.

Plant Physiology

Variable fluorescence, water potential, transpiration loss, and stomatal conductance were measured for the shrub, winged sumac (*Rhus copallinum*). Variable fluorescence is a measure of photosynthetic efficiency, and has been found to correlate with environmental stressors (see citations in Duda et al. 2003). Water potential was measured as water stress using a pressure bomb (Duda et al. 2003). The effect of habitat disturbance on plant physiology could not be statistically determined, because of the very high level of variability among plants at a given disturbance class, and even within individual plants. The high variability also appeared to be affected by many environmental influences, including prescribed burns. The selection of species for this experiment, as well as, inherent sampling bias, are subjected to the identical problems discussed for the DI guild.

We also examined the Hurst coefficient of growth ring widths in loblolly pine (*Pinus taeda*) to determine if habitat disturbance decreased a tree’s ability to buffer stress conditions, and as predicted by theory, there is a loss of complexity in growth patterns. The Hurst coefficient

declined with increasing disturbance, indicating that there is a loss of buffering ability due to mechanical soil disturbance.

Spatial Organization in Plant Communities

DFA analysis derives a metric, “alpha”, to assess random versus non-random community structural patterns. Random patterns produce an $\alpha = 0.5$, see Methods. In our analysis, all alpha values exceeded 0.5, regardless of habitat disturbance class and community response to prescribed fires. This indicates that the structure of these communities is non-random. Examining the number of times alphas; for a given species, year, and fire history were higher (vs. lower) in High disturbance versus less disturbed sites, we find no apparent trend. Using the same approach to compare alphas between years, there was a small rise (2002 was a much wetter year than 2001), but it was not significant. Changes in alpha between fire histories also did not show any trend. Finally, under the presumption that alpha values are invariant among species, unlikely but perhaps worth considering as a possibility, we used 2-way ANOVA with disturbance as a fixed effect and fire history as a random factor to examine differences in alpha. Again, there was no difference for either effect ($F_{90,1}=0.53$, $p=0.60$ for fire history; $F_{90,1}=0.27$, $p=0.70$ for disturbance class; $F_{90,1}=0.35$, $p=0.56$ interaction).

There are at least two reasons why these results might be so weak. First, because of the spatial extent of the study sites, it was impossible to construct transects of adequate length (statistically), and transects invariably crossed patches of different levels of disturbance. Second, ground cover can be expected to react to disturbance much more quickly than the overlying canopy. Therefore, whatever stresses originally had been imposed by military activities might have been dissipated quickly by community reorganization. In other words, the disruption of autocorrelation patterns might have been ameliorated over time by changes in species composition and population densities. The physical alterations from a given disturbance regime may have become the norm to a newly self-organized system. Each site probably represents a distinct disclimax, or has been displaced to a different successional stage or path. The fact that alpha values show no significant differences among sites suggest the former, because displacement from a characteristic trajectory reflects disrupted organization, and therefore, differential drops in alpha towards 0.5. The lack of evidence for drops in alpha values between unburned and just burned sites suggests that fire must also be a part of the “normal environment”. This can be expected because of the frequent prescribed burns at Fort Benning, and the historical role of fire disturbance in southeastern Sandhills physiography.

Local Heterogeneity in Phase I Sites

An important problem that surfaces is the large degree of inter-site variability inherent in landscape mosaics when trying to classify disturbance patches into disjunct disturbance classes. A-horizon depth and soil compaction are a good example. Figure 29 shows these EI metrics as disturbance class means. These metrics clearly characterize the disturbance gradient with Low and Medium disturbance classes being more similar than the High class. However, when actual site means were examined a slightly different picture emerged, because the Sally Branch Medium and High sites had deeper A-horizons than the Bonham Creek sites (Figure 30). Sally Branch Medium and High sites are topographically lower than Bonham Creek sites and must experience less erosion or also acquire soils from upslope. The disturbance gradient pattern

holds very well for the Bonham Creek watershed. Site M3, with a high component of maturing longleaf pine, despite showing signs of military training activities, had patchy (note magnitude of standard error) deep A-horizons. The trend is evident in Figure 30 that soil compaction increases with disturbance class, but again there are some deviations. M2 demonstrated unusually low soil compaction. The soils at this site possessed the highest sand content in the Medium disturbance classes, while M1 possessed the highest clay content among all nine sites. Clayey soils are more susceptible to compaction, while the converse is true of sandier soils. Site H2 possessed some higher quality patches, and this is reflected in lower mean soil compaction and wider standard error.

Phase II Research (2003)

The primary motivation of Phase II research was to validate the Ecological Indicators (EIs) identified in Phase I in a more extensive disturbance gradient, including relatively relative pristine areas, and a greater variety of upland plant communities and soil types. Eight EIs were identified as promising in Phase I: General Habitat Metrics, General Ground Cover, Floristics Ground Cover, Soil Chemistry, Nutrient Leakage, Soil Mineralization Potential, Microbial Community Dynamics, and Ground/Litter Ant Communities. However, Nutrient Leakage and Soil Mineralization Potential require a great deal of effort, time, laboratory analyses, and specialized equipment and expertise for monitoring; and are not readily applicable for large sample validation experiments such as our Phase II. However, these Ecological Indicators would be very useful for long-term monitoring of specific fixed sites. Therefore, six EISs that were identified in Phase I were further evaluated at 40 sites in the more extensive disturbance gradient and complex ecosystems of Phase II.

The General Habitat EIS was particularly important for two reasons: 1) its simple and ecosystem relevant metrics reliably and with high statistical significance quantified the landscape disturbance gradient, and 2) its metrics could be measured in the field by minimally trained field personnel using simple and inexpensive equipment. The General Habitat metrics were: Soil A-Horizon Depth, Soil Compaction, Litter Cover (100-Bare Ground), Canopy Cover, Basal Area, and Tree Density. Soil Organics, a metric in the Soil Chemistry EIS and also highly responsive to the disturbance gradient, requires simple laboratory analysis.

An important effort in Phase II was to combine individual indicator metrics to develop an analytical and observer unbiased Site Comparison (or Condition) Index (SCI). Twelve indicator metrics were selected based on performance in Phase I and consensus by SEMP team leader researchers (Table 10). Eight of these differed highly significantly ($P < 0.001$) along the 10-class disturbance gradient. The Normalized Difference Vegetation Index (NDVI) is often used to assess and monitor landscape condition, because it measures net primary productivity, and correlates strongly with LAI (leaf area index), FPAR (fraction of absorbed photosynthetically active radiation), field-measured net primary production, measures of chlorophyll, and albedo (e.g., Franklin 2001). Figure 31 shows NDVI values for the 40 sites relative to the 10 disturbance classes (Bob Lozar, U.S. Army – ERDC – CERL, provided the data). NDVI was only able to distinguish between the most pristine and highly disturbed sites, although there was a clear trend in the decline of NDVI between DC6 to DC10.

A-horizon depth characterizes the disturbance gradient extremely well (Figure 32). There was a smooth, linear, monotonic decrease of the A-horizon with increasing disturbance. DC3 was the single exception, but it was not significantly different from either DC4 or DC2. The consistent pattern in this figure was analytically verified by the pairing of adjacent DCs, resulting in five statistically significant groups based on A-horizon depth.

The pattern of soil compaction for the 40 sites (Figure 33) was similar to A-horizon depth, but there were seven statistically significant disturbance classes, and the most pristine and most disturbed sites were dramatically separated from the other classes. Statistical groupings differed from that displayed by A-horizon depth. DC2 contains the site with the highest clay content (13.6%). Soils with higher clay content typically demonstrate higher levels of soil compaction.

Litter cover was selected from ten ground cover metrics using discriminant analysis (Figure 34). DF1 had a correlation of 0.95 with “bare ground”, and litter cover = 100 – (bare ground) at the 40 sites. Although canopy cover, basal area, and tree density are typically highly correlated; they each represent different components of vegetation structure and forest development. Therefore, all three were included in SCI development.

Site Comparison (Condition) Index

Figure 35 presents the SCI scores (means with standard errors) for the 10 disturbance classes. Note that SCI scores declined smoothly and linearly as habitat disturbance increased. The SCI based on eight statistically significant and ecologically important soil and vegetation parameters modeled the 10-class disturbance gradient extremely well. The relatively pristine sites and very degraded sites clearly separated themselves from the other sites. There were two minor exceptions. DC2 and DC4 had slightly lower SCI scores than expected. These results are robust and gratifying for several important reasons. Virtually identical patterns were obtained with the three “intermediate SCIs” that were averaged to produce the “final SCI”. Additionally, the final weighed composite SCI was similar in pattern to A-horizon depth (Figure 32) and soil compaction (Figure 33). These two indicators were identified in Phase 1 research as being the most important habitat variables defining the 9-site/3-class land-use disturbance gradient. The robustness in analyses can be attributed to the high multicollinearity inherent in habitat metrics that attempt to quantify a disturbance gradient. Scores from the analytical unbiased SCI reproduced almost perfectly the disturbance ranking assigned by a very experienced observer, thus non-subjective uniformity of ranking was achieved.

The 40 sites were ranked by their respective SCI scores (Figure 36). The histogram of SCI scores for the SCI ranked 40 sites revealed a ***sigmoid logistic decay function***, analytically demonstrating that relatively few sites were either very high quality or very severely degraded, and suggesting a “threshold effect” of rapidly declining SCI values as disturbance increases from “pristine sites” or as it approaches severely degraded sites. Discrepancies between SCI and Disturbance Class rankings revealed interesting ecosystem patterns that are currently under investigation.

The two highest ranked sites based on the SCI were mesic forests with complex and predominantly deciduous vegetation, and they were also in DC1 (Figures 37 and 38). A pristine xeric site also in DC1 was ranked relatively low by the SCI (9th), presumably because the site

had sparser vegetation development with less structure than the mesic sites (Figure 39). The site with the lowest SCI score (40th) was in the central portion of a major training area (Delta), possessed a great deal of bare ground, and was classified as a DC10 (Figure 40).

The largest discrepancy between SCI and disturbance class ranking was a relatively pristine (DC2) maturing Longleaf Pine Forest with simple vegetation structure (i.e., physiognomy) in rocky rolling hills with unusual wind-eroded sandstone outcrops (Figure 41). This site was ranked 19th by the SCI.

These preliminary results in developing a Site Comparison (Condition) Index from a wide variety of upland vegetation communities in the complex physiographic setting at Fort Benning are indeed encouraging. Nevertheless the data were collected at a single location in the Fall-Line Sandhills. Additional data is required from a larger geographic area and an even greater variety of vegetation communities and soil types (especially clayey), both in the Southeast and in other regions of the United States.

Phase II Evaluation of Ecological Indicator Systems

The results from developing the SCI identified seven highly significant Ecological Indicator metrics that were easy and economical for land managers to monitor: Soil A-Horizon Depth, Soil Compaction, Soil Organics, Litter Cover (100-Bare Ground), Canopy Cover, Basal Area, and Tree Density. The NDVI did not add additional information to these metrics, and additionally was only available as a site-averaged parameter. The seven indicator metrics were collected on each of the four transects at the 40 sites. A SCI was calculated for each transect using these seven indicator metrics in a similar fashion that was used for the 40-site SCI scores (Figure 36) and described in the Methods section. The highest possible score was a “100”, and it was remarkable that one transect achieved this score. The lowest possible value was “0”, and would only be achieved in a concrete parking lot with no vegetation and no wind-blown leaves. Figure 42 illustrates the histogram for the SCI scores for the 160 transects. The 160 ranked transects were divided into five new Disturbance Classes, each containing 32 transects: Low, Low-Med, Medium, Med-High, and High. It is important to remember that these new disturbance classes were analytically determined by six General Habitat and one Soil Chemistry indicator metrics. Note that the disturbance gradient is completely and smoothly characterized. As in the case of the 40 sites, note also that the histogram of SCI scores for the 160 transects revealed a ***sigmoid logistic decay function***, analytically demonstrating that few transects were either very high quality or very severely degraded, suggesting a “threshold effect” of rapidly declining SCI values as disturbance increases from pristine patches or as SCI values approach severely degraded sites.

Phase II Landscape Heterogeneity

Phase II research was designed for implementation in a heterogeneous landscape setting. The analytically derived SCI scores for each of the 160 transects at the 40 sites clearly demonstrated that our Ecological Indicators were evaluated across a very broad and continuous landscape disturbance gradient, with sampled transect habitats ranging from pristine to extremely degraded (Figure 42).

The 40 sites consisted of 10 diverse upland forest community types spanning the complete range of Fort Benning Forest stands and early succession vegetation. These 10 forest classes were

identified by cluster analysis and nonmetric multidimensional scaling, using basal area of tree species as the ordination variable (see Methods section):

Oak-Hickory Deciduous Forest: Mesic, very diverse	N=1
White/Southern Red/Post Oak – Shortleaf/Loblolly Pine	N=1
Mixed Pine – Southern Red oak	N=2
Mixed Pine – Oak-Hickory: Loblolly Pine dominant	N=7
Mixed Pine – Oak: Longleaf Pine dominant	N=6
Loblolly/Shortleaf Pine – Hardwoods	N=6
Pure Loblolly Pine – Hardwoods: Piedmont Forest	N=2
Longleaf Pine Forest: high basal area	N=5
Turkey/Sand Post Oak – Pine Savanna: Xeric	N=3
Mixed Pine – Oak: lower basal area, Disturbed all or some Early Succession	N=7

Despite the broad range of forest community types, the soils were surprisingly relatively similar (Figure 43). Over two-thirds (68.1 percent) of the soils were loamy-sand, while only two transects (1.3 percent) were classified as sandy-clay-loam soils. We recognized many patches of clayey soils in the field, but apparently on the scale of 100 m transects, these clayey pockets “averaged out” to sandy-loam or loamy-sand soils. In some areas of the installation the roads are very clayey, and when wet, they make it a challenge to keep the vehicle on the road. This also adds to the perception of higher clay content in the surrounding landscape. However, the roads are subjected to a great deal of erosion, and the sandy particles and soil layers are readily washed away, exposing the more tenacious clay layers which underlie much of the installation’s sandy soils. Similarly, based on field observations, we would have predicted that sand soils comprised more than 17 transects (10.6 percent).

General Ground Cover Guild

The General Ground Cover Guild consisted of 11 general categories of ground cover: forbs (including legumes), legumes, grass, woody plants, ferns, yucca, cactus, pine seedlings, total pines, total ground cover, and number of species (or morpho-species) of ground cover. These categories are easily identified by surveyors who are not experienced with the complex plant taxonomy of the Southeast. The four discriminant functions (DFs) clearly separated the five disturbance classes (DCs). DF1 characterized the disturbance gradient with Low (woody cover and species richness) and High (grass cover) DCs clearly separated from moderate DCs (Figure 44). Note that the moderate DCs were similar but showed a clear trend. DF2 clearly shows an interesting and unexpected “intermediate disturbance” pattern, separating DCs into three groups based on relative disturbance (Figure 45). The moderate DCs were characterized by higher ground cover, especially forbs and cacti. DF3 had a sine wave pattern, and the Low-Med DC was separated from the rest based on yucca and pine cover (Figure 46). DF4 shows the separation of the Med-High DC based on lower woody, grass, and total cover (Figure 47). The discriminant function structure matrix for this analysis is provided in Table 11.

Floristics Ground Cover Guild

The Floristics Ground Cover Guild consisted of 10 principal components (PCs) that were extracted with a principal components analysis of 239 species (or morpho-species) of ground

cover plants (see Methods). Two DFs were sufficient to separate the five DCs. DF1 again characterized the disturbance gradient with three classes delineated: Low and Low-Med, Med and Med-High, and High (Figure 48). The High DC was significantly separated from the other DCs. The disturbance gradient was based primarily on PCs 4 and 5. DF2 again demonstrated a very clear “intermediate disturbance” pattern (Figure 49). Low disturbance sites were characterized by woody and fern ground cover and many species, while High disturbance sites were associated with grasses and sand blackberry, and moderate disturbed sites were characterized by broomsedge and woody ground cover especially early succession species. The discriminant function structure matrix for the Floristics Ground Cover analysis is provided in Table 12. Table 13 illustrates the primary taxa associated with Low, Moderate, and High disturbance classes.

Ground/Litter Ant Community Guild

DF1 separated the High and the Med-High DCs from each other and the less disturbed sites on the basis of four species of ants (Figure 50). *Dorymyrmex smithi*, *D. bureni*, and *Pheidole bicarinata* were characterized with disturbance, and *Myrmecina americana* with less disturbance. *Dorymyrmex smithi* is a widespread species associated with open canopy and bare ground, presumably because it requires relatively hot nest chambers. *Pheidole bicarinata* is a sand specialist found in the sand dunes of the Great Lakes and in the Mojave Desert. *Myrmecina americana* has small colonies and usually builds obscure nests under stones in moist shady areas. DF2 possess an intermediate disturbance shape as in the two previous guilds (Figure 51). A number of species are associated with moderately disturbed sites: particularly *Aphaenogaster floridana*, *Formica pallidifulva*, *Paratrechina arenivaga*, *Hypoponera* sp1, *Solenopsis invicta* (exotic fire ant), and *Forelius pruinosus*. Species partial to low disturbance sites contrasted in DF2 include: *Aphaenogaster rudis* complex, *Camponotus castaneus*, and *Temnothorax pergandei*. DF3 (Figure 52) and DF4 (Figure 53) both assist in separating Low-Medium and Med-High DCs from their adjacent DCs along the disturbance gradient.

Microbial Community Function and Dynamics Guild

The Microbial Community Function and Dynamics guild could not be evaluated at this time, because major errors were found in the database regarding the association of laboratory results with specific field transects. The error is currently being corrected.

Synthesis and Association of all Ecological Indicators

The other component of Phase II is the synthesis and modeling of all derived indicator metrics into statistical combinations that will be useful for land management and landscape disturbance ecological theory. This component of the research will be completed when AJK has all the data to work with.

Discussion, Conclusions, and Major Points

This research has made important scientific and land management advancements in five areas:

- 1) the identification of individual and classes (guilds) of Ecological Indicators (EIs) that quantify and characterize landscape disturbance;
- 2) the use of this information to construct Site Comparison or Site Condition Indices (SCIs);

- 3) new insights into the relationships among landscape disturbance, biodiversity patterns, ecosystem processes, and the intermediate disturbance hypothesis;
- 4) detailed identification of species-habitat/environment and landscape disturbance relationships;
- 5) the clarification of complex ecosystem and physiological processes.

This report essentially deals with the first two, because these were the primary objective of the proposed research. Numbers three and four are currently being investigated with statistical modeling, especially examining the relationships between biodiversity patterns and landscape disturbance. These analyses are directed primarily at understory (ground cover) and canopy (trees) vegetation and selected invertebrates, especially ant communities. Number five is directly addressed by several publications produced by our team, and additional analyses are continuing.

The use of multivariate (MANOVA) and univariate (ANOVA) analyses of variance in conjunction with discriminant analysis (DA) proved to be a powerful approach for the identification and association of EIs in this research. DA extracts discriminant functions (DFs) that are weighed linear combinations of the original predictor variables (e.g., indicator metrics), and therefore, identifies and quantifies the relative importance of these metrics in separating pre-established groups or classes, in this case disturbance classes. Recall, that DA “does not know” any ranking or ordinal nature of the groups. DA “only knows” that there are N-groups that need to be distinguished by a variable set common to all groups. DF1 possesses the greatest discriminating power in separating the groups. DF2 has the next highest power and is orthogonal (independent and uncorrelated) to DF1, and so forth with the remaining DFs. If DF scores of sites or sample transects are ranked correctly in a disturbance gradient (i.e., Low to High disturbance classes), then the variables and their weighing coefficients are an analytical representation of the disturbance gradient. If DF1 accomplishes this, it can be interpreted that the primary separation of the groups is due to disturbance and we have identified the metrics and their relative importance. For example, in Phase I with three disturbance classes, DF1 for the following variable sets was able to accomplish this: General Habitat (Figure 3), Trees (canopy vegetation) (Figure 4), General Ground Cover (Figure 5), and Floristic Ground Cover (Figure 6). Other Ecological Indicator Systems were also able to do this. A group of statistically derived variables, weighted or unweighted, that characterized disturbance classes or a disturbance gradient was called an Ecological Indicator Guild.

Eight of the eleven researched Ecological Indicator Systems in Phase I research were very successful at discriminating among three disturbance classes (High, Medium, Low) using DA. These eight guilds were: General Habitat Metrics, General Ground Cover, Ground Cover Floristics, Soil Chemistry, Nutrient Leakage, Soil Mineralization Potential, Microbial Community Dynamics, and Ground/Litter Ant Communities. Plant Physiology and Spatial Organization in Plant Communities were unable to measure effects due to habitat disturbance. Developmental Instability (DI) of selected plant species was also unable to reliably and consistently distinguish among disturbance classes. DI is the phenotypic asymmetry response to stress in the early embryonic development of an organism. DI was overly sensitive to a wide range of environmental perturbations, including drought, fire (and nutrient pulses), herbivory, and gall parasitism. An inherent problem of DI is that the test species must be present in the entire disturbance gradient. Therefore, the species must possess unusually broad life history

characteristics, physiological tolerances, or genetic polymorphism. Additionally, DI analyses require a large number of statistical contrasts and *a posteriori* comparisons, raising statistical validity issues in sampling and interpretation. An important consideration is that the test species may be selecting similar micro-habitats along the entire disturbance gradient. Military training disturbance is patchy and produces fragmented landscapes, which nevertheless possess higher quality micro-habitats, even in heavily used training ranges. On the other hand, micro-habitat disturbance patches occur in relatively pristine areas because of disturbance by feral hogs, tree-fall gaps, and the effects from locally severe prescribed burns. The clarification of these problems and issues would require the simultaneous use of laboratory and field transplant experiments, an expensive and resource intensive procedure.

The eight successful EI guilds encompass a very broad range of ecological attributes and ecosystem processes, including: physical and chemical properties of soils, simple economically obtained properties of vegetation, understory and canopy floristics, biodiversity metrics, microbial dynamics assessed by how bacteria and fungi partition substrate utilization, nutrient dynamics and leakage, and the structure (species composition and relative abundance) of an ecologically important animal community – ants. A critical feature of these eight EI guilds was their robustness to persistent and major background disturbance perturbations at Fort Benning: weather (e.g., severe drought), prescribed burns, and soil disruption by feral hogs. These covariates were purposely not included in the extraction of EI guilds to assess if their confounding effects were overridden by the underlying disturbance gradient.

All eight successful EI guilds in Phase I, differing widely in tracking ecosystem condition and responses, demonstrated that the Low and Medium disturbance classes were similar to each other, but differed a great deal from the highly disturbed sites. This indicates that the Medium sites may be well on their recovery trajectory from past military training activities. Nevertheless, Low and Medium sites were also successfully differentiated by all eight EI guilds. DA results from these guilds were consistent. Therefore, DA consistently provided a quantitative assessment of the relative ecological differences among the three disturbance classes (i.e., the relative locations of the three disturbance classes in discriminant space).

A-horizon depth and soil compaction were the only EI metrics among all habitat parameters that successfully and significantly ($P < 0.001$) distinguished among the three disturbance classes of Phase I. Indeed, these two EIs and soil mineralization potential (consists of two metrics) were the only metrics that *individually* could distinguish the three disturbance classes. These identified metrics have profound assessment and monitoring implications. Soil is considered the major template for maintaining ecological processes and landscape sustainability (Dylis 1968, Herrick 2000, Schoenholtz et al. 2000, Johnston and Crossley 2002, Coleman et al. 2004). The A-horizon forms at the soil surface by accumulation of humus, and is the layer of highest biological activity, decomposition, and nutrient recycling (Perry 1994, Ellis and Mellor 1995). Two-thirds of the earth's entire biodiversity live in terrestrial soils and underwater sediments (Baskin 2005, pg 3). Soil compaction has many negative impacts on ecosystem processes including: reduced seed germination and root growth, retarded aeration and water infiltration, increased runoff and erosion, decreased microbial activity and nutrient dynamics, increased difficulty in invertebrate and vertebrate burrowing activities, and discouraging the development of biologically active surface crusts and litter mixing.

The Ground Cover Floristic guild consisted of 24 species (plus an unknown morpho-species forb) of ground cover plants extracted from 67 taxa. Ground cover includes shrubs and tree seedlings <2 m in height. These species were: 11 forbs, 7 tree seedlings, 5 shrubs, a woody vine (poison ivy), and brackenfern. All of these species are abundant and widespread in the Southeast. Therefore, their ability to successfully identify the disturbance gradient and separate the disturbance classes on DF1 (Figure 6) makes this an important EI guild.

The Microbial Community Dynamics guild, although successful in separating the disturbance classes, was a significant challenge for statistical inference and interpretation. Because both bacteria and fungi respond to and closely track moisture, temperature, and seasonal availability of litter, detritus, and nutrients; assessing habitat disturbance within this environmentally noisy background will remain a sampling and analysis challenge. Nevertheless, the pattern obtained in the matrix of Table 4 was very encouraging. There are 112 cells in this 14 x 8 matrix, but only 18 cells are populated. This directly indicates that seven substrate guilds (derived from an original 95), and the way they are utilized (i.e., total activity or functional richness) independently by bacteria and by fungi to characterize the disturbance gradient are rather specific, and different in uplands versus lowlands.

The Soil Chemistry guild needs to be closely analyzed and integrated with the microbial guild. Soil Organic Content showed promise in Phase I as an important indicator of habitat disturbance. Analysis demonstrated that nitrate has low concentrations at Low disturbance sites, presumably because of more rapid nutrient uptake by more abundant vegetation or stronger and more stable links to mycorrhizal associations. Higher soil organic matter and lower pH was associated with less disturbed sites. The lower pH is due to the presence of humic acids resulting from more active decomposition processes. It was surprising that microbial biomass carbon did not differ among disturbance classes. This may be a terrestrial example of the “paradox of the plankton” where marine or limnetic *biomass* trophic pyramids are reversed because of the higher turn-over rates (i.e., energy transfers) of phytoplankton compared to zooplankton. If this is indeed the case, the disturbance classes differ in microbial activity rates (as demonstrated in the microbial guild), while maintaining approximately the same biomass, a most interesting observation.

The Nutrient Leakage guild was subjected to unequal sample sizes among years, sites, seasons, and habitats (uplands and lowlands), because of drought conditions and physical damage to lysimeters by prescribed burns and wildlife, especially feral hogs. Lowland sites exhibited more consistent and greater ion concentrations than upland sites. Moderately disturbance lowland sites retained ions (sodium, potassium, magnesium, and sulfate) better than either less or higher disturbed sites. Highly disturbed upland sites leached more nitrate than less disturbed sites.

The Soil Mineralization Potential guild was very successful at assessing relative habitat disturbance (Figure 23), and shows promise as an indicator for assessing and monitoring forest ecological condition, see Kovacic et al. (2006) for more interesting details.

Ant communities are gaining interest as biological indicators of disturbance and ecological conditions (Agosti et al. 2000, Andersen et al. 2002, Andersen and Majer 2004). The Ground/Litter Ant Community guild with 28 species (103,203 individuals) was very successful

at discriminating among the three disturbance classes. *Dorymyrmex smithi* comprised 87% of all individuals. This species requires warm nests and prefers habitats with open canopy and bare soils, and therefore, dominated the highest disturbed sites and the discriminant analysis. Nevertheless, the removal of the species for subsequent analyses had no effect on analysis results, indicating the robustness of the ant community as an effective and reliable EI guild. Five species of ants (554 individuals) were particularly successful at discriminating the disturbance gradient: *Aphaenogaster floridana*, *Camponotus castaneus*, *Letptothorax texana*, *Paratrechina parvula*, and *Solenopsis molesta* (native fire ant). The abundant imported fire ant (*Solenopsis invicta*) was present in the 28 species analysis, but did not contribute significantly to disturbance class discrimination. A great deal of detailed information is available in Graham et al. (2004, 2005).

It is important to recall that the Fort Benning landscape has been subjected to a wide variety of landscape disturbances: historical agricultural activities (including associated infrastructure), historical major and recent managed timber harvest, recent mechanized U.S. Army mechanized infantry training, and frequent prescribed burns. Historical environmental disturbances although quantitatively unaccountable, undoubtedly significantly alter, often appreciably, current ecosystem structure, dynamics, and processes. Present day plant community species composition and species richness in northeastern France are the direct result of agricultural intensity during the period AD 50-250 (Dupouey et al. 2002). Therefore, soil degradation from past land-use may be irreversible on historical time scales. Current field measures of ecosystem condition and properties and their reference to disturbance represent the cumulative reflection of all the historical and current anthropogenic and natural disturbance regimes subjected to the landscape with no hope of unraveling all the details. Nevertheless, the careful selection of relatively pristine reference sites statistically contrasted to a broad landscape disturbance gradient has identified important Ecological Indicators of habitat disturbance, with the opportunity to analytically associate indicator metrics with ecosystem structure, function, and processes; and therefore, providing important monitoring capabilities for land managers.

The individual EI metrics identified in Phase I research were validated in a much broader landscape context in Phase II. Forty sites (including the original nine from Phase I) were selected throughout Fort Benning representing relatively pristine to severely degraded military training areas in all available upland plant communities and forest types. These sites were classified into 10 disturbance classes before field data were collected, based on a visual assessment of disturbance to vegetation and soils. A-horizon depth, soil compaction, and DF1 of general ground cover characteristics (dominated by bare ground) were plotted on the 10-class disturbance gradient; clearly verifying the utility of these EIs (Figures 32, 33, 34). These three EI metrics were far more effective at characterizing this disturbance gradient than the more traditionally used NDVI (normalized difference vegetation index) derived from satellite imagery (Figure 31).

A Site Comparison (or Condition) Index was constructed from seven EI metrics: A-horizon depth, soil compaction, soil organic content (correlates with carbon), litter cover (100-bare ground), canopy cover, basal area, tree density; and the NDVI. Statistically, the NDVI did not contribute any additional information to the SCI. Weighting coefficients were developed for the EI metrics and NDVI based on statistical procedures (see Methods section). SCI scores for the

40 sites were plotted against the 10 disturbance classes (Figure 35). The SCI modeled the disturbance gradient monotonically and smoothly. The unbiased analytically derived SCI scores reproduced almost perfectly the ranking assigned by a very experienced observer, thus non-subjective uniformity of ranking was achieved.

The histogram of SCI scores for the SCI ranked 40 sites revealed a sigmoid logistic decay function (Figure 36), analytically demonstrating that relatively few sites were either very high quality or very severely degraded, and suggested a “threshold effect” of rapid decline in SCI values as disturbance increased from “pristine sites” or as severely degraded sites were approached. Discrepancies between SCI and Disturbance Class (DC) rankings revealed interesting ecosystem patterns. Deciduous forests with their high canopy cover and tree biomass and complex vegetation layers scored the highest with both the SCI and DC ranking (Figures 37, 38). On the other hand, a pristine xeric scrub oak – longleaf pine savanna (DC1) with relatively open canopy and less complex vegetation was ranked ninth with the SCI (Figure 39). The lowest SCI ranked site was in the center of the Delta Training Ranges, and was highly degraded possessing a great deal of bare ground (Figure 40). A relatively pristine longleaf pine forest in rocky rolling hills with very simple vegetation structure was ranked 19th (in the middle of the SCI gradient), but was in DC2 (Figure 41). This site is one of Fort Benning’s protected *Unique Ecological Areas* - Arkansas Oak Rock Hills.

The use of indices to classify or characterize landscape parcels raises an interesting caveat. This is exactly analogous to the calculation of a diversity index, a frequently used index for environmental monitoring and environmental impact assessment. The diversity index consists of two metrics: species richness (i.e., number of species) and evenness (the relative abundances among individual species). Even though there is a high positive correlation between the index and species richness with the accumulation of many samples, one can never be sure which of the two components of the index is more important when comparing any two specific samples. Two samples with the identical species diversity index, may nevertheless, differ dramatically in community structure. One community may possess a very large number of species with highly skewed species-abundance patterns. In other words, there are a few dominants, but most species are very rare. The other community may have relatively few species, but each species possesses similar abundances. These are compositionally, and undoubtedly functionally as well, dramatically different communities, but are described as identical by a diversity index. Similarly, and more meaningful to a land manager, high basal area can be achieved by either relatively few giant trees or a high density of very small trees. The basal area metric alone cannot distinguish between these two extreme possibilities.

The SCI has been useful and unbiased in analytically quantifying landscape disturbance, and was instrumental in identifying the relationship between biodiversity elements and habitat disturbance (Krzyśik et al. 2005a). However, the SCI raises the same ambiguity as the diversity index, especially when trying to compare across different community physiognomies (Figures 37 to 41, discussed above). A-horizon and soil compaction are highly negatively correlated at the 40 sites (Spearman’s rho: -0.72, $P < 0.001$). However, land managers are often more interested in comparing two specific sites, than knowing summary metrics for a large sample of parcels. Extreme values in either A-horizon or soil compaction can disguise moderate values in both. Historical soil A-horizon losses from agriculture or timber harvest may be associated with low

soil compaction, because of minimal disturbance by tactical or other OHVs. Level topography not plowed with uneroded soils may have deep A-horizons, but be heavily compacted by recent tactical vehicle maneuvers. The sensitivity of soils to compaction is highly dependent on clay content. The bottom line is that each metric in an index carries unique and important environmental information that is only “averaged” in the construction of an index. Land managers and ecologists must be equally cognizant of the benefits and limitations of indices. The continued refinement of SCIs is encouraged, but they must be used with the concurrent responses of the individual metrics that comprise them.

SCI scores, based on the seven indicator metrics (six General Habitat metrics and a Soil Chemistry metric, NDVI was not used) were also calculated for the 160 transects at the 40 sites, and the resulting histogram also revealed a sigmoid logistic decay function (Figure 42). The 160 transects were divided into five disturbance classes: Low, Low-Med, Medium, Med-High, High. Discriminant analysis with the interpretation of DF1 demonstrated that General Ground Cover (Figure 44) and Floristics Ground Cover (Figure 48) indicator guilds clearly characterized the landscape disturbance gradient, even with the use of a wide variety of plant communities. The DA result of the ant communities was not as direct on the disturbance gradient. Ant community structure on DF1, as characterized by species composition and relative abundance, was similar in Low to Medium disturbance classes, and differed at both Med-High and High disturbance (Figure 50). The three Low to Medium disturbance classes were clearly separated by DF3 and DF4 (Figures 52, 53). This result indicates that although all five disturbance classes could be separated by DA based on ant community structure, there was no monotonic change in ant community structure based on the disturbance gradient. This may be attributed to the broad variety of plant communities represented in the disturbance gradient. In other words, ant community species composition and relative abundance was not only responding to the disturbance gradient, but also to the nature of the plant community (forest type and succession stage).

We have seen above in the three DAs that DF1 has the most discriminating set of weighed indicator metrics that characterize the overall disturbance gradient. A most interesting, important, and unexpected result is the examination of DF2, the second most important DF for separating the five disturbance classes, and uncorrelated with DF1. Note that all three guilds: General Ground Cover (Figure 45), Floristics Ground Cover (Figure 49), and Ant Communities (Figure 51) validate the intermediate disturbance hypothesis, based on our analytical description of a broad landscape disturbance gradient in a diverse assemblage of plant communities. This result is of significant importance to ecological theory, restoration strategies, and land management approaches concerning the organization and succession of ecological communities. Additionally, we have also demonstrated the intermediate disturbance hypothesis directly with species richness and the diversity index of ant communities (Graham et al. 2005, Krzysik et al. 2005b), and six measures of ground cover: woody, forbs, legumes, *Opuntia*, *Yucca*, and total (Krzysik et al. 2005b). Species-habitat and landscape disturbance relationships are further being investigated and modeled with NMS (nonmetric multidimensional scaling), CCA (canonical correspondence analysis), SEM (structural equation modeling), neural networks, and several other techniques.

Our research results in identifying Ecological Indicators and classifying their metrics into guilds in a wide variety of upland vegetation communities in the complex physiographic ecotone and disturbance regimes at Fort Benning are indeed encouraging. Nevertheless, the data were collected at a single location in the Fall-Line Sandhills. Additional data is required from a larger geographic area and an even greater variety of vegetation communities and soil types (especially clayey), both in the Southeast and in other regions of the United States.

Ecological Indicator Design Criteria

- Ease of use for land managers
- Cost effective
- Ecological relevance & value
- Reflect ecosystem dynamics and physiological stress
- Quantifiable with statistical estimates of accuracy & precision
- Robust & multi-scale
- EcoRegion application
- Global methodology extension
- Symmetry: track degradation & recovery/restoration
- Reasonable response times
- Reliable, consistent, unambiguous
- Incorporation of natural variance
- Known sensitivity to temporal sampling window
- Association with suites of stressors

Table 1. Ecological Indicator Design Criteria developed before field data were collected to guide the selection of Ecological Indicator Systems and their associated metrics.

Bonham Creek (6 Sites), 2000-2001-2002		
FACTOR	Bacteria (P)	Fungi (P)
Year (2000-2001-2002)	<0.001	<0.001
Season (Spring-Fall)	<0.001	NS 0.56
Habitat (Upland-Lowland)	<0.001	<0.001
Disturbance (Low-Medium-High)	<0.001	<0.001
FACTOR INTERACTIONS		
Year x Season	<0.001	NS >0.05
Year x Habitat	<0.001	NS 0.12
Season x Habitat	NS 0.13	NS 0.99
Year x Disturbance	0.003	<0.001
Season x Disturbance	NS 0.51	NS 0.17
Habitat x Disturbance	NS 0.12	0.003
Bonham Creek and Sally Branch (9 sites), 2002		
FACTOR		
Season (Spring-Fall)	0.018	NS 1.00
Habitat (Upland-Lowland)	<0.001	<0.001
Disturbance (Low-Medium-High)	0.007	<0.001
FACTOR INTERACTIONS		
Season x Habitat	0.019	NS 1.00
Season x Disturbance	NS 0.29	NS 1.00
Habitat x Disturbance	NS 0.65	<0.001

Table 2. Multivariate analysis of variance (MANOVA) significance table of bacteria and fungi Microbial Variables for two data sets: Bonham Creek (6 sites), 2000-2001-2002; and Bonham Creek and Sally Branch (9 sites), 2002. Factors are: year, season, habitat, and disturbance class. Note that there are two analyses: bacteria and fungi MANOVAS. Significance based on: Wilk's Lambda, Hotelling's Trace, and Pillai's Trace. See Table 3 for Tamhane's T2 multiple comparisons test for MANOVA.

Data Set	Bacteria	Fungi
Bonham Creek (6 Sites) 2000-2001-2002	Low > High (8) Med > High (9)	High > Low (4) High > Med (2) Med > Low (1)
Bonham Creek and Sally Branch (9 Sites) 2002	Low > High (5) Med > High (7)	High > Low (9) High > Med (7) Med > Low (4)

Table 3. Tamhane's T2 multiple comparisons test for MANOVA of Table 2. Significance is based on P < 0.05. Numbers in Parentheses represent number of occurrences out of a possible maximum of 14 (number of Microbial Variables, each for bacteria and fungi).

Microbial Variable Substrate Guild and *Response	BACTERIA				FUNGI			
	Upland		Lowland		Upland		Lowland	
	DF1	DF2	DF1	DF2	DF1	DF2	DF1	DF2
Simple Carbohydrates A					X			
Simple Carbohydrates R	X		X					
Complex Carbohydrates A					X		X	
Complex Carbohydrates R				X		X		
Amines/Amides A				X				
Amines/Amides R			X					
Amino Acids A					X			
Amino Acids R	X							X
Carboxylic Acids A	X							
Carboxylic Acids R	X							
Polymers A							X	
Polymers R							X	
Nucleotides A		X						
Nucleotides R						X		

*A = Total Activity

*R = Functional Richness

Table 4. Association of bacteria and fungi primary Microbial Variables (Substrate Guilds and Responses) with discriminant functions (DF) 1 and 2 for upland and lowland habitats. These primary Microbial Variables were common to both data sets: Bonham Creek (6 sites, 2000-2001-2002) and Bonham Creek and Sally Branch (9 sites, 2002).

Bonham Creek (6 Sites), 2000-2001-2002			
FACTOR	Complete (P)	Upland (P)	Lowland (P)
Year (2000-2001-2002)	<0.001	<0.001	<0.001
Season (Spring-Fall)	<0.001	<0.001	<0.001
Habitat (Upland-Lowland)	<0.001	-----	-----
Disturbance (Low-Medium-High)	<0.001	<0.001	<0.001
FACTOR INTERACTIONS			
Year x Season	<0.001	<0.001	<0.001
Year x Habitat	NS >0.082	-----	-----
Season x Habitat	0.030	-----	-----
Year x Disturbance	<0.001	<0.001	<0.001
Season x Disturbance	<0.001	<0.001	0.001
Habitat x Disturbance	<0.001	-----	-----
Bonham Creek and Sally Branch (9 sites), 2002			
FACTOR			
Season (Spring-Fall)	<0.001	<0.001	<0.001
Habitat (Upland-Lowland)	<0.001	-----	-----
Disturbance (Low-Medium-High)	<0.001	<0.001	<0.001
FACTOR INTERACTIONS			
Season x Habitat	0.023	-----	-----
Season x Disturbance	<0.001	<0.001	<0.001
Habitat x Disturbance	<0.001	-----	-----

Table 5. Multivariate analysis of variance (MANOVA) significance table of Soil Chemistry for two data sets: Bonham Creek (6 sites), 2000-2001-2002; and Bonham Creek and Sally Branch (9 sites), 2002. Factors are: year, season, habitat, and disturbance class. Note that there are three analyses: complete design, upland, and lowland MANOVAS. Significance based on: Wilk's Lambda, Hotelling's Trace, and Pillai's Trace. See Table 6 for Tamhane's T2 multiple comparisons test for MANOVA.

Bonham Creek (6 sites), 2000-2001-2002		
Soil Chemistry	Upland (P)	Lowland (P)
Nitrate (NO ₃ ⁻)	M>L 0.043, H>L 0.021	M>L 0.010
Ammonium (NH ₄ ⁺)	NS >0.058	NS >0.33
Soil Organics	L>H <0.001, M>L 0.012 M>H <0.001	L>M 0.032
Microbial Biomass Carbon	NS >0.34	NS >0.12
Moisture	M>H 0.002	NS >0.82
pH	M>L 0.033, H>L <0.001	H>L <0.001, H>M <0.001

Bonham Creek and Sally Branch (9 sites), 2002		
Soil Chemistry	Upland (P)	Lowland (P)
Nitrate (NO ₃ ⁻)	M>L 0.009	NS >0.42
Ammonium (NH ₄ ⁺)	NS >0.34	NS >0.16
Soil Organics	L>H <0.001, M>H <0.001	NS >0.11
Microbial Biomass Carbon	NS >0.20	NS >0.46
Moisture	M>H 0.021	NS >0.33
pH	H>M 0.011	H>L <0.001, H>M <0.001

Table 6. Tamhane's T2 multiple comparisons test for MANOVA of Table 5.

MANOVA (separate analysis for each habitat)		
FACTOR	Upland (P)	Lowland (P)
Year (2000-2001-2002)	<0.001	<0.001
Disturbance (Low-Medium-High)	0.002	<0.001
FACTOR INTERACTIONS		
Year x Disturbance	<0.039	<0.001
MANOVA (complete design)		
FACTOR	All	
Year (2000-2001-2002)	<0.001	
Habitat (Upland-Lowland)	<0.001	
Disturbance (Low-Medium-High)	<0.001	
FACTOR INTERACTIONS		
Year x Habitat	NS >0.66	
Year x Disturbance	0.004	
Habitat x Disturbance	0.002	

Table 7. Multivariate analysis of variance (MANOVA) significance table of Nutrient Leakage anions, cations, and pH. Upland and lowland sites were analyzed separately (above), as well as the complete MANOVA (below) with year, habitat, and disturbance class as factors. Significance based on: Wilk's Lambda, Hotelling's Trace, and Pillai's Trace. Tamhane's T2 multiple comparisons test for disturbance classes is shown in Table 8.

Anions & Cations	Upland	Lowland
Sodium	NS > 0.81	L>M <0.001, H>M 0.005
Potassium	NS > 0.95	L>M 0.015, H>M <0.001
Lithium	NS > 0.92	NS > 0.35
Calcium	NS > 0.23	NS > 0.12
Magnesium	NS > 0.61	L>M 0.011, H>M 0.019
Ammonium (NH ₄ ⁺)	NS > 0.24	NS > 0.45
Nitrate (NO ₃ ⁻)	H>L 0.036, H>M 0.044	NS > 0.41
Phosphate	NS > 0.54	NS > 0.69
Sulfate	NS > 0.065	L>M <0.001, H>M <0.001
Chlorine	L>H 0.020	NS > 0.62
Fluorine	M>L 0.048, H>L 0.039	NS > 0.82
pH	NS > 0.91	NS > 0.12

Table 8. Tamhane's T2 multiple comparisons test for MANOVA of Table 7. Upland and lowland habitat analyzed separately.

Species	Code	13 Species		12 Species		6 Species		5 Species	
		DF1	DF2	DF1	DF2	DF1	DF2	DF1	DF2
Aphaenogaster ashmeadi	AphAsh								
Aphaenogaster floridana	AphFlo	0.35	0.14	0.42	0.24	0.39	-0.24	0.48	0.38
Aphaenogaster texana	AphTex								
Brachymyrmex musculus	BraMus	-0.085	0.34	-0.15	0.31				
Camponotus castaneus	CamCas	0.24	-0.21	0.33	-0.15	0.27	0.32	0.37	-0.25
Camponotus socius	CamSoc								
Crematogaster ashmeadi	CreAsh								
Crematogaster atkinsoni	CreAtk								
Crematogaster minutissima	CreMin	0.28	-0.32	0.39	-0.25				
Dorymyrmex bureni	DorBur								
Dorymyrmex smithi	DorSmi	-0.52	-0.17			-0.58	0.3		
Forelius pruinosus	ForPru	0.048	-0.26	0.091	-0.24				
Formica pallidifulva	ForPal								
Leptothorax texana (davisii)	LepTex	0.19	0.42	0.19	0.48	0.2	-0.67	0.21	0.76
Monomorium viride	MonVir								
Paratrechina arenivaga	ParAre								
Paratrechina parvula	ParPar	0.42	-0.17	0.55	-0.057	0.47	0.24	0.63	-0.097
Paratrechina vividula	ParViv	0.013	0.3	-0.019	0.3				
Pheidole bicarinata	PheBic								
Pheidole crassicornis	PheCra	0.34	0.051	0.43	0.14				
Pheidole dentata	PheDen	0.099	0.37	0.081	0.4				
Pheidole metallescens	PheMet	0.098	-0.28	0.16	-0.26				
Pheidole morrisi	PheMor								
Pheidole pilifera	PhePil								
Prenolepis imparis	PreImp								
Solenopsis invicta	SolInv								
Solenopsis molesta	SolMol	0.17	-0.17	0.24	-0.13	0.19	0.26	0.27	-0.2
Trachymyrmex septentrionalis	TraSep								

Table 9. The 28 ant species used in discriminant analysis of the Ground/Litter Ant Community Guild, and the pooled within-disturbance class correlations between species and standardized canonical discriminant functions: 28, 13, 12, 6, and 5 species pools are analyzed. Twelve rare species were eliminated from the original pool of 40 species. Note the large negative effect of *Dorymyrmex smithi*, and the corresponding shift in positive correlations when it is deleted in a subsequent analysis.

SEMP Researcher Proposed Metric	Ecological Indicator Phase I & II Evaluation	Statistical Significance (P) (Based on Simple Linear Regression)
Soil A-Horizon Depth	A-Horizon Depth	<0.001
Soil Compaction	Soil Compaction	<0.001
Soil-Sediment Carbon	Soil Organics Microbial Biomass Carbon	<0.001 0.006
Soil-Sediment Nitrogen	Ammonium (NH ₄ ⁺) Nitrate (NO ₃ ⁻)	0.66 0.038
Surface Cover (satellite)	NDVI¹	<0.001
Canopy Cover	Canopy Cover	<0.001
Vegetation Structure	Basal Area Tree Density Litter Cover² Total Ground Cover	<0.001 <0.001 <0.001 0.21
Species Composition	Not Evaluated (requires taxonomic expertise by land manager)	-----

Table 10. Selection of Ecological Indicators for the SCI. The 8 proposed metrics by SEMP team leader researchers are in the first column. Our team's identified 12 Ecological Indicators are in the second column. The eight indicators selected for the SCI are in red and bold, based on P<0.001.

¹NDVI was provided by Bob Lozar, U.S. Army-ERDC-CERL.

²Litter Cover (100 – Bare Ground) was selected by Discriminant Analysis.

General Ground Cover Metric	Discriminant Function			
	1	2	3	4
Woody	-.480(*)	.445	.269	.463
NSp_GC (5.8 sqm)	-.450(*)	.427	-.036	-.003
Grass	.447(*)	.051	.079	.431
Forbs	.024	.728(*)	.009	-.194
Legumes	-.093	.572(*)	-.140	-.064
Total_GC	-.232	.544(*)	.219	.309
Opuntia	-.037	.490(*)	-.346	.194
Yucca	-.051	.220	.533(*)	-.055
PinesAll	-.009	.323	.328(*)	-.259
PineSeedling	-.040	.315	.316(*)	-.196
Ferns(**)	-.263	-.005	-.037	-.281(*)

Table 11. Discriminant Function structure matrix of General Ground Cover Guild. Values are pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions.

*** Variables are ordered by absolute size of correlation within the discriminant function.**

**** This variable was eliminated from the analysis because it did not pass the tolerance test, indicating that it does not possess additional information for discrimination.**

PC	Discriminant Function			
	1	2	3	4
PC5	-.537(*)	-.282	.301	.282
PC4	.517(*)	.036	.348	-.030
PC8	-.151	.619(*)	.483	-.045
PC7	.244	-.245(*)	-.095	-.151
PC3	.082	.201(*)	.138	-.106
PC10	-.094	.403	-.576(*)	-.166
PC2	.028	.168	-.196(*)	.002
PC9	.085	.264	-.108	.723(*)
PC1	.193	-.025	.197	.424(*)
PC6	-.057	.089	.349	-.393(*)

Table 12. Discriminant Function structure matrix of Floristics Ground Cover Guild.
See Table 11 for interpretation.

Low Disturbance (from DF1)	Moderate Disturbance (from DF2)	High Disturbance (from DF1)
Vaccinium staminium (W)	Andropogon virginicus (G)	Paspalum notatum (G)
Pteridium aquilinum (Fern)	Rhus radicans (W)	Rubus cuneifolius (W)
Vaccinium arboreum (W)	Diospyros virginiana (W)	Helianthemum sp. (F)
Quercus falcata (W)	Pityopsis graminifolia (F)	Panicum laxiflorum (G)
Rhus radicans (W)	Rhus copallina (W)	Panicum aciculare (G)
Liquidambar styraciflua (W)	Crataegus flava (W)	Ipomea pandurata (F)
Diospyros virginiana (W)		Festuca ovina (G)
Vitis rotundifolia (W)		Plantago aristata (F)
Quercus margaretta (W)		Lechea villosa (F)
Vaccinium myrsintes (W)		Agrostis hyemalis (G)

Table 13. Ground cover taxa associated with Low, Moderate, and High disturbance sites based on discriminate analysis. Taxa are ranked on the relative magnitude of their varimax rotated principal component loadings. DF1 was strongly associated with PCs 4 and 5, and DF2 primarily with PC8 (Table 12).

* = also associated with less disturbed sites in Phase I research at the nine sites in mixed pine/hardwoods forest. *Coreopsis lanceolata* (F) was associated with Low DCs in both Phase I and II research.

** = associated with High disturbance sites in Phase I research.

F = forb, G = grass, W = woody

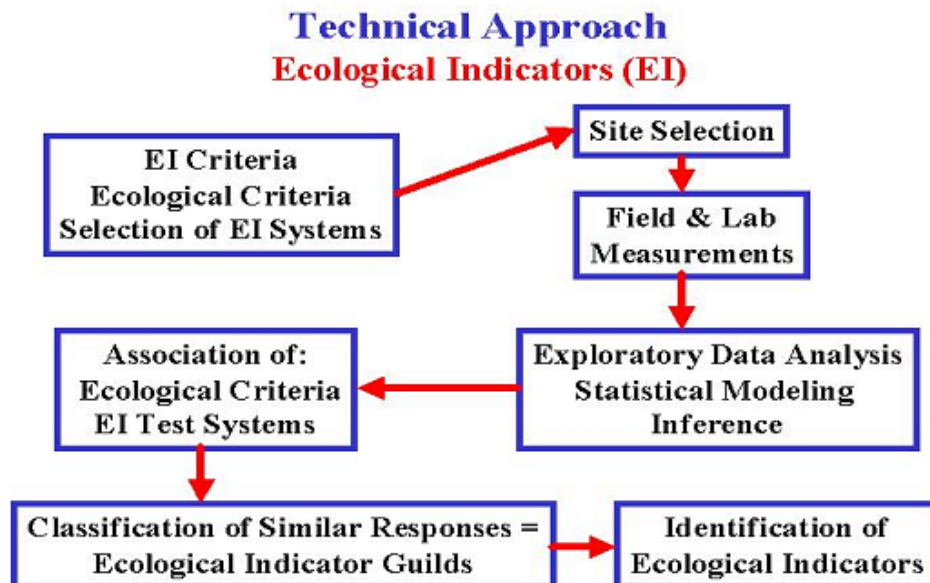


Figure 1. Technical approach for the identification of Ecological Indicators.

Site Selection Phase I

Disjunct Landscape Disturbance Gradient

3 sites selected in each disturbance class

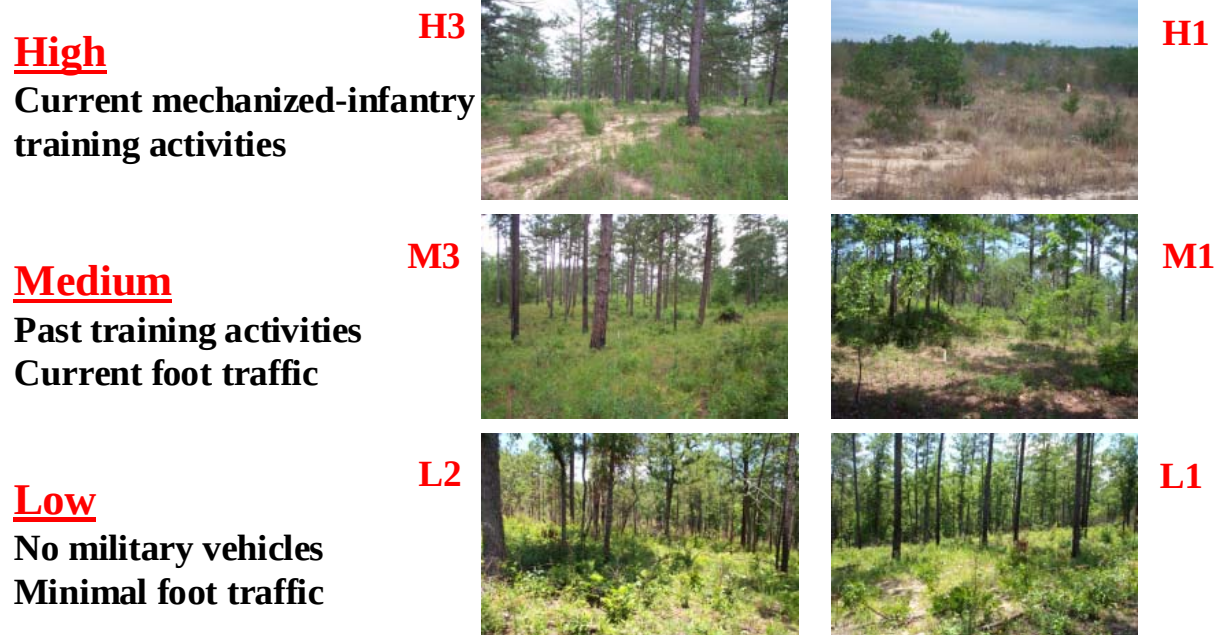


Figure 2. Phase I research High, Medium, and Low disturbance classes with two sites shown in each disturbance class. The other three sites are not shown.

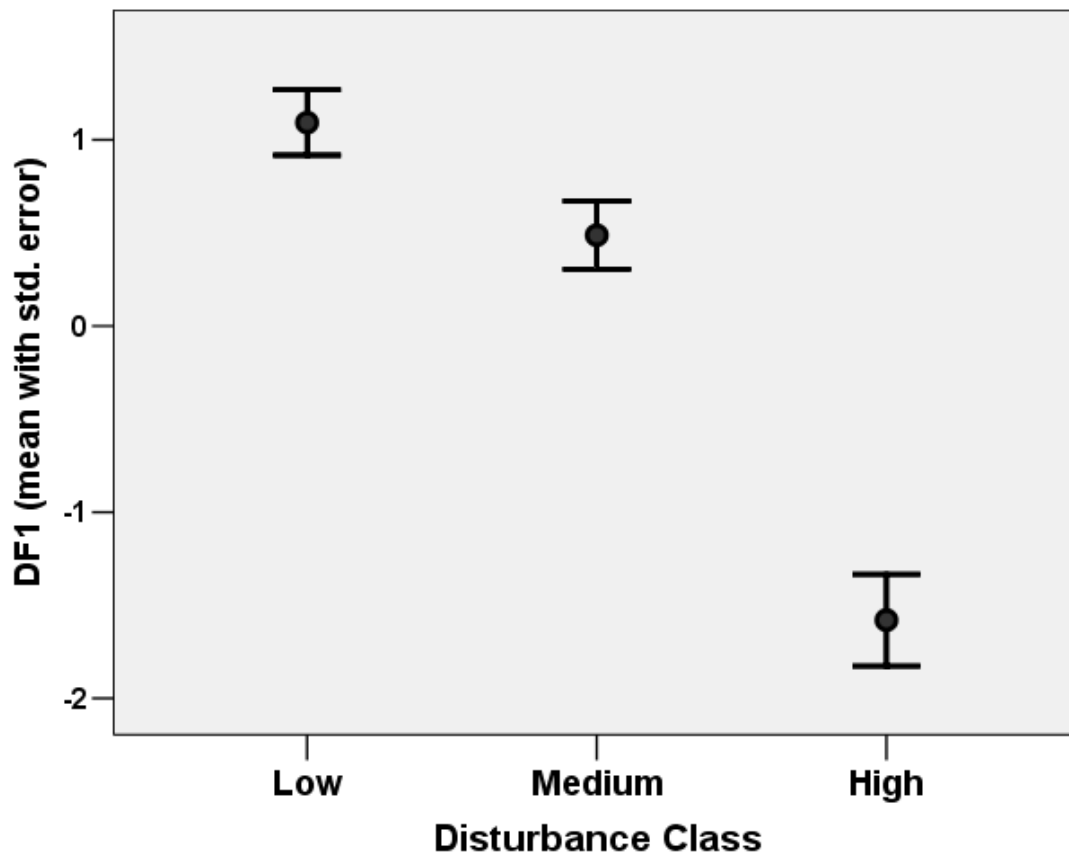


Figure 3. Discriminant Analysis scores for General Habitat variables: A-horizon depth, soil compaction, canopy cover, and basal area. DF1 explains 99.5% of discriminating variance. Bonham Creek and Sally Branch (9 sites), 2001-2002.

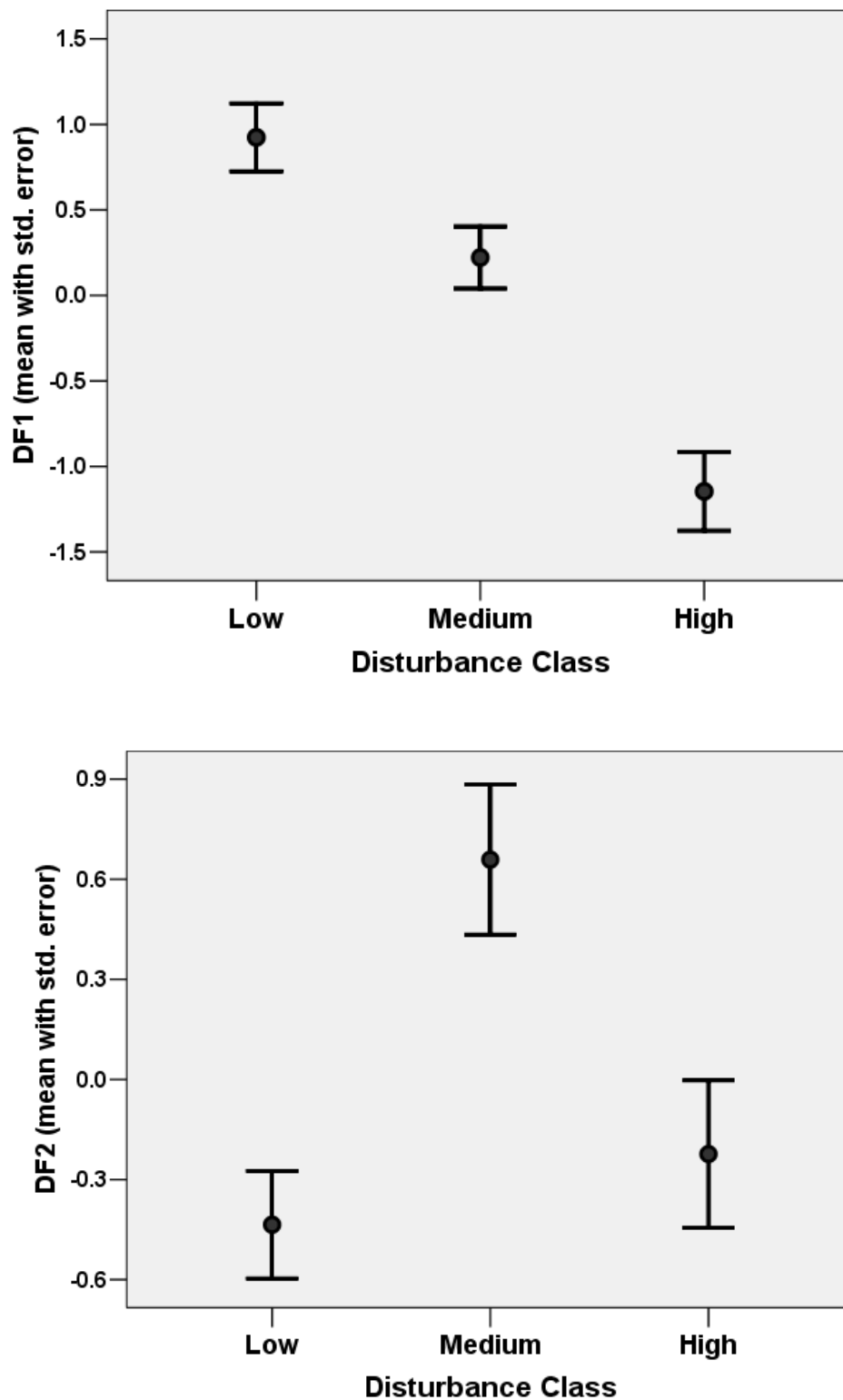


Figure 4. Discriminant Analysis scores for General Habitat tree variables: tree density, tree species richness, DBH (mean), and number of trees/species. DF1 (above) explains 77% and DF2 (below) 23% of discriminating variance. Bonham Creek and Sally Branch (9 sites), 2001-2002.

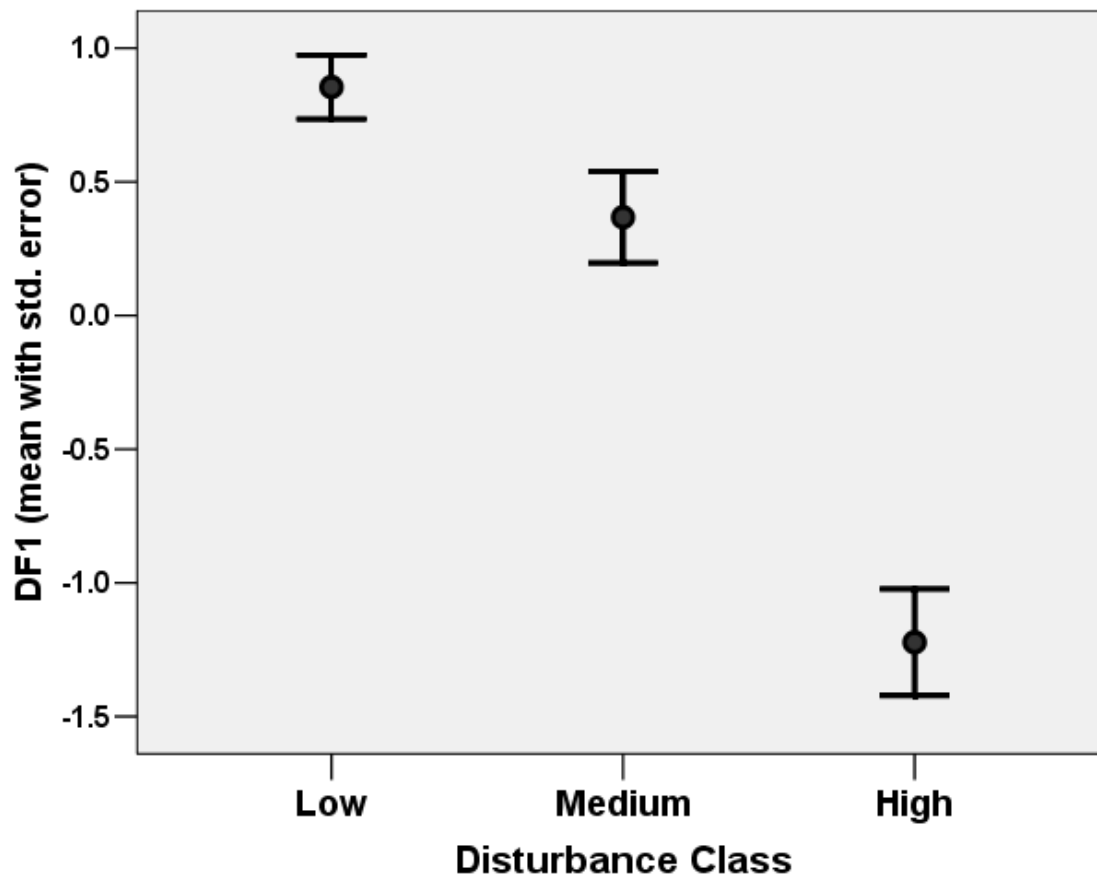


Figure 5. Discriminant Analysis scores for General Ground Cover variables: litter cover, bare ground, forb cover, woody vegetation cover, and grass cover. DF1 explains 94% of discriminating variance. Bonham Creek and Sally Branch (9 sites), 2001-2002.

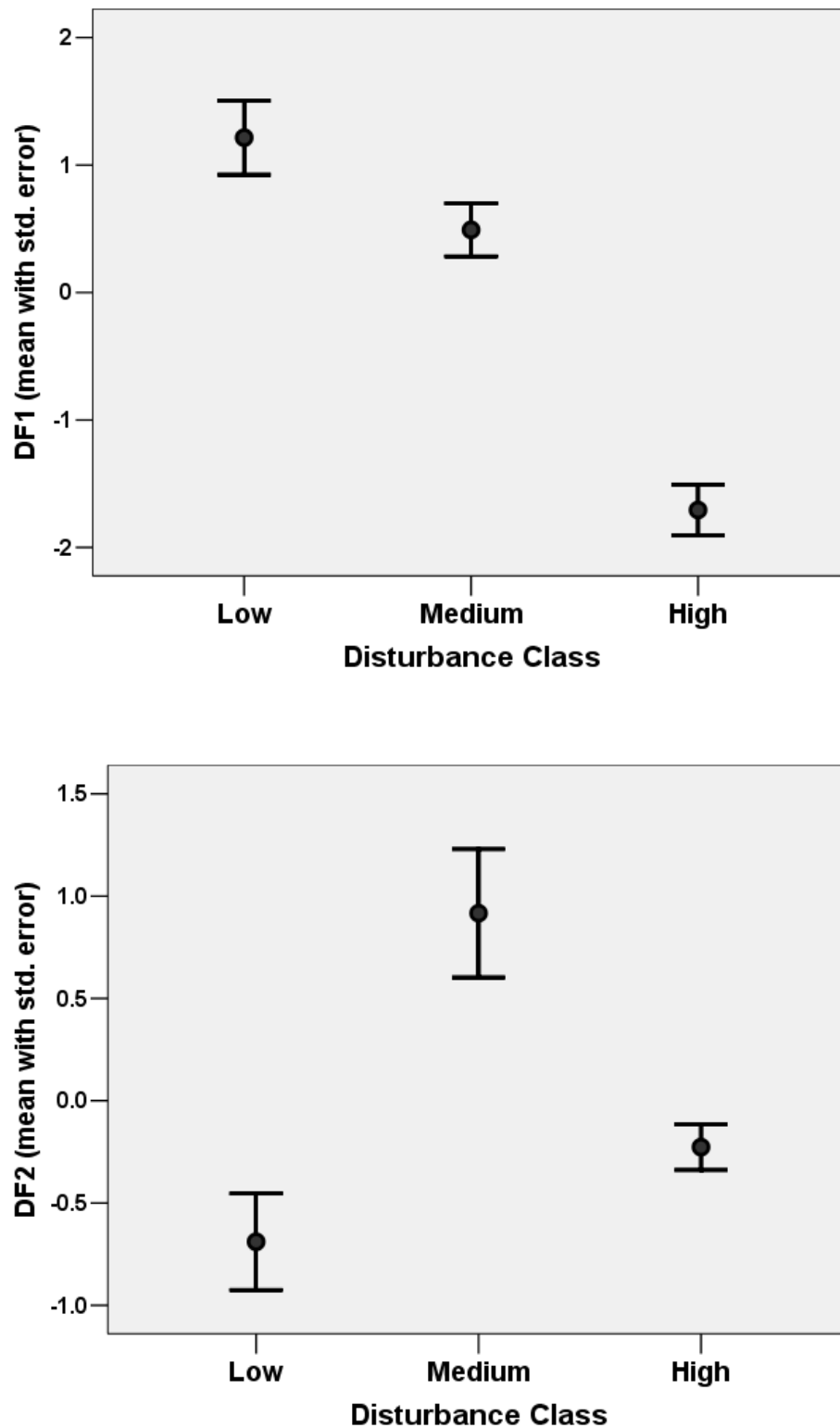


Figure 6. Discriminant Analysis scores for Ground Cover Florestics variables. The six variables are principal components from a PCA of 67 ground cover plant taxa, see Methods. DF1 (above) explains 77% and DF2 (below) 23% of discriminating variance. Bonham Creek and Sally Branch (9 sites), 2002.

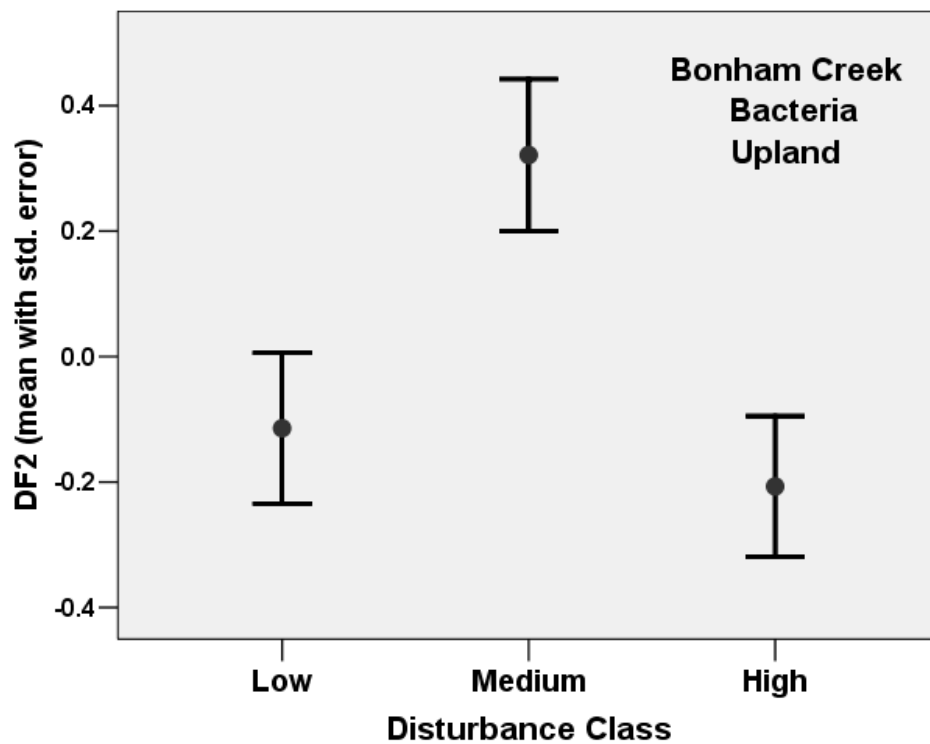
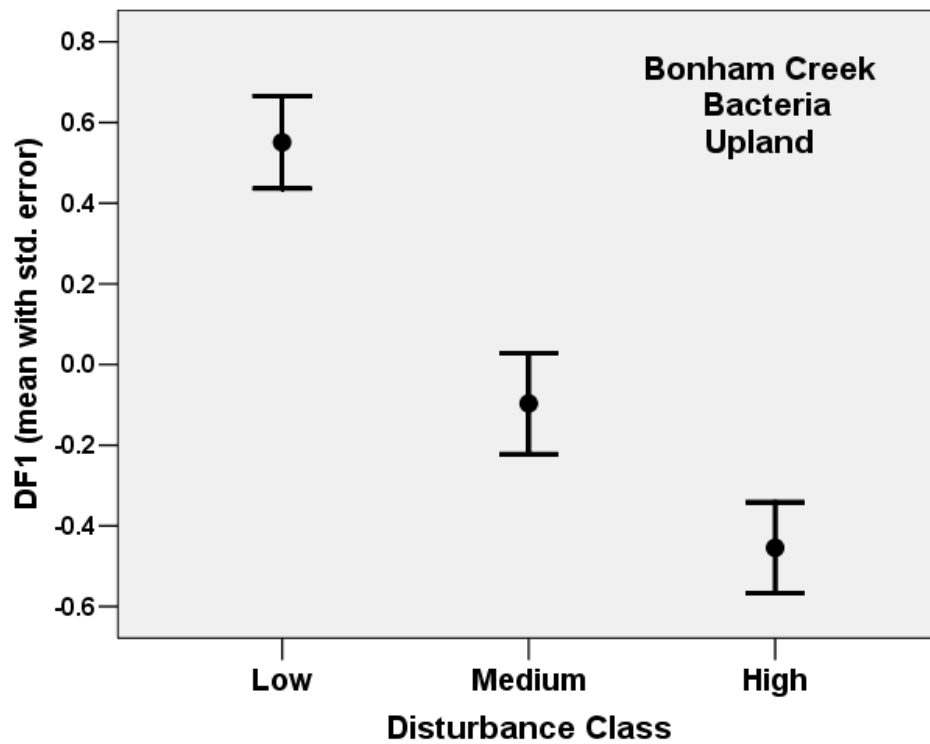


Figure 7. Bacteria Upland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek 6 sites, 2000-2001-2002.

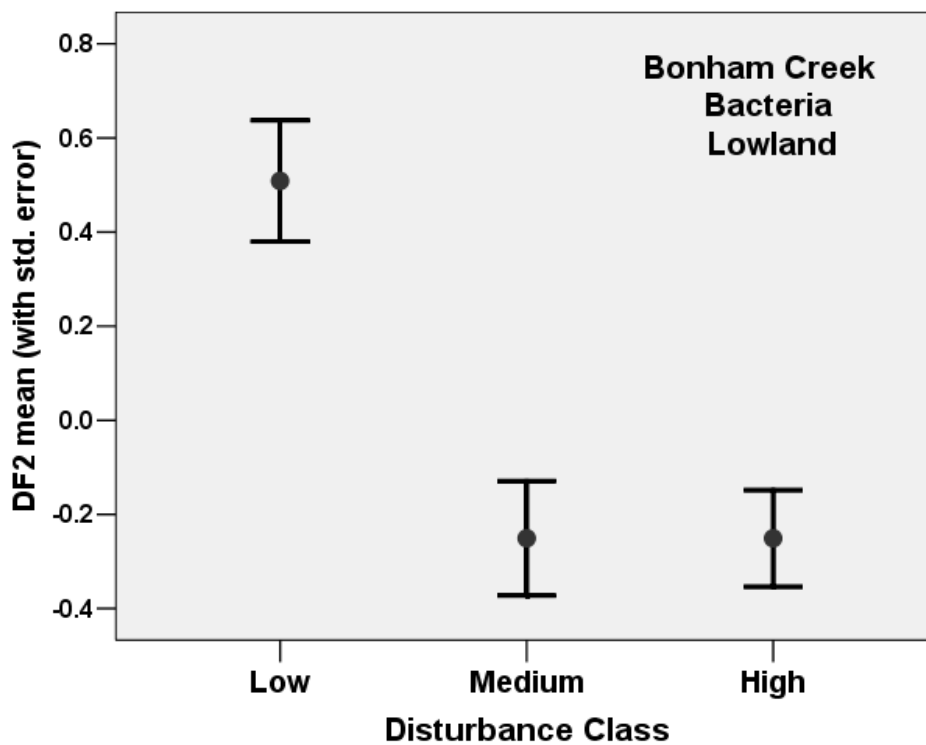
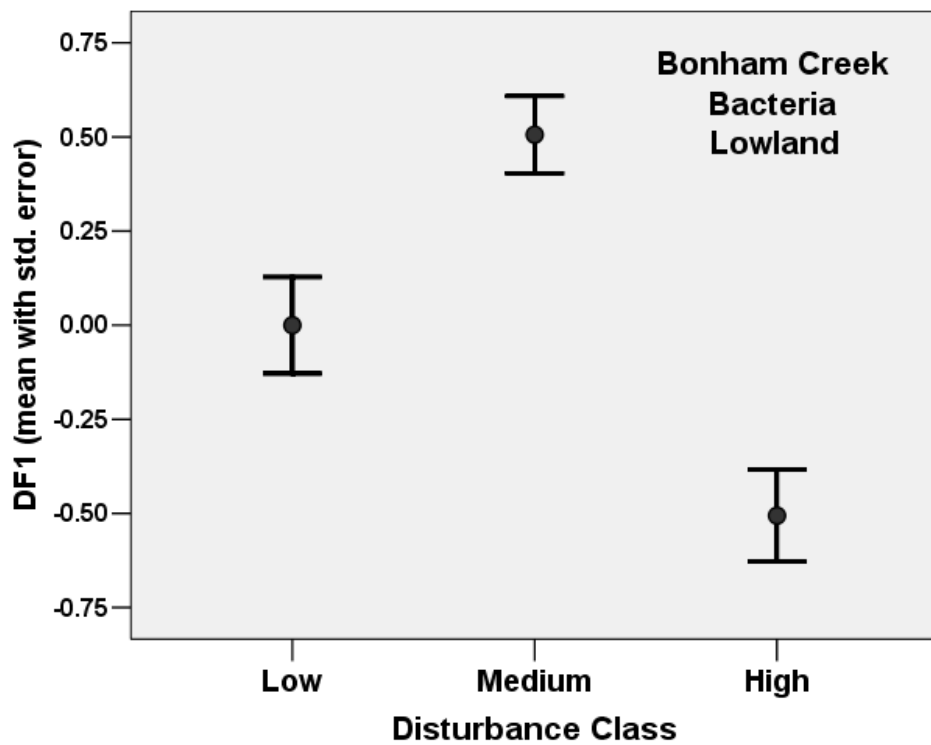


Figure 8. Bacteria Lowland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek 6 sites, 2000-2001-2002.

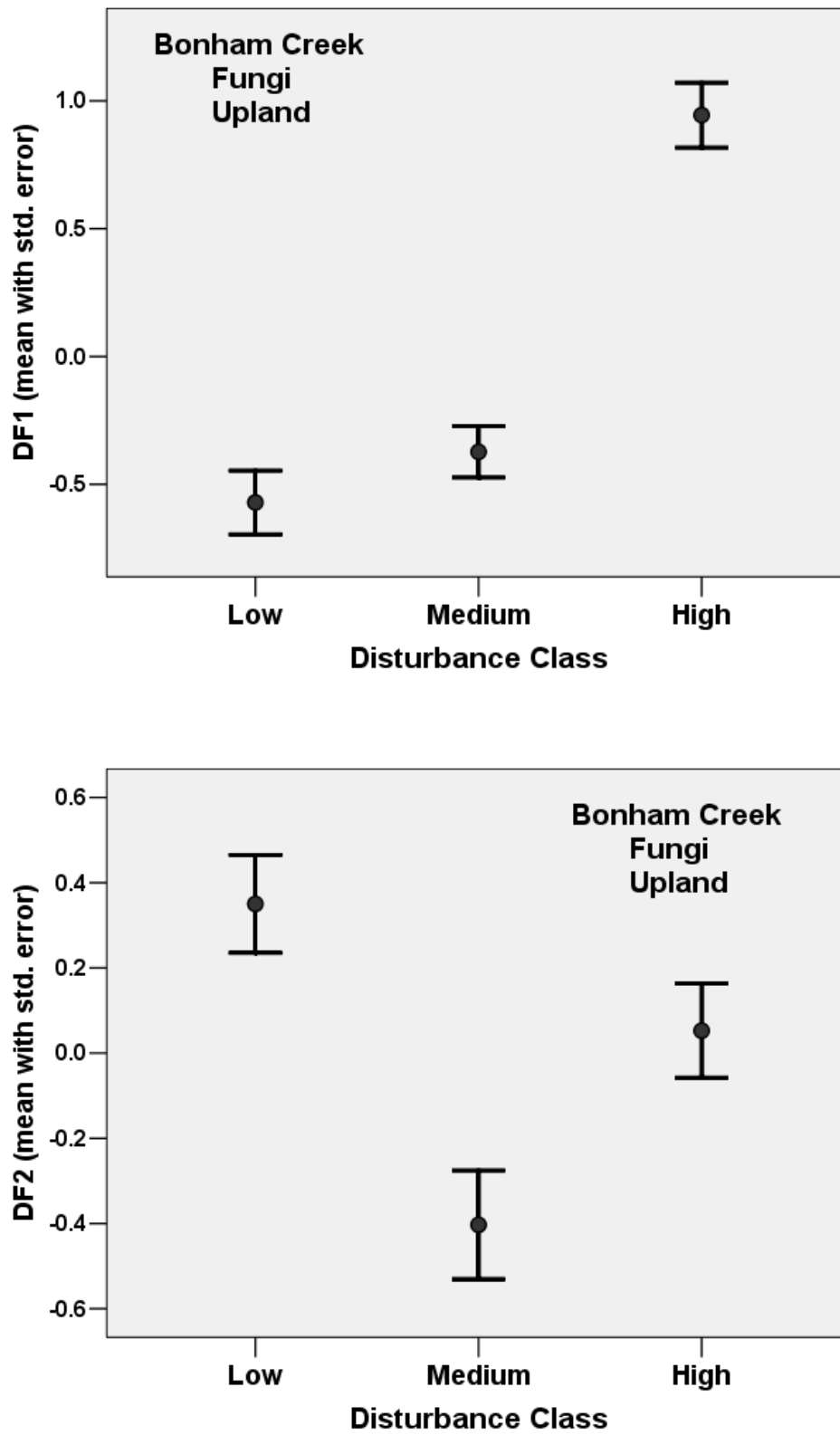


Figure 9. Fungi Upland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek 6 sites, 2000-2001-2002.

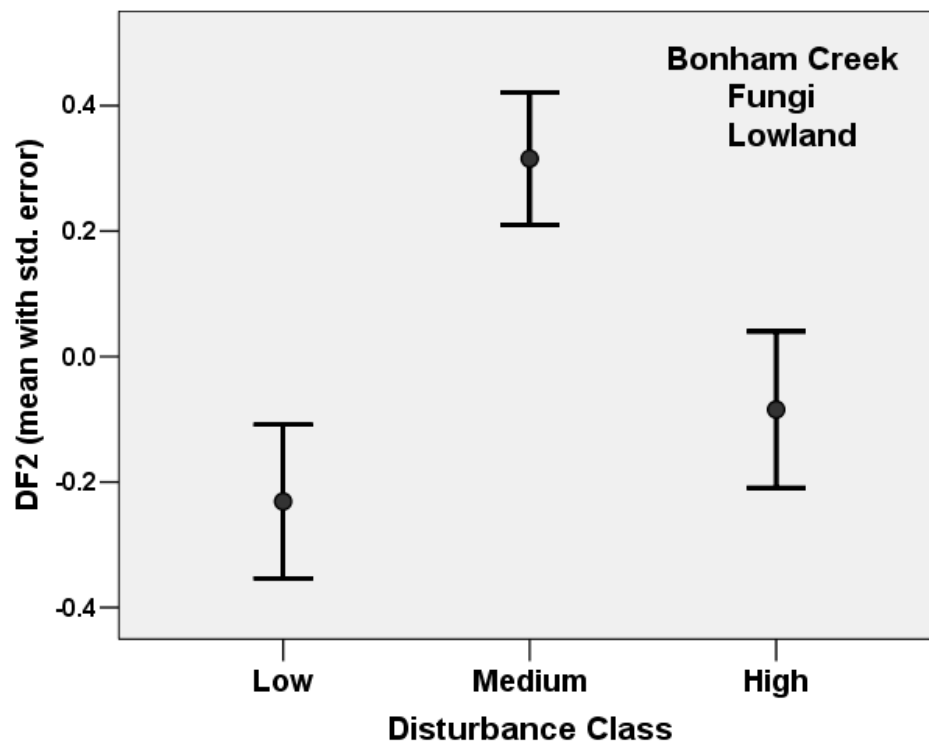
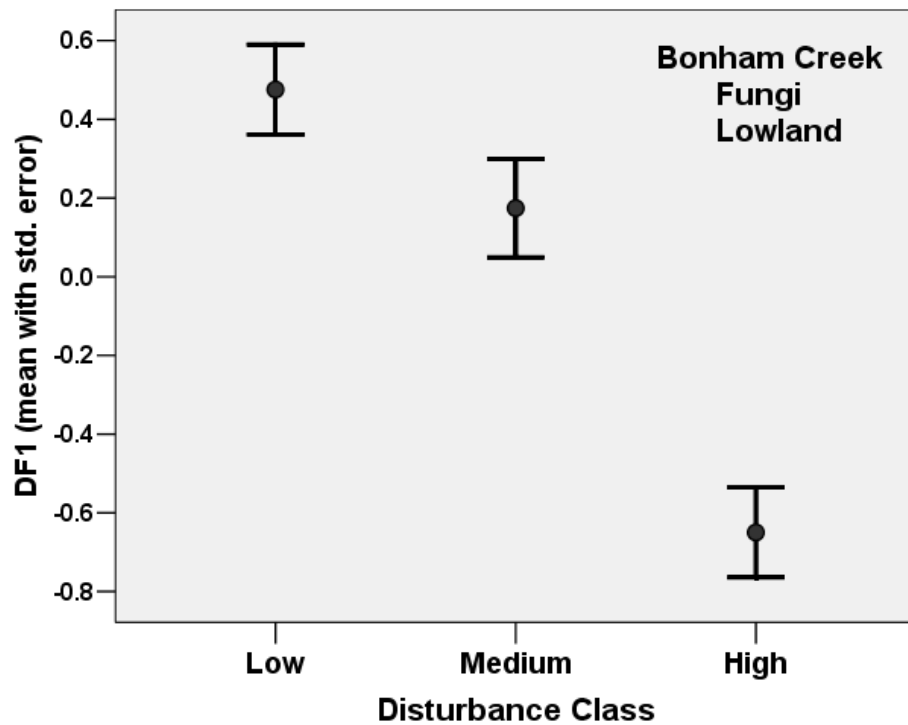


Figure 10. Fungi Lowland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek 6 sites, 2000-2001-2002.

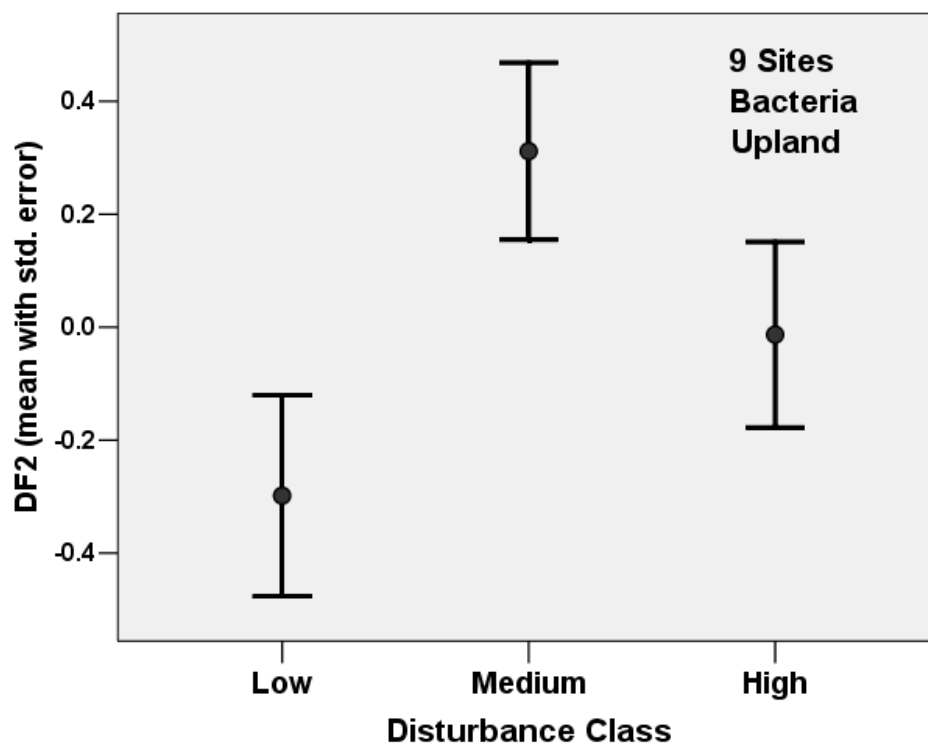
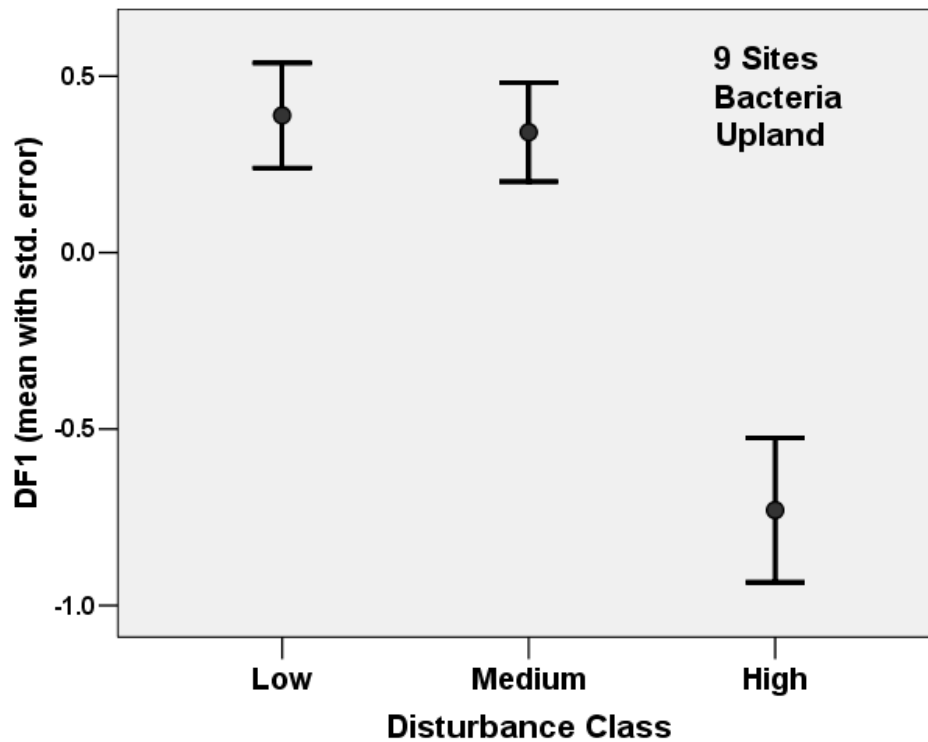


Figure 11. Bacteria Upland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek & Sally Branch 9 sites, 2002.

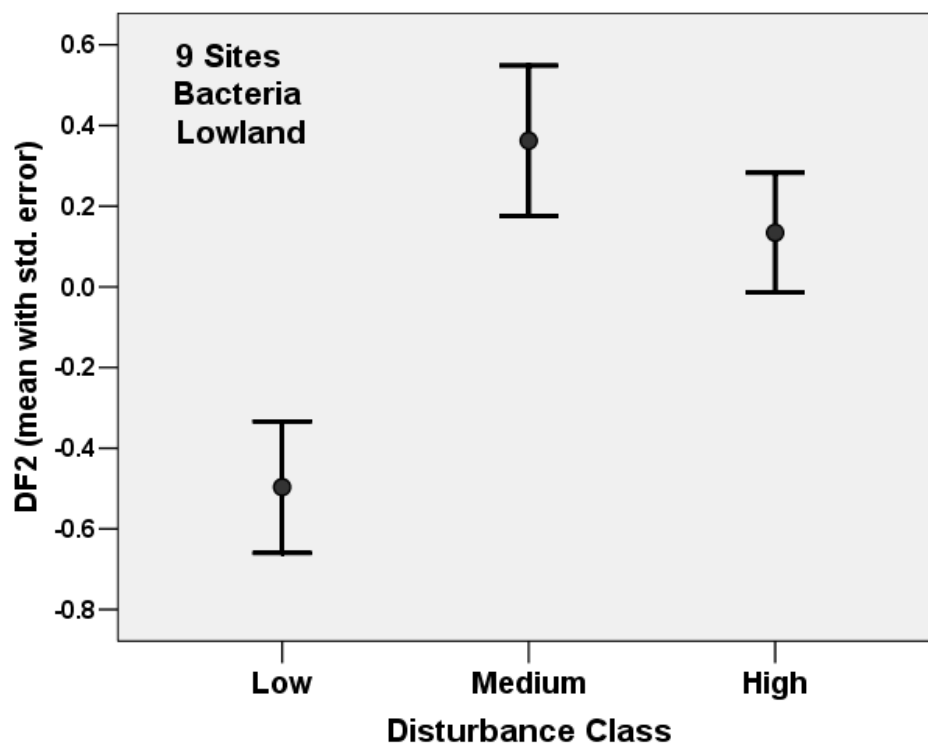
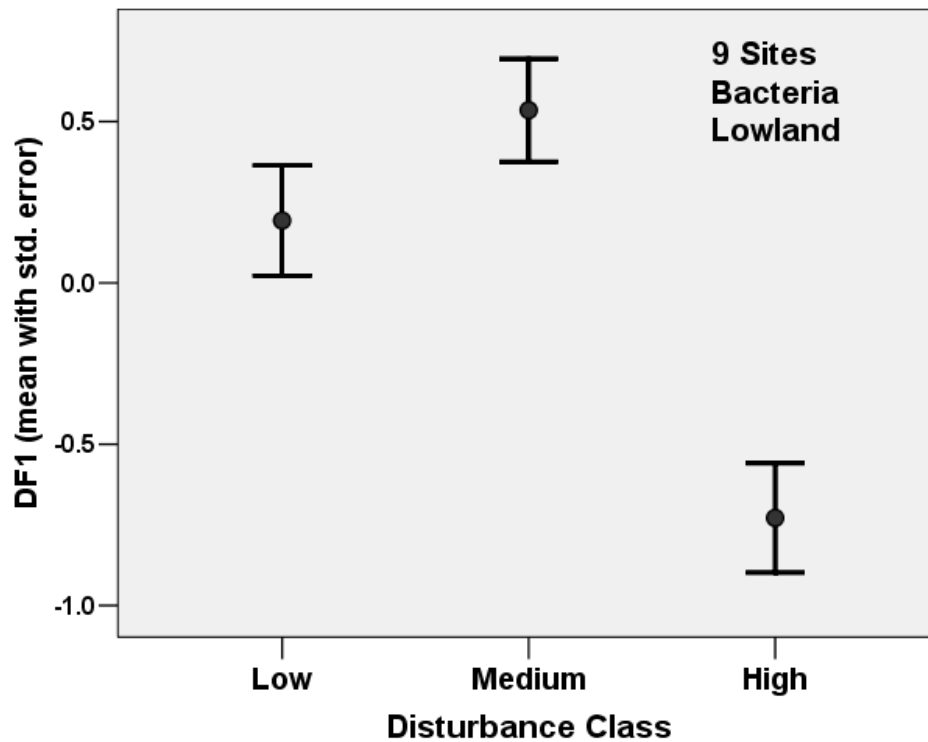


Figure 12. Bacteria Lowland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek & Sally Branch 9 sites, 2002.

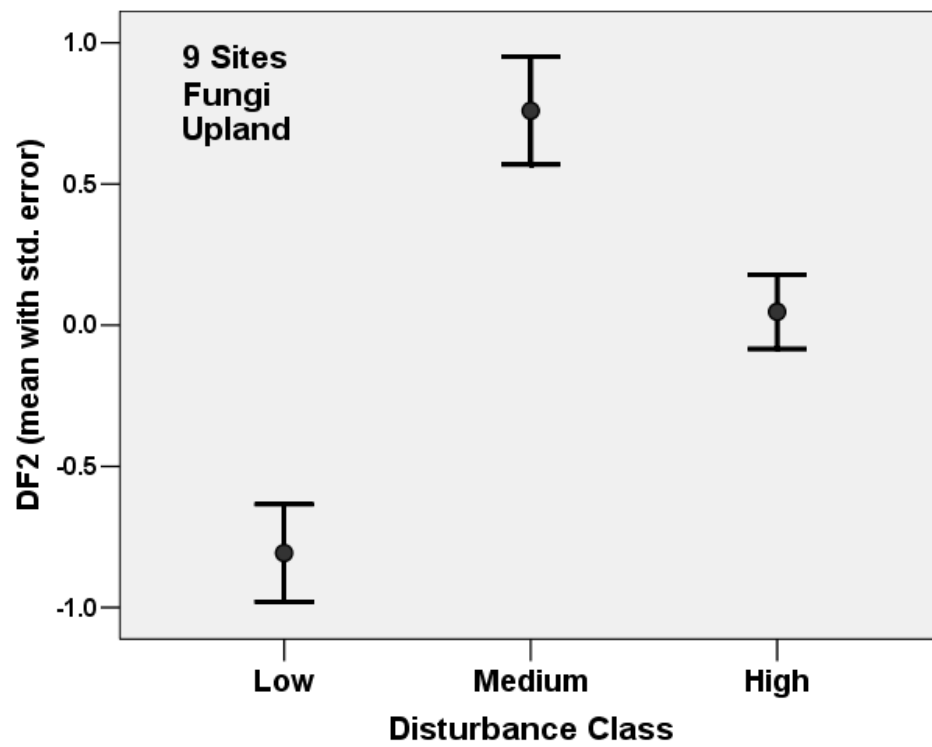
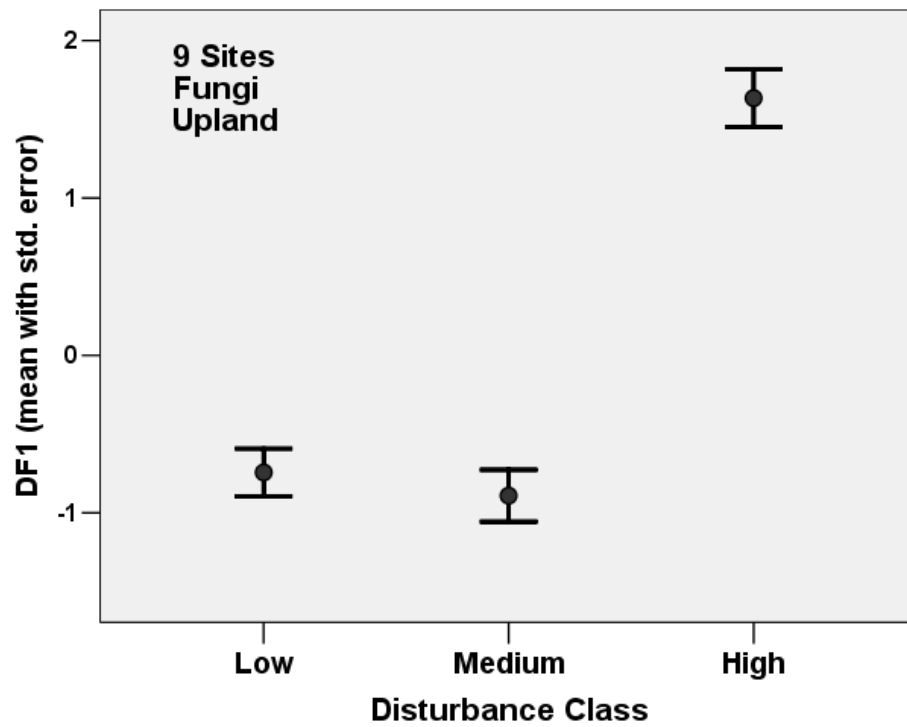


Figure 13. Fungi Upland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek & Sally Branch 9 sites, 2002.

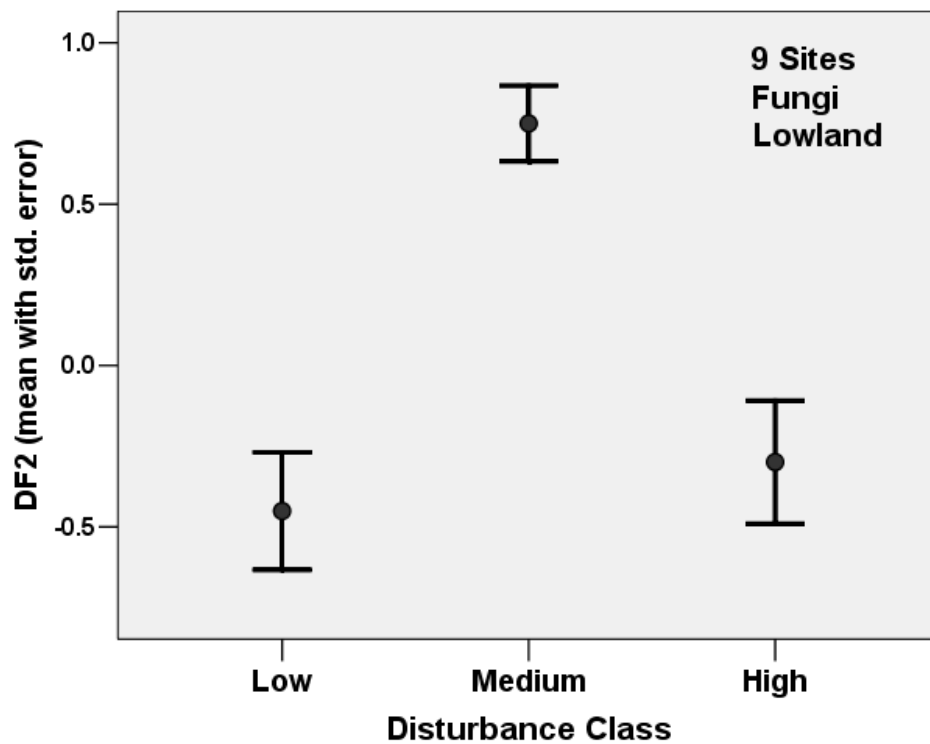
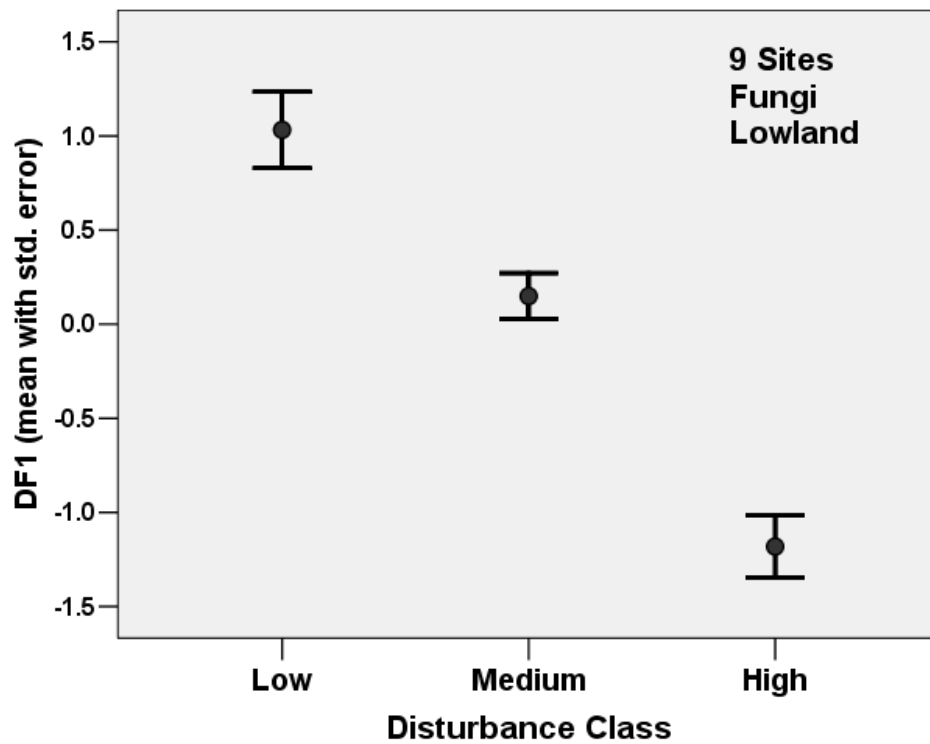


Figure 14. Fungi Lowland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek & Sally Branch 9 sites, 2002.

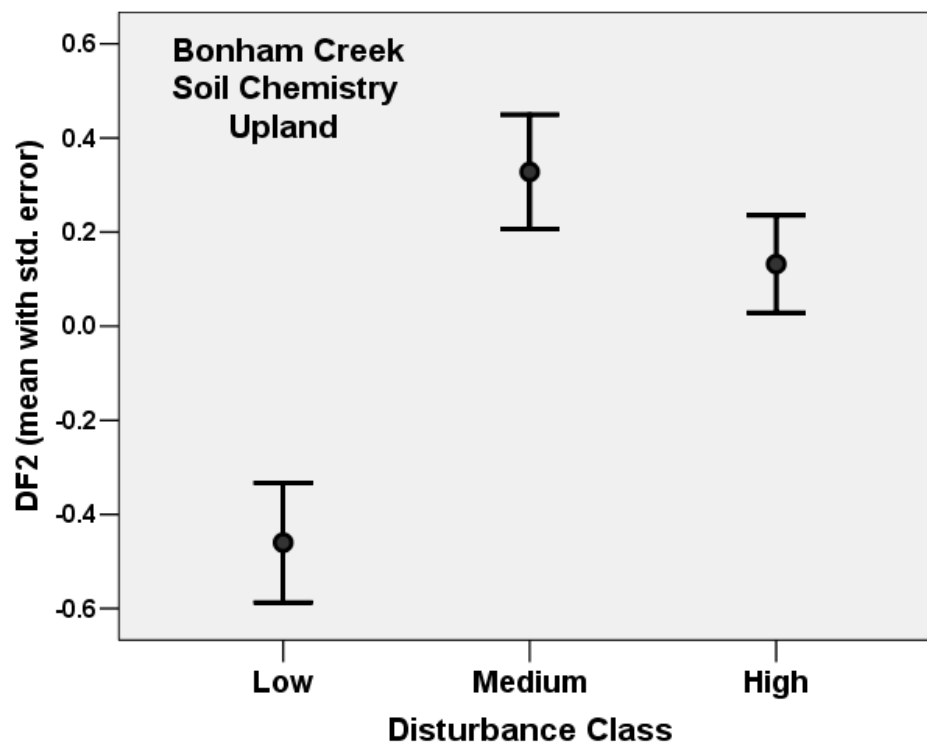
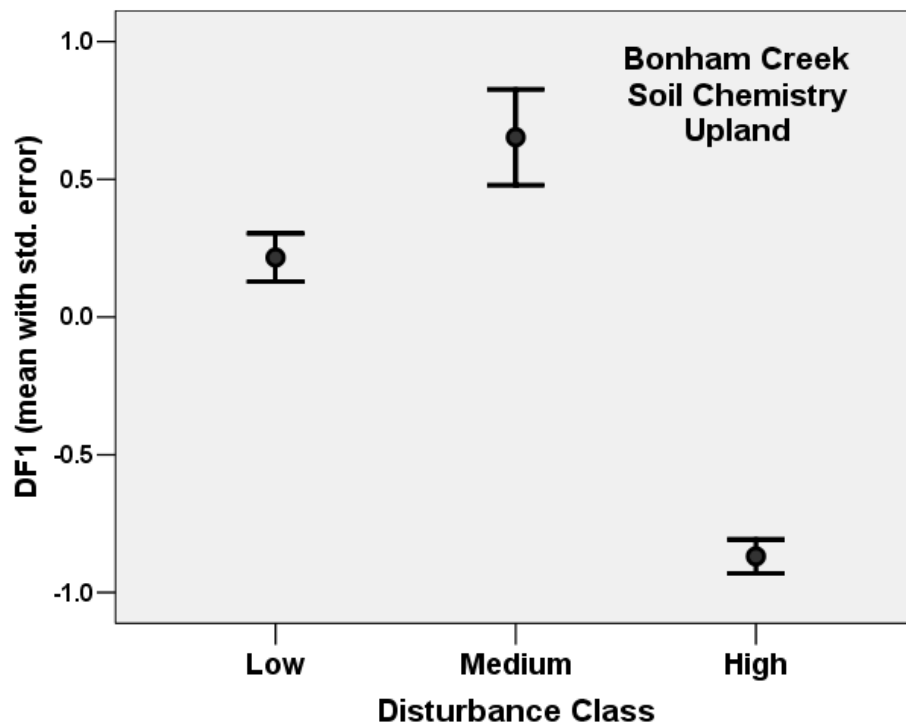


Figure 15. Soil Chemistry Upland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek 6 sites, 2000, 2001, 2002.

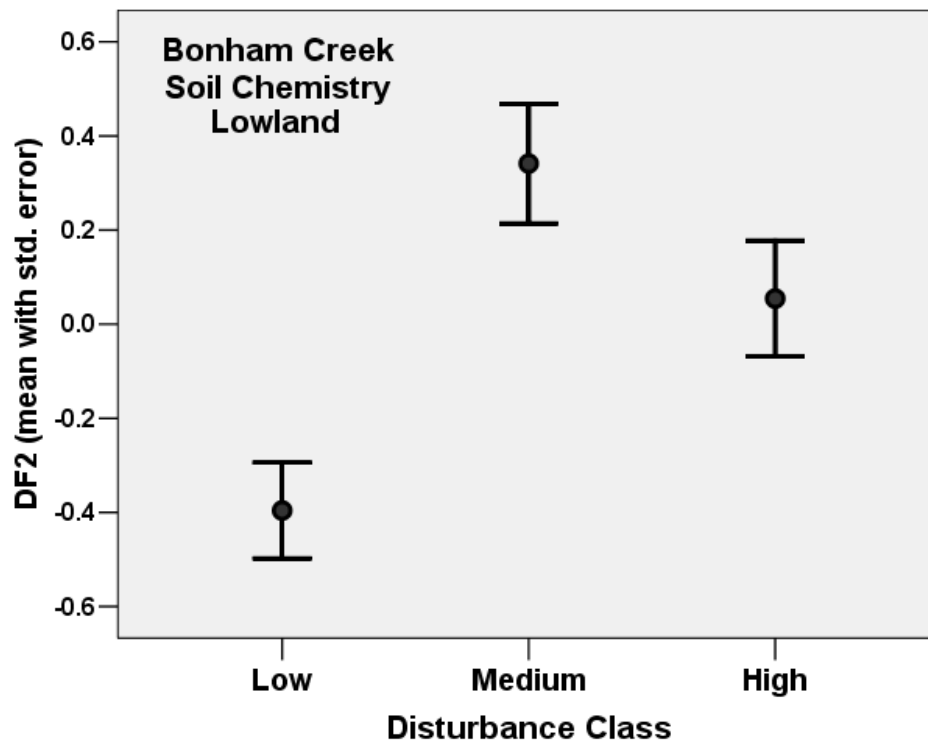
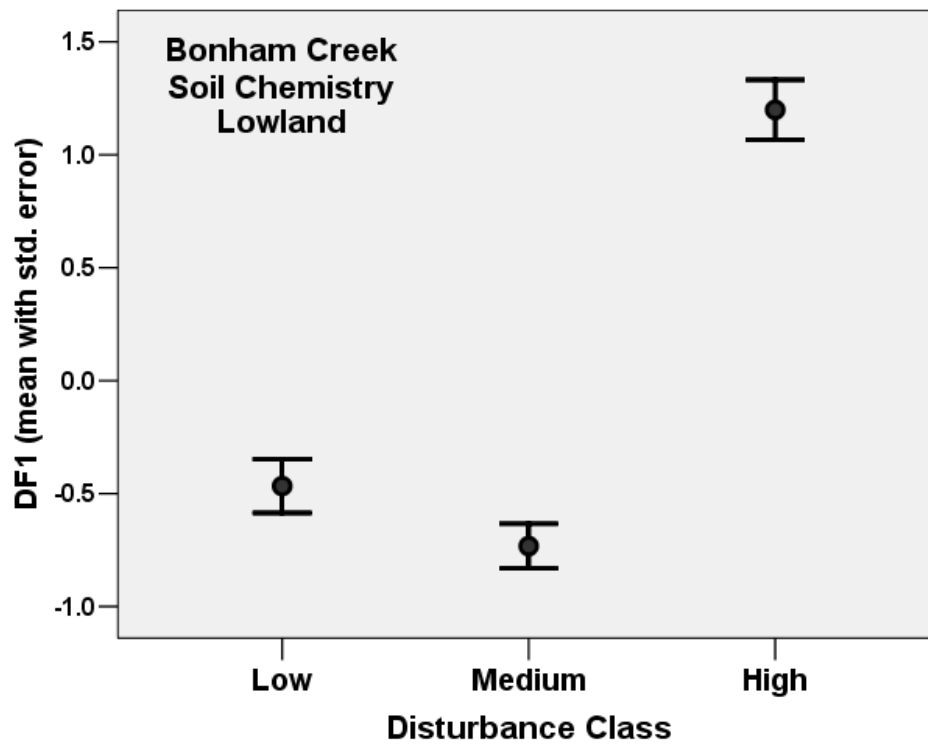


Figure 16. Soil Chemistry Lowland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek 6 sites, 2000, 2001, 2002.

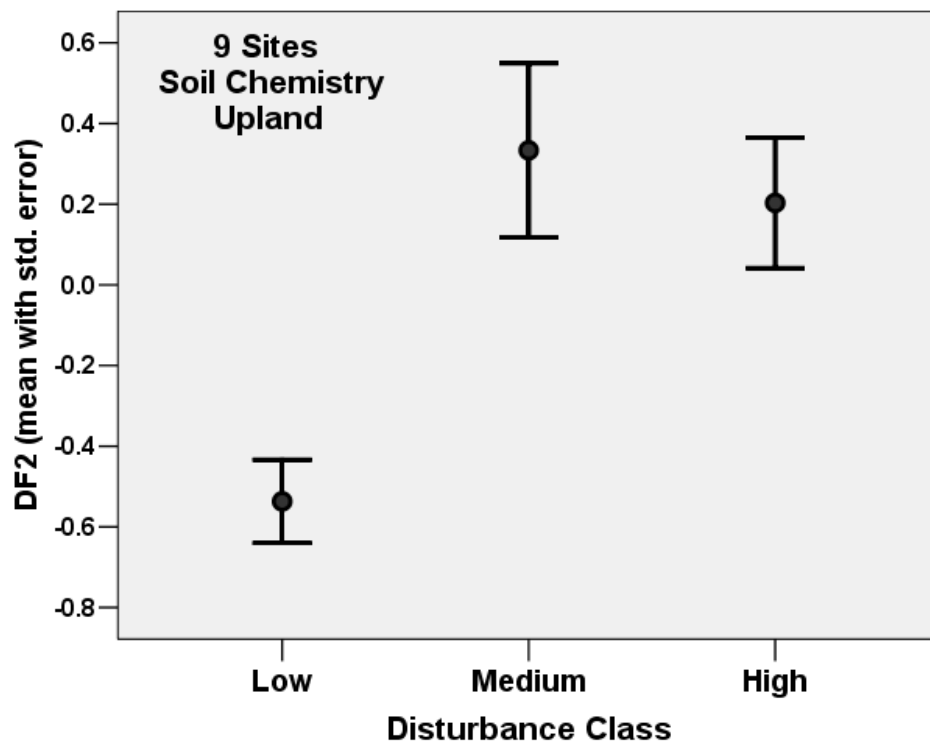
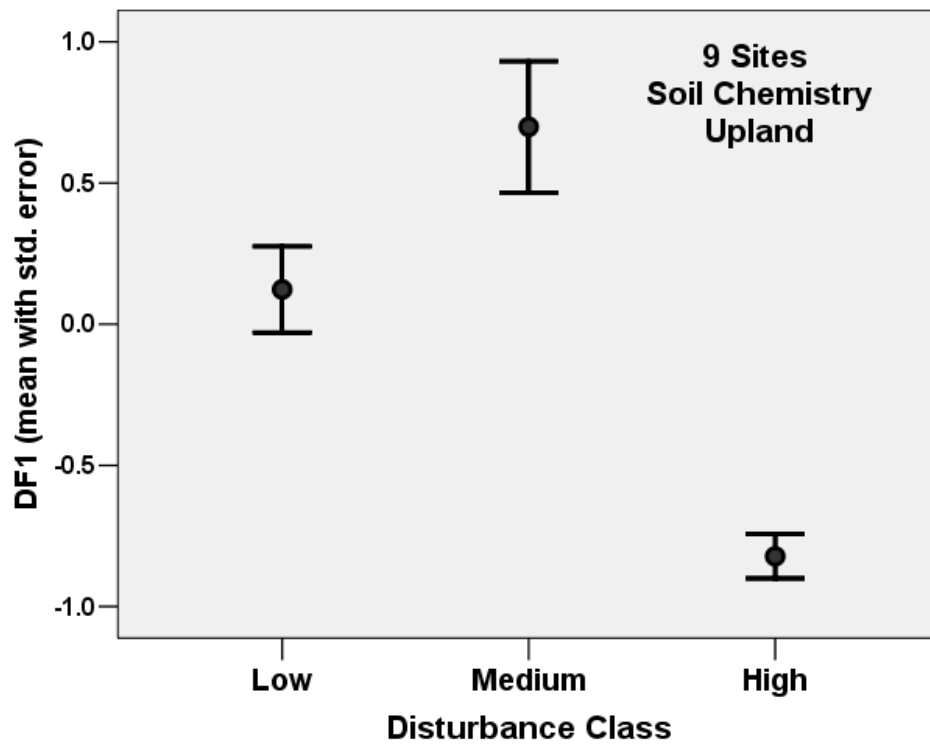


Figure 17. Soil Chemistry Upland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek and Sally Branch 9 sites, 2002.

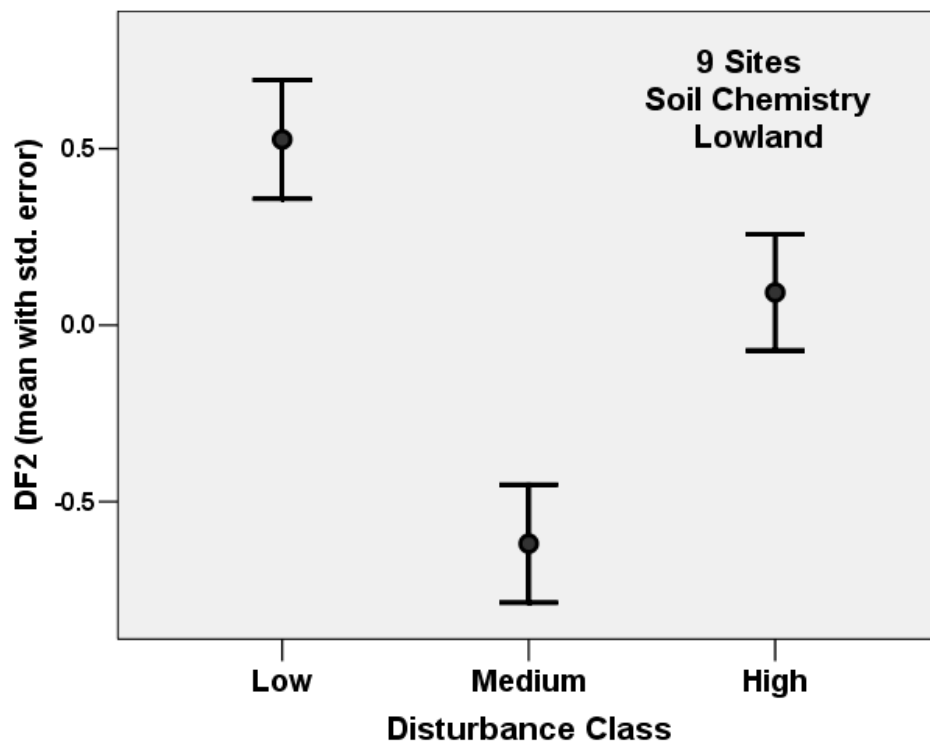
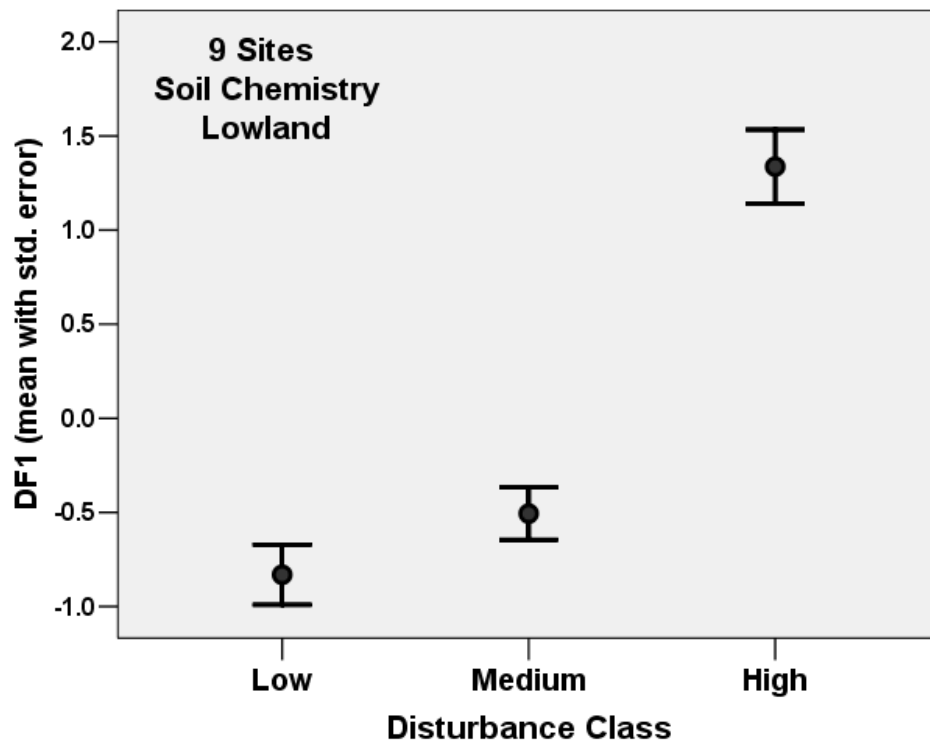


Figure 18. Soil Chemistry Lowland Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek and Sally Branch 9 sites, 2002.

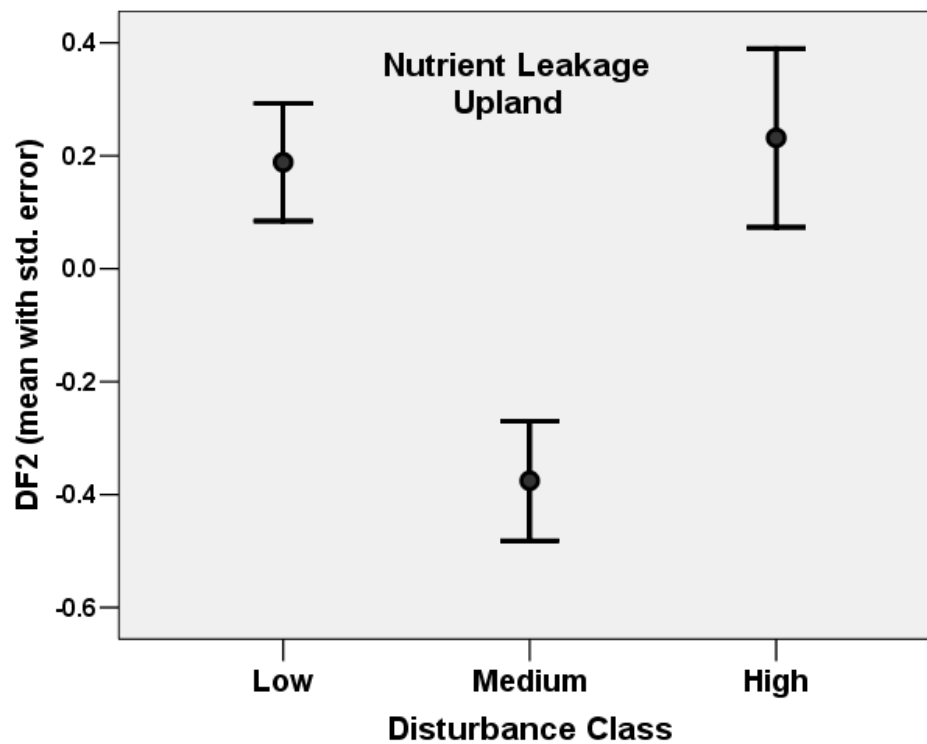
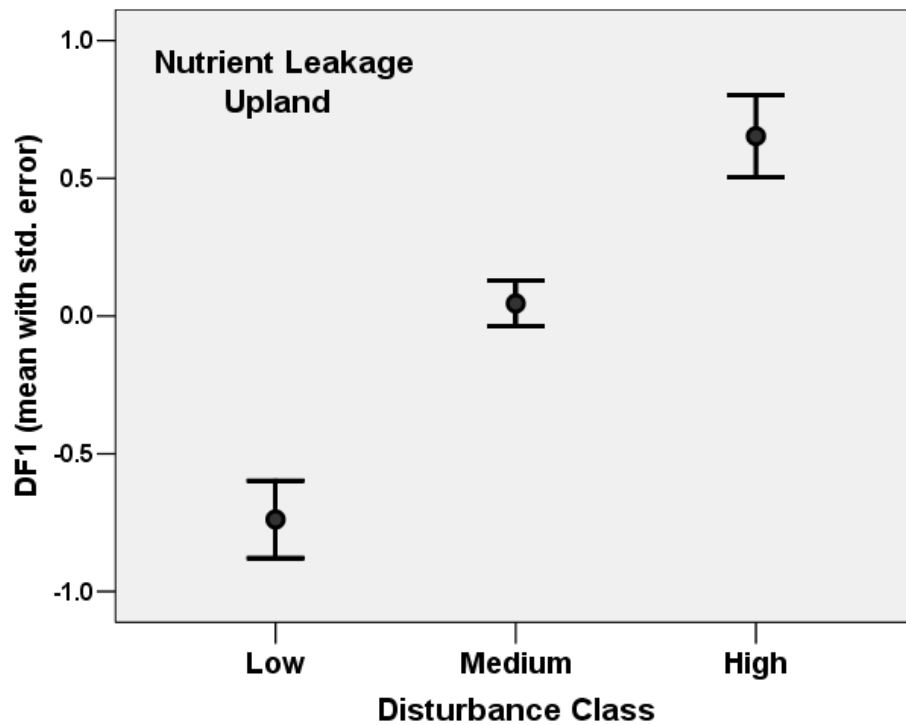


Figure 19. Nutrient Leakage Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek and Sally Branch (9 sites, 2000-2001-2002). Upland. Total sample size = 192.

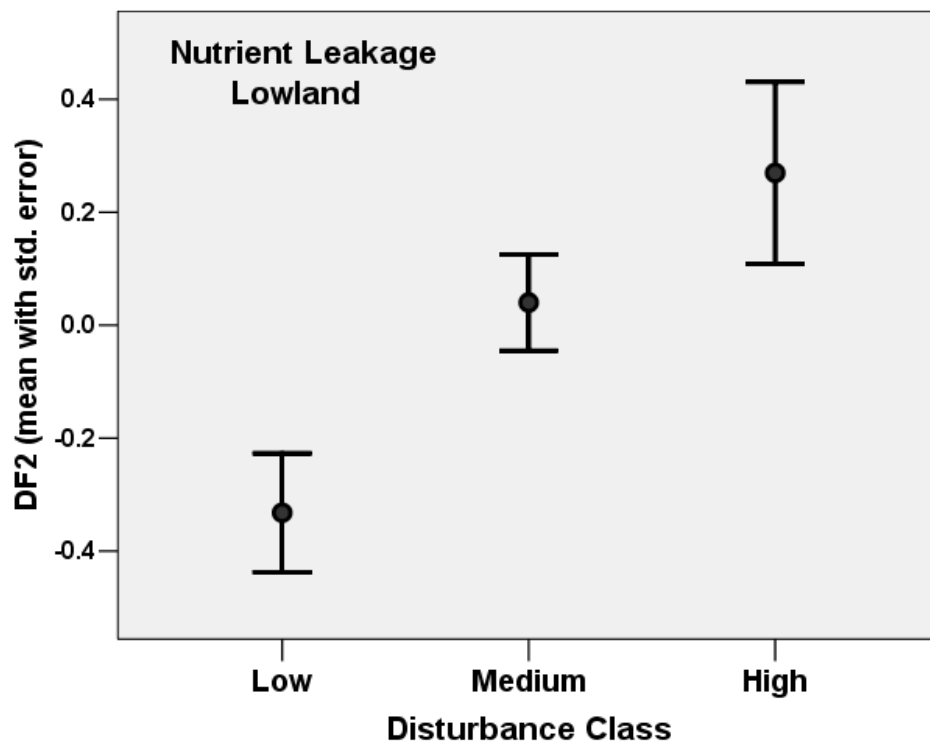
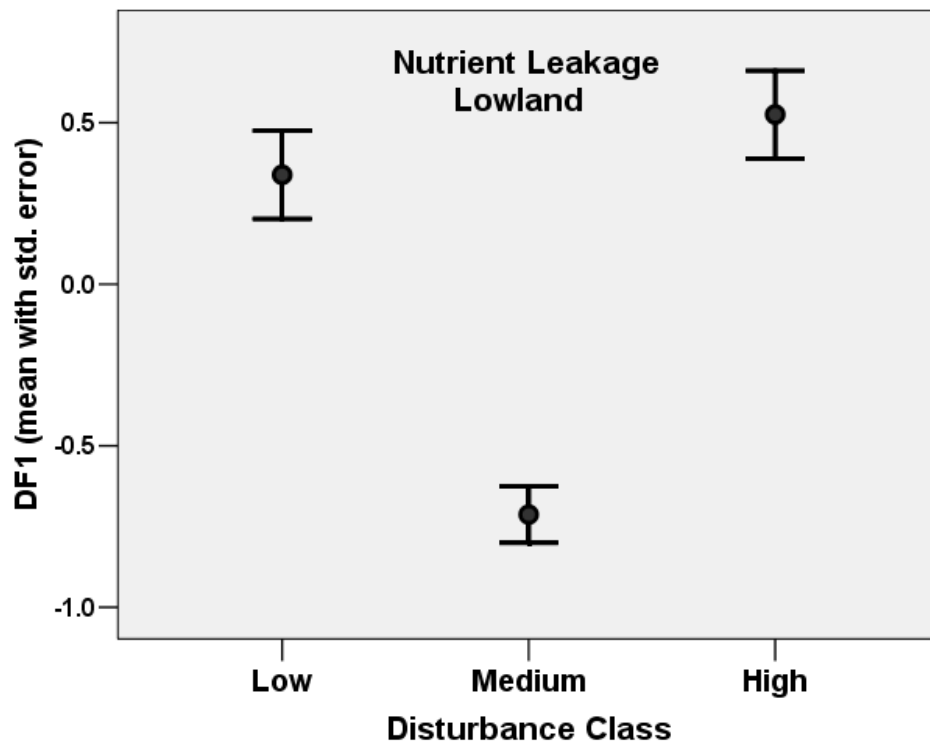


Figure 20. Nutrient Leakage Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek and Sally Branch (9 sites, 2000-2001-2002). Lowland. Total sample size = 214.

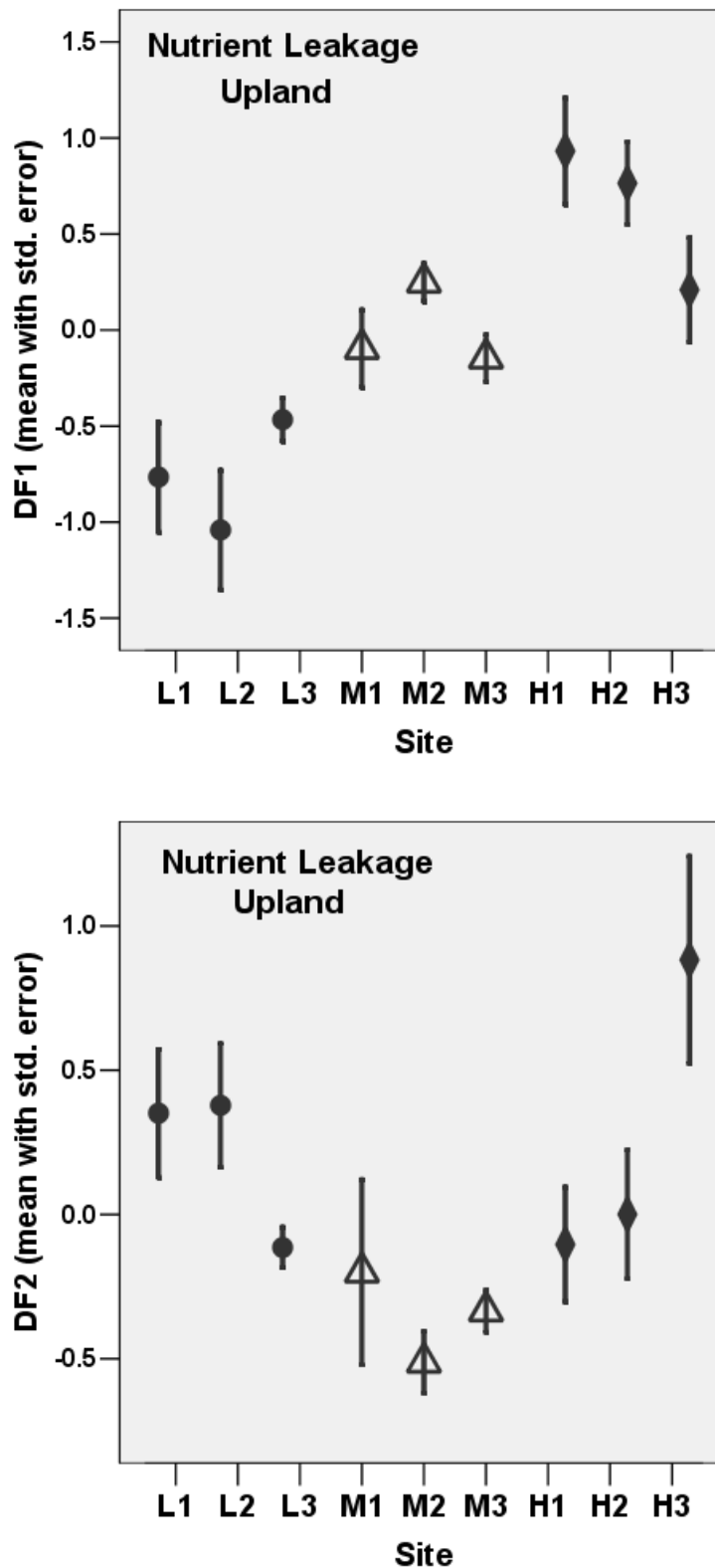


Figure 21. Nutrient Leakage Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below) shown for each site. Bonham Creek and Sally Branch (9 sites, 2000-2001-2002). Upland. Total sample size = 192.

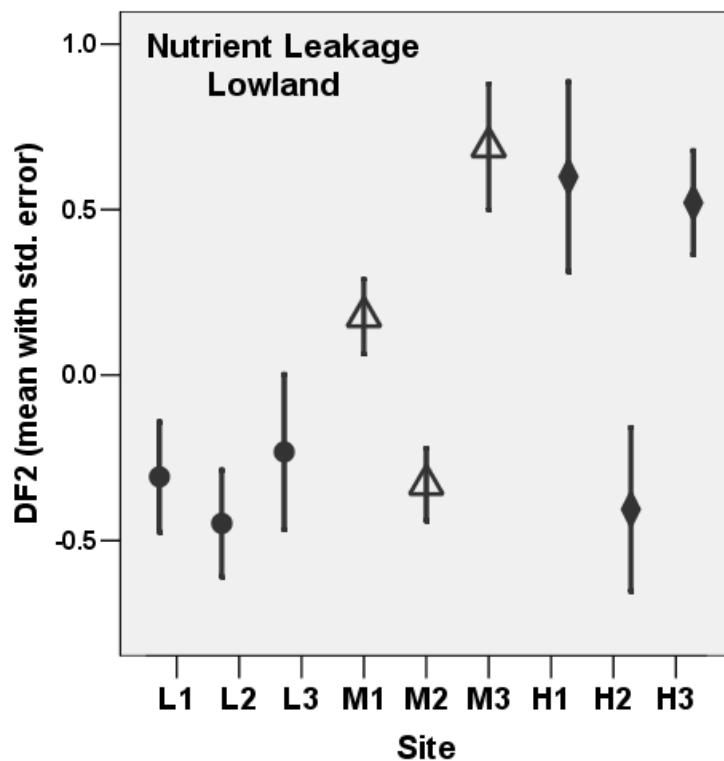
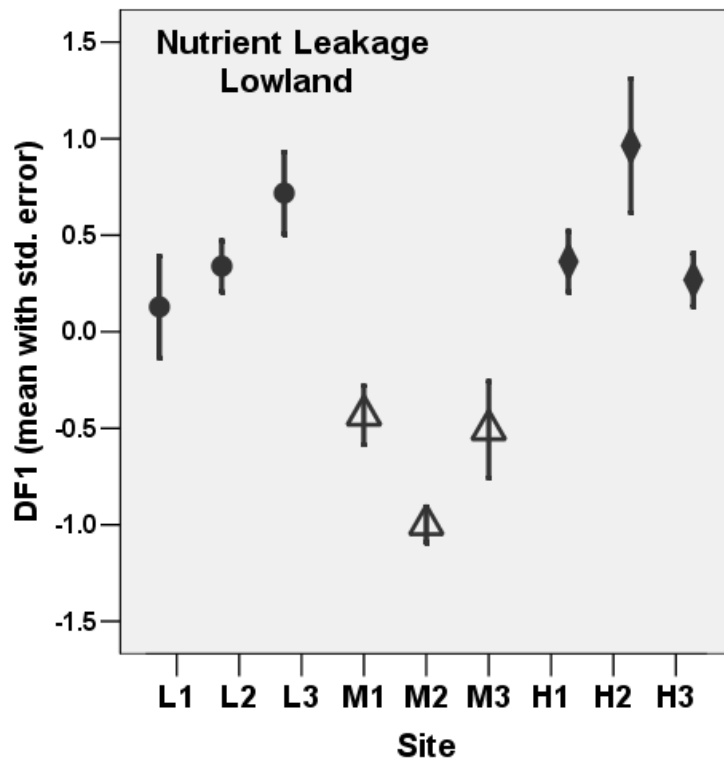


Figure 22. Nutrient Leakage Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below) shown for each site. Bonham Creek and Sally Branch (9 sites, 2000-2001-2002). Lowland. Total sample size = 214.

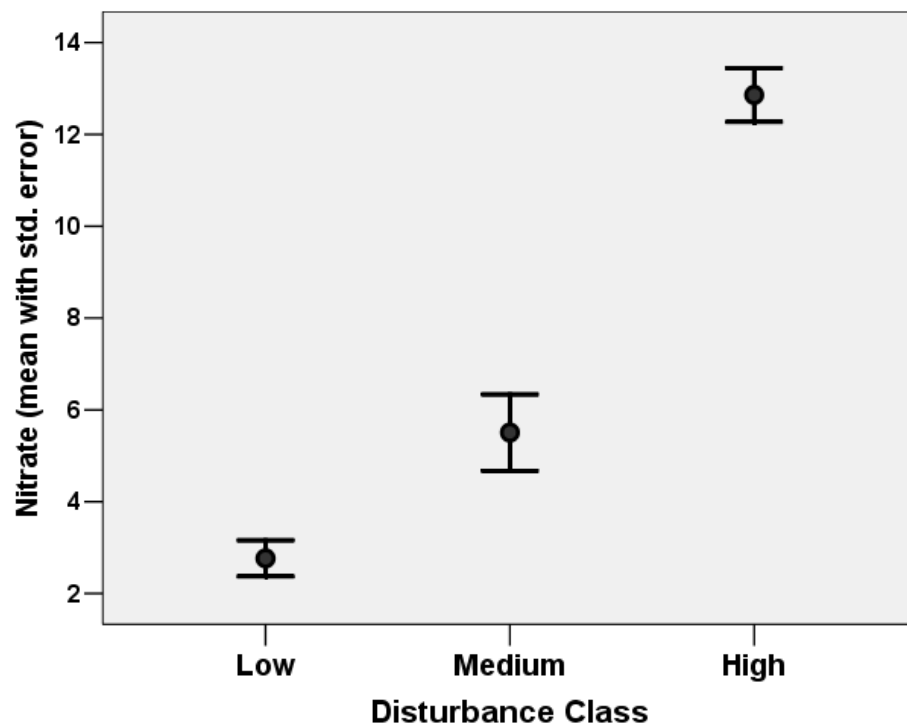
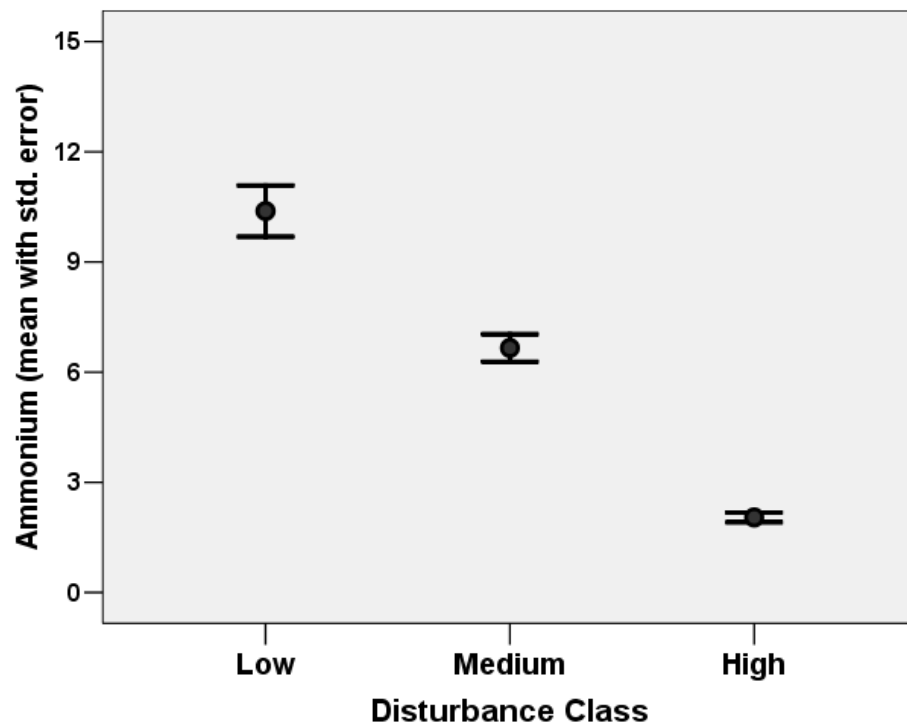


Figure 23. Soil Mineralization Potential experiment. Ammonium (NH_4^+) (above) and nitrate (NO_3^-) (below) soil concentrations after laboratory incubation for four days. Concentrations are N-mg/kg.

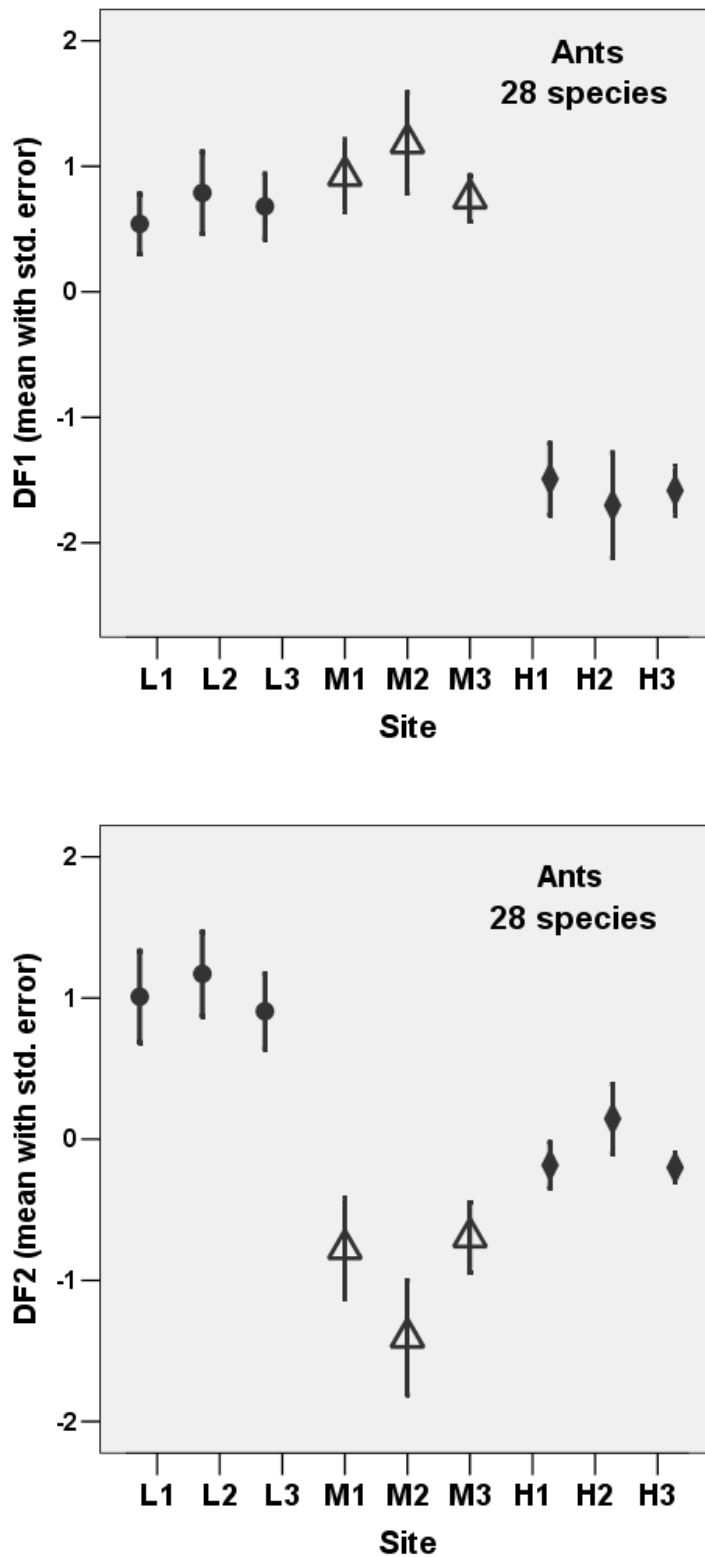


Figure 24. Ground/litter Ant community (28 species) Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek and Sally Branch (9 sites, 2000-2001-2002). Total number of ants = 103,203 and 87% of individuals are *Dorymyrmex smithi* (89,429).

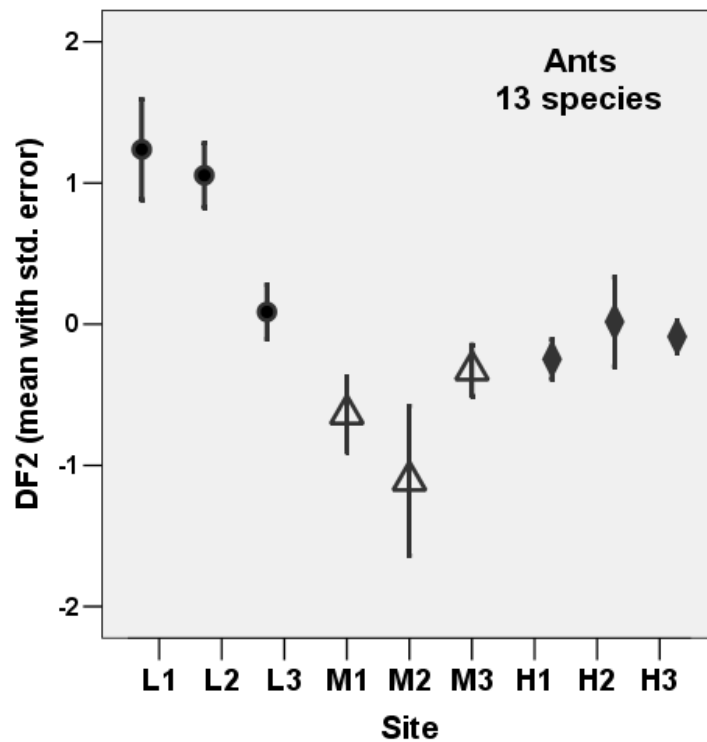
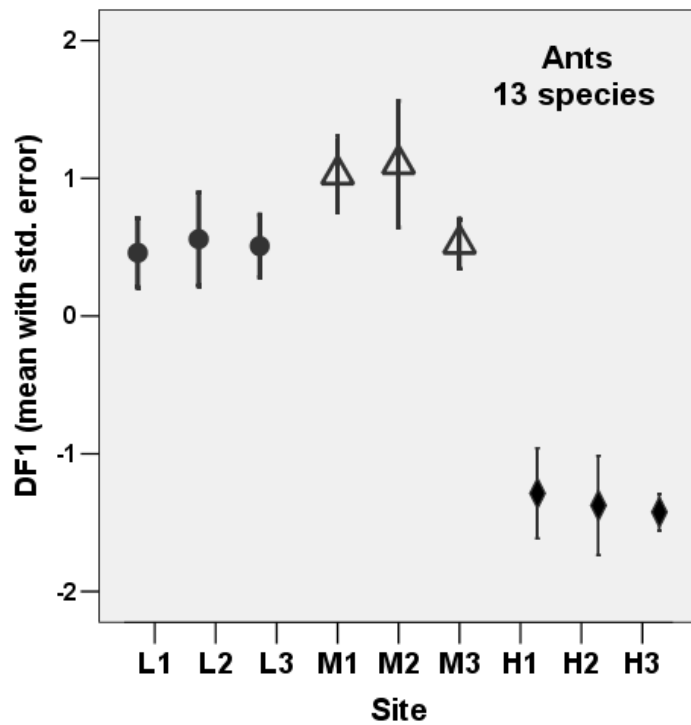


Figure 25. Ground/litter Ant community (13 species) Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek and Sally Branch (9 sites, 2000-2001-2002). Total number of ants = 92,739 and 96% of individuals are *Dorymyrmex smithi* (89,429).

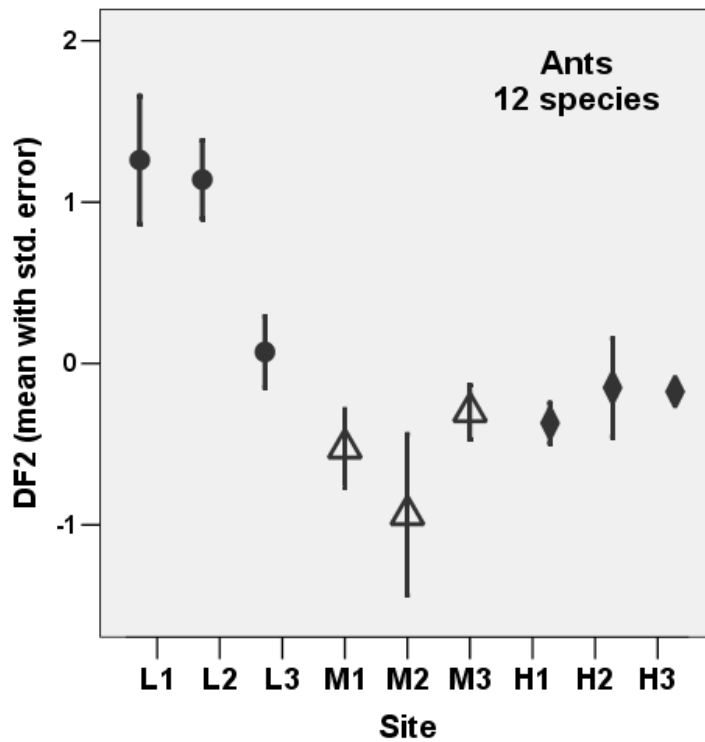
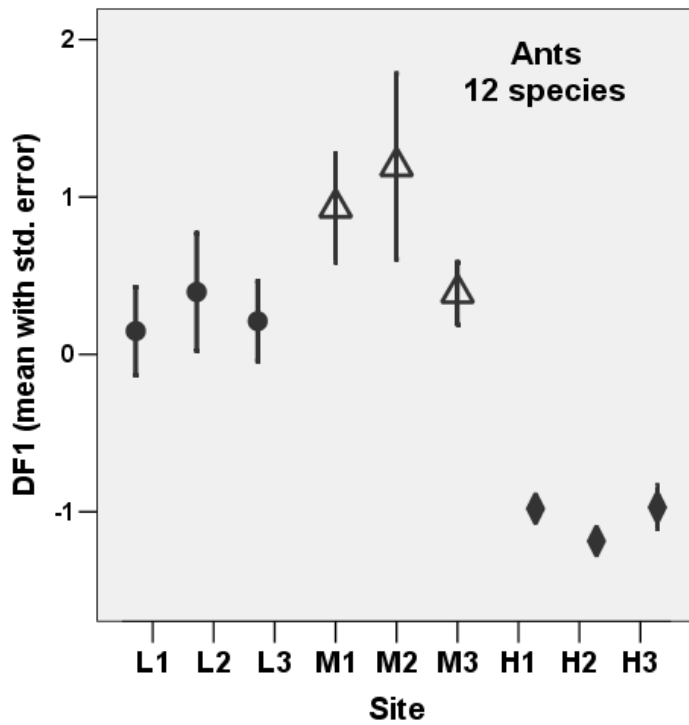


Figure 26. Ground/litter Ant community (12 species, *Dorymyrmex smithi* deleted)
Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2
(below). Bonham Creek and Sally Branch (9 sites, 2000-2001-2002).
Total number of ants = 3310, 3.2% of original 28 species.

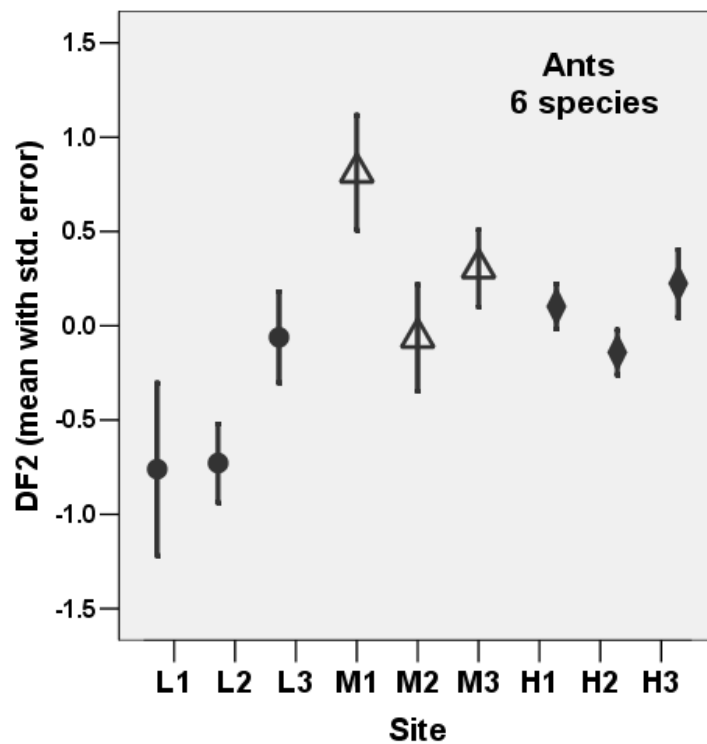
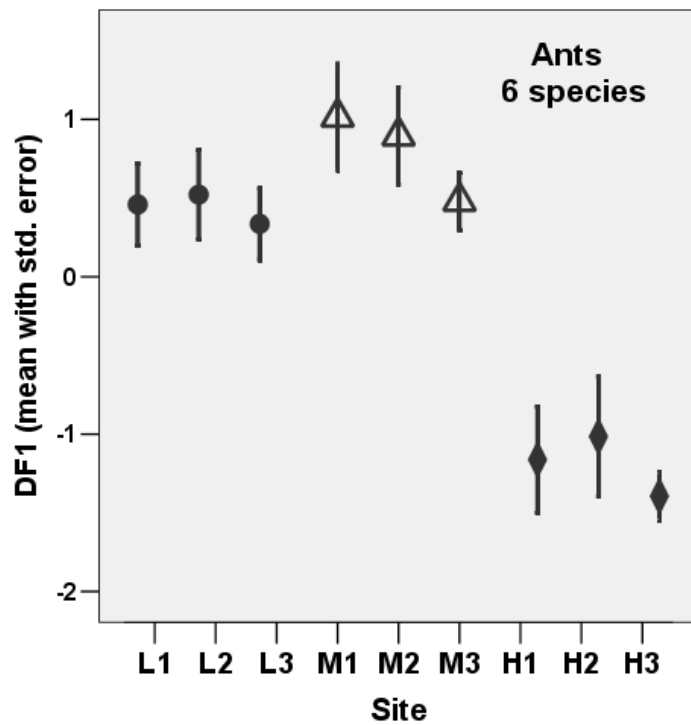


Figure 27. Ground/litter Ant community (6 species) Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2 (below). Bonham Creek and Sally Branch (9 sites, 2000-2001-2002). Total number of ants = 89,983 and 99% of individuals are *Dorymyrmex smithi* (89,429).

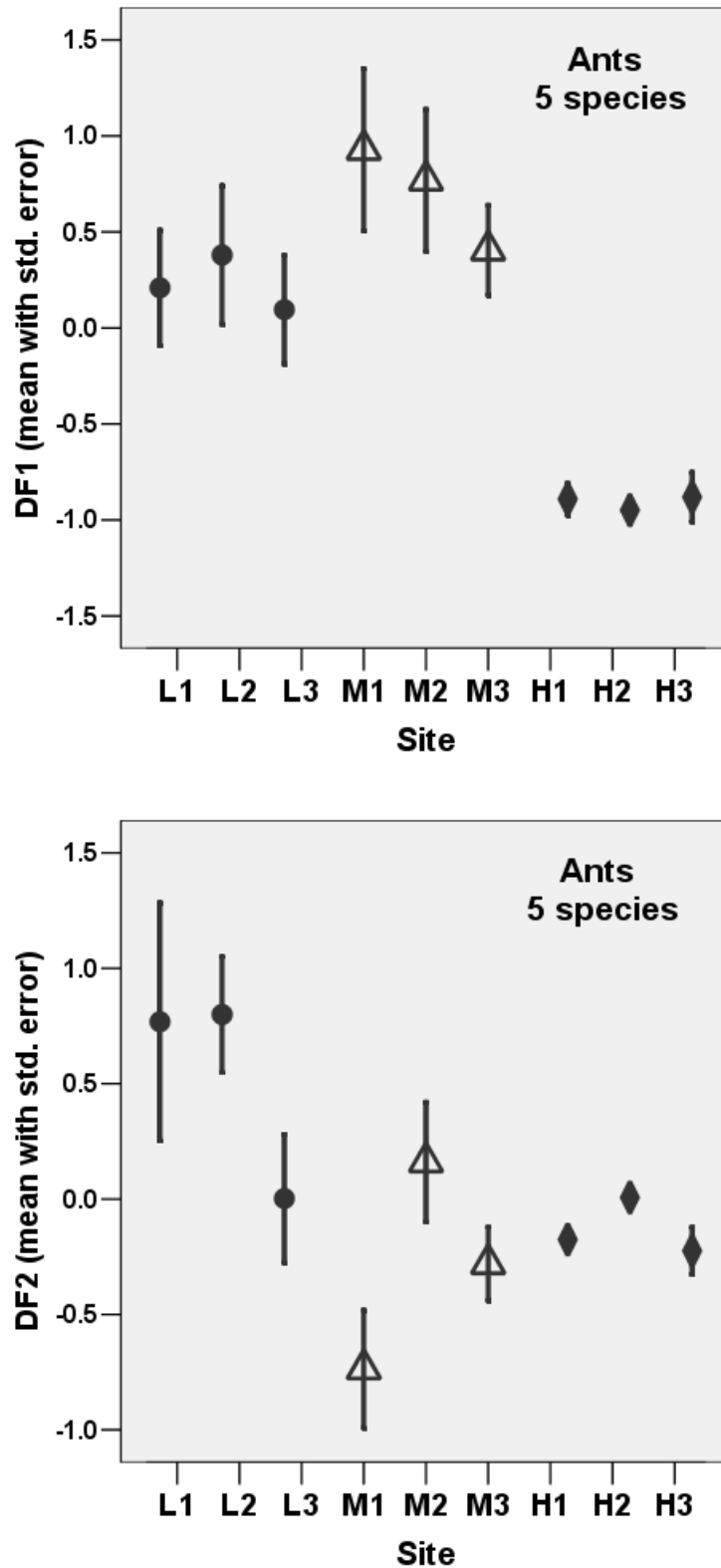


Figure 28. Ground/litter Ant community (5 species, *Dorymyrmex smithi* deleted)
Discriminant Analysis scores, discriminant function 1 (above) and discriminant function 2
(below). Bonham Creek and Sally Branch (9 sites, 2000-2001-2002).
Total number of ants = 554, 0.54% of original 28 species.

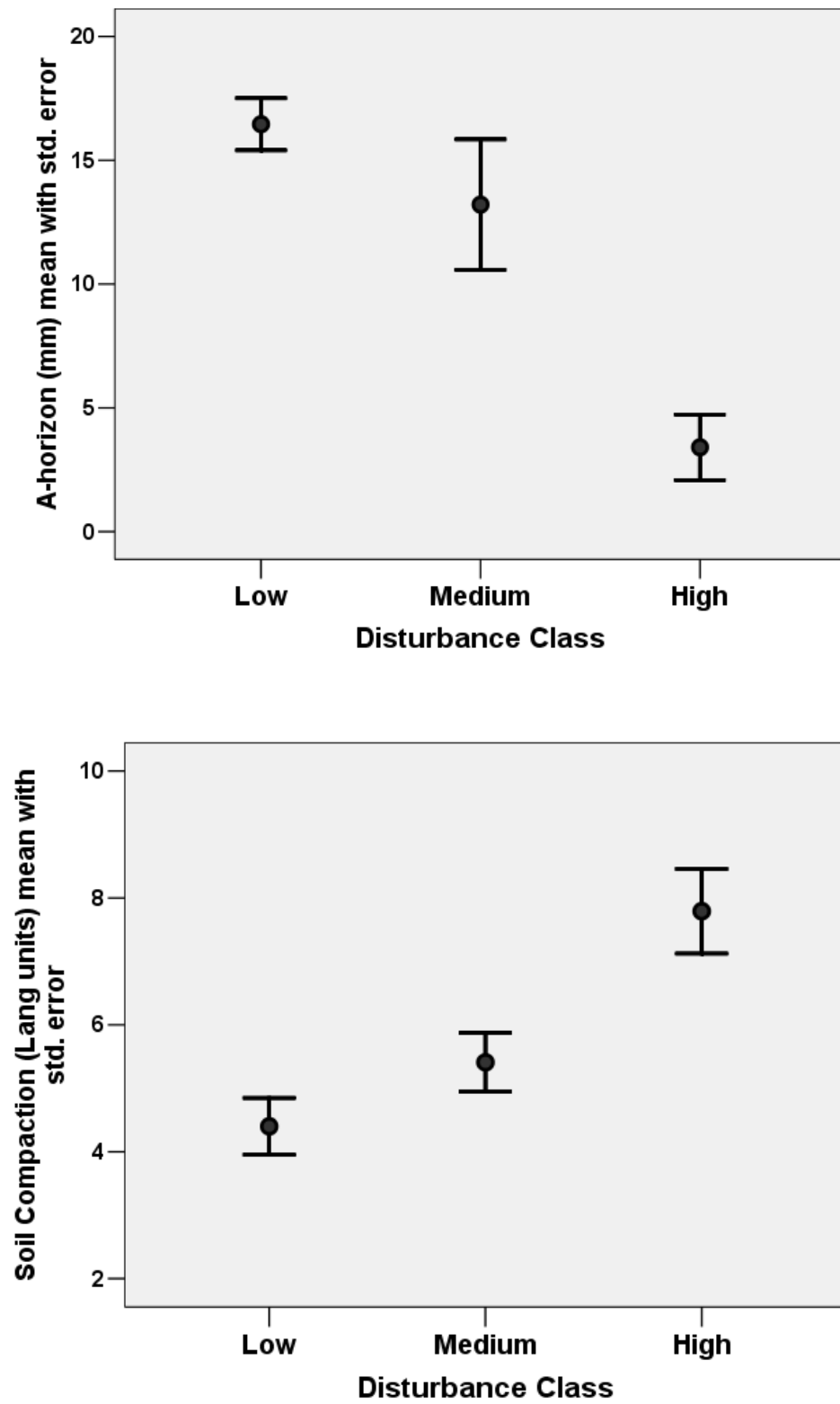


Figure 29. Soil A-horizon depth (above) and soil compaction (below) of the nine Phase I sites, shown as disturbance class means; Bonham Creek and Sally Branch, 2001-2002.

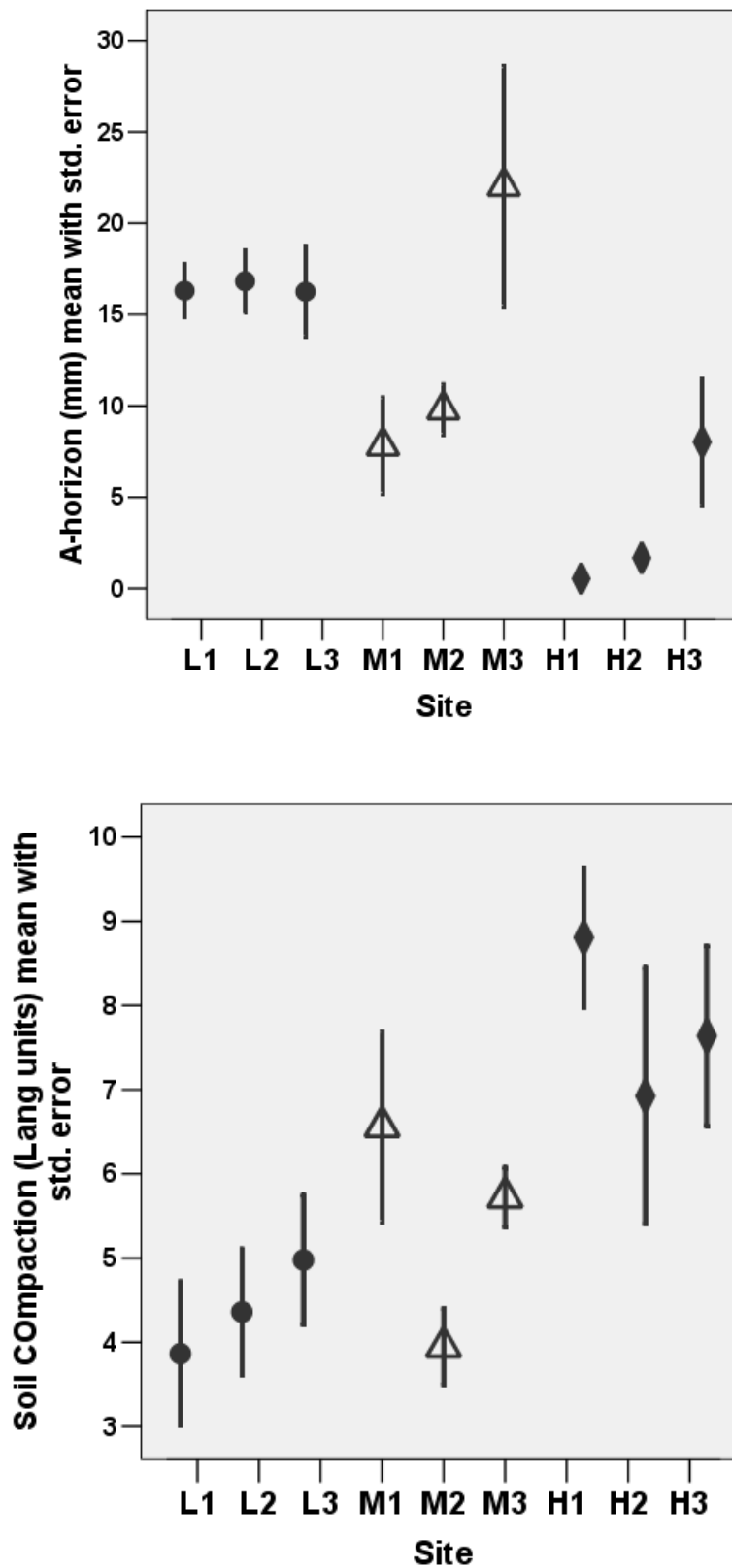


Figure 30. Soil A-horizon depth (above) and soil compaction (below) of the nine Phase I sites, shown as site means; Bonham Creek (numbers 1 & 2) and Sally Branch (number 3), 2001-2002.

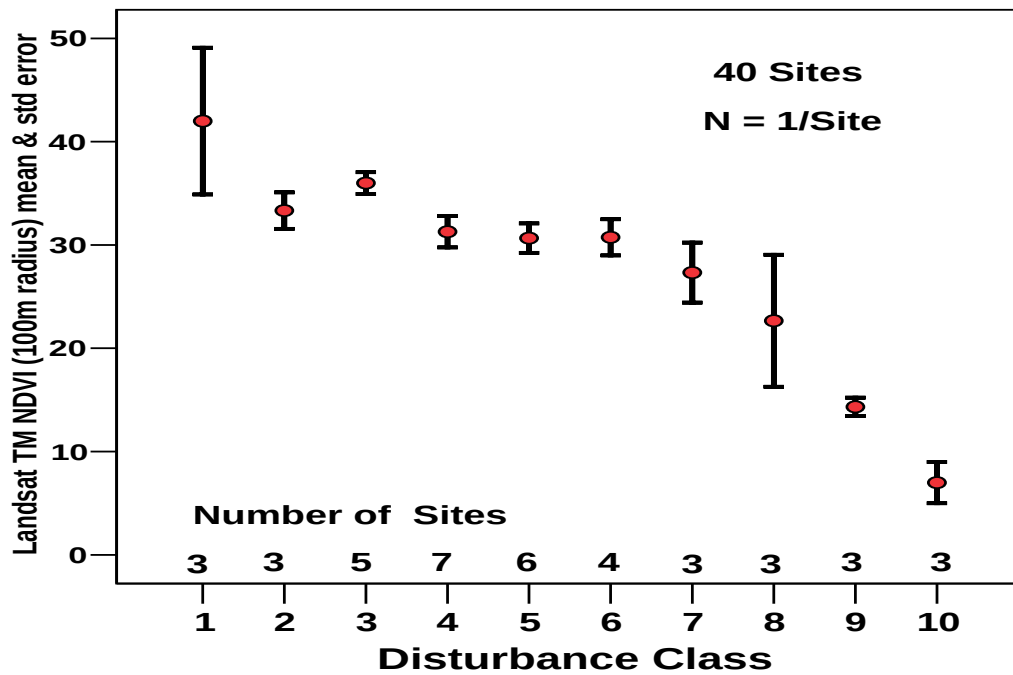


Figure 31. NDVI (mean and standard error) at the 40 sites based on disturbance class.

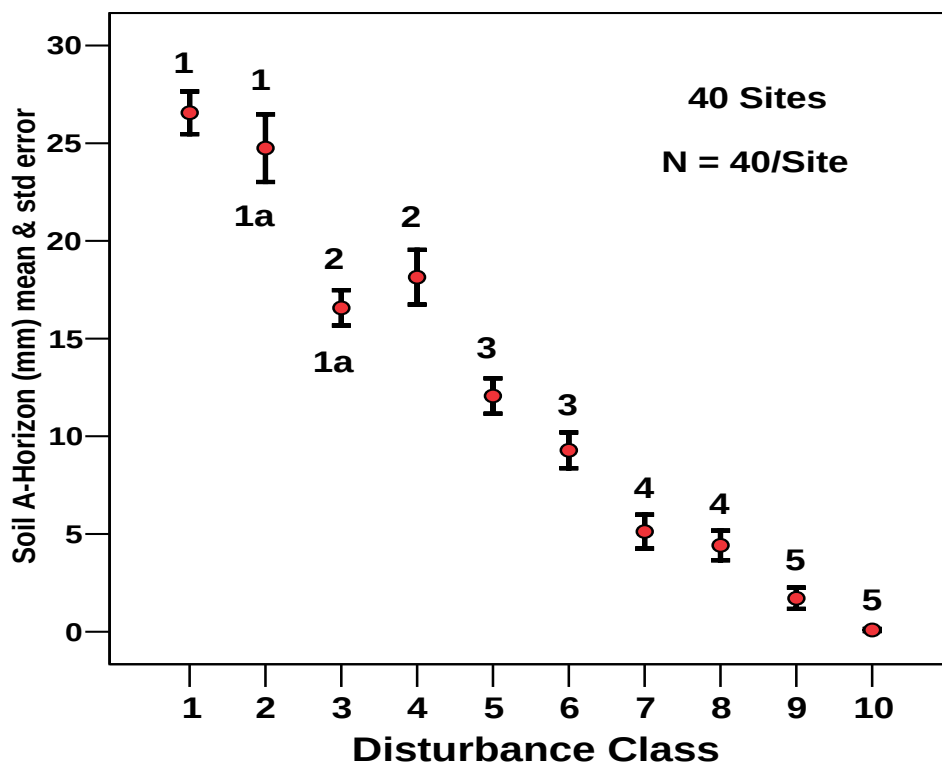


Figure 32. Soil A-horizon depth (mean & standard error) at the 40 sites based on disturbance class. See Figure 31 for number of sites in each disturbance class. Numbers represent statistically similar disturbance classes.

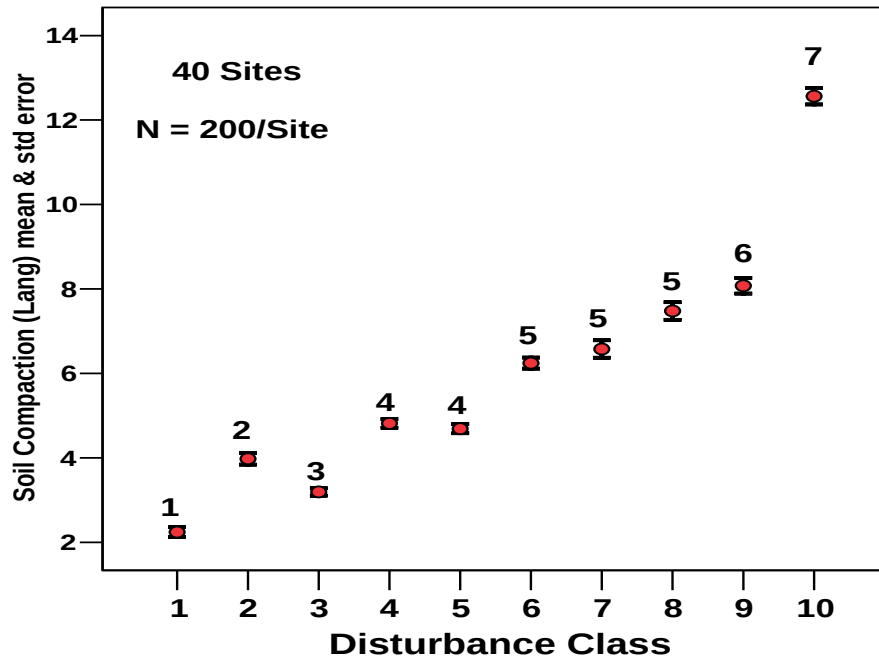


Figure 33. Soil compaction (mean and standard error) at the 40 sites based on disturbance class. See Figure 31 for number of sites in each disturbance class. Numbers represent statistically similar disturbance classes.

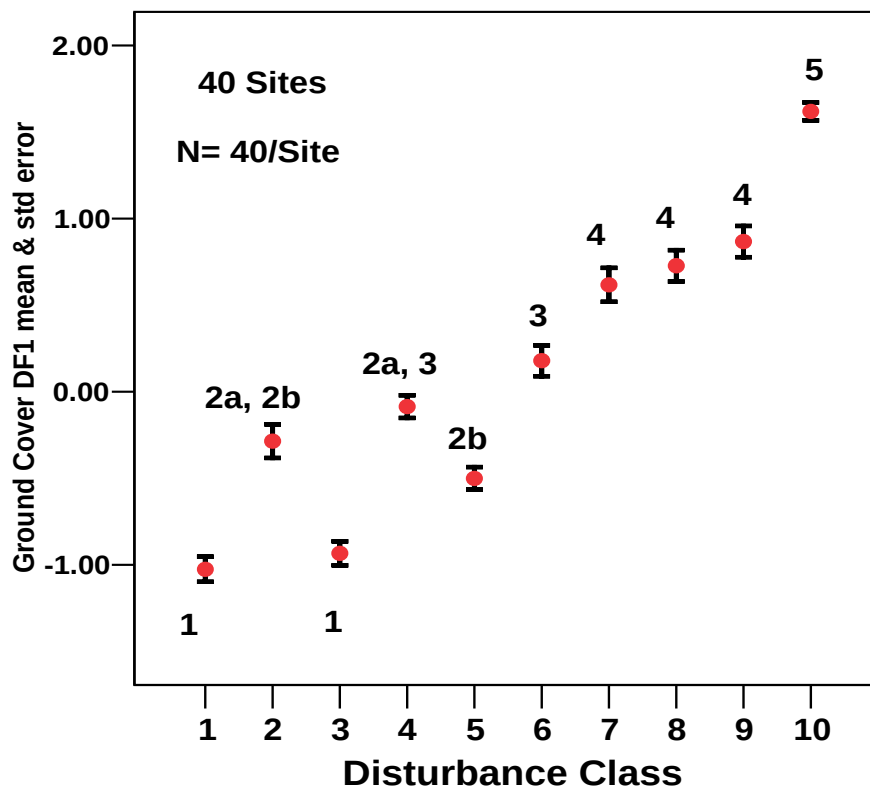


Figure 34. Ground cover DF1 scores (mean and standard error) at the 40 sites based on disturbance class. DF1 was primarily associated with Bare Ground. Numbers represent statistically similar disturbance classes.

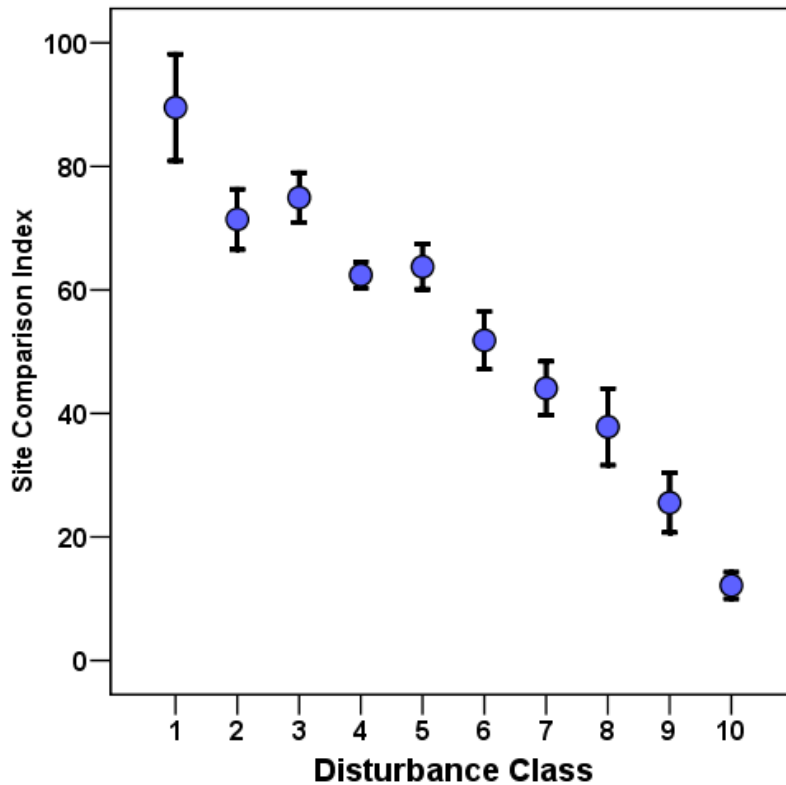


Figure 35. Site Comparison Index for the 10 disturbance classes. The standardized SCI was based on 7 Ecological Indicator metrics and NDVI (the metrics of Table 10 in bold and red), all weighed by statistical criteria. Note that the ordinate is a relative scale. See Figure 31 for number of sites in each disturbance class.

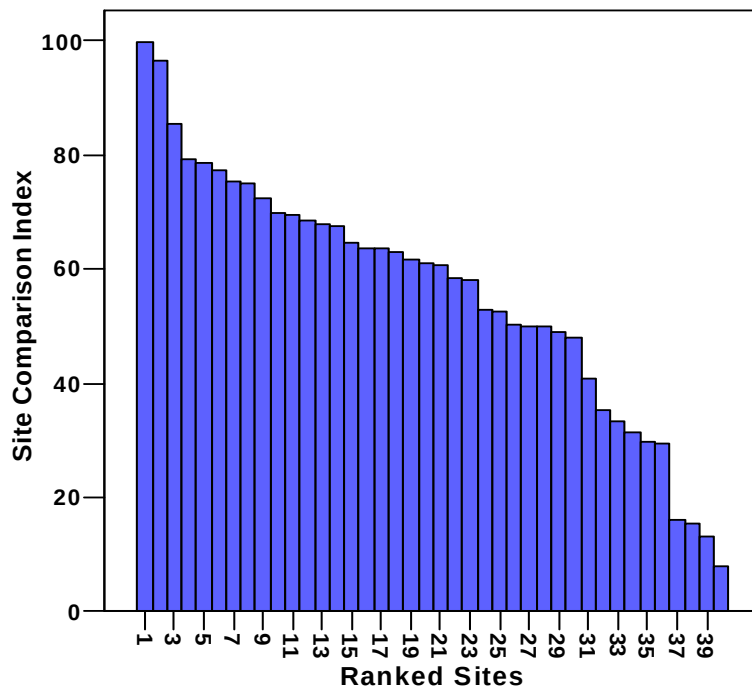


Figure 36. Site Comparison Index scores for the 40 sites ranked by their respective SCIs.



**Figure 37. Highest SCI rank (B2)
Mesic Southern Red Oak – Mixed Pine Forest
DC1, 71% deciduous**



**Figure 38. Second highest SCI rank (E5)
Mesic Oak – Hickory Forest
DC1, 97.5% deciduous**



**Figure 39. A xeric but pristine site (K13)
Scrub Oak – Longleaf Pine Savanna
DC1, but ranked 9th by SCI**



**Figure 40. Lowest SCI rank (D15-1)
Delta Training Area, Mixed Pine – Hardwoods
DC10**



**Figure 41. Relatively pristine
Longleaf Pine Forest (F4)
in rocky rolling hills with
simple vegetation structure.
DC2, ranked 19th by SCI.**

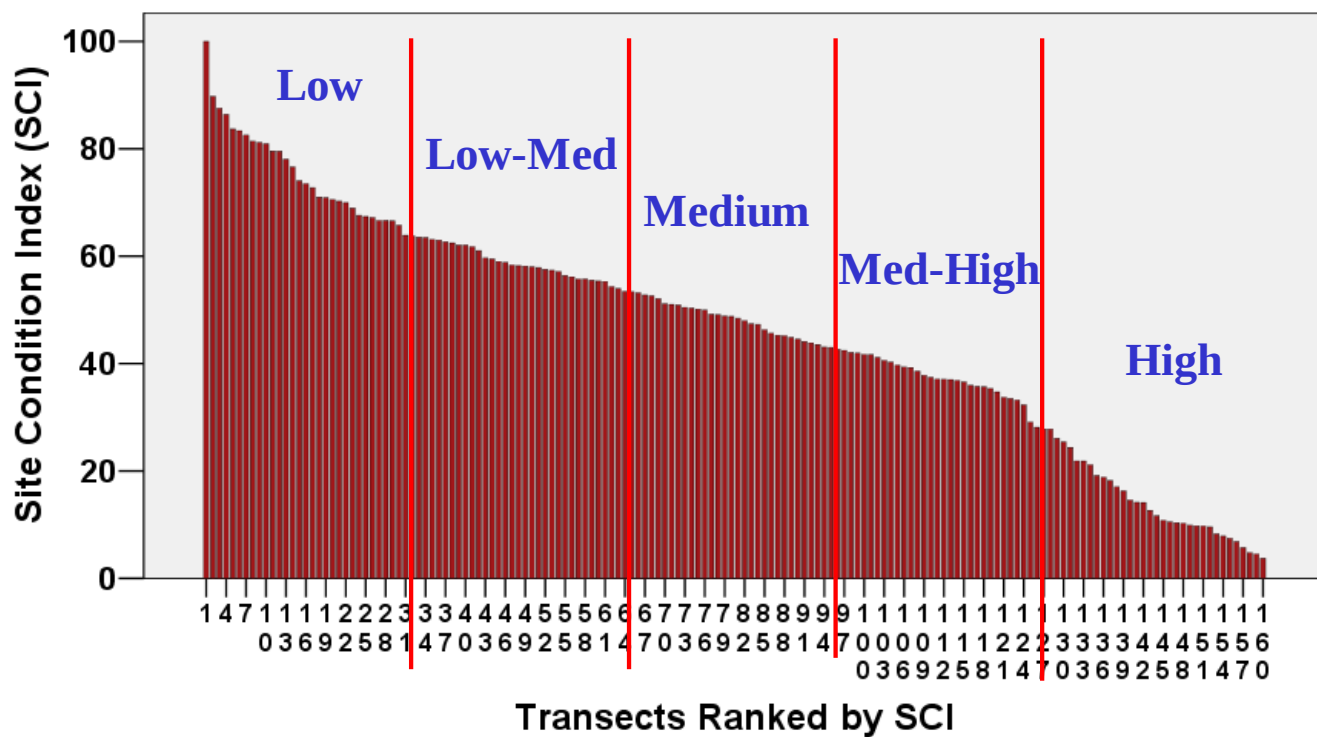


Figure 42. 160 Transects Ranked by the Site Condition Index. These 160 transects were reduced to FIVE new Disturbance Classes with 32 transects in each class.

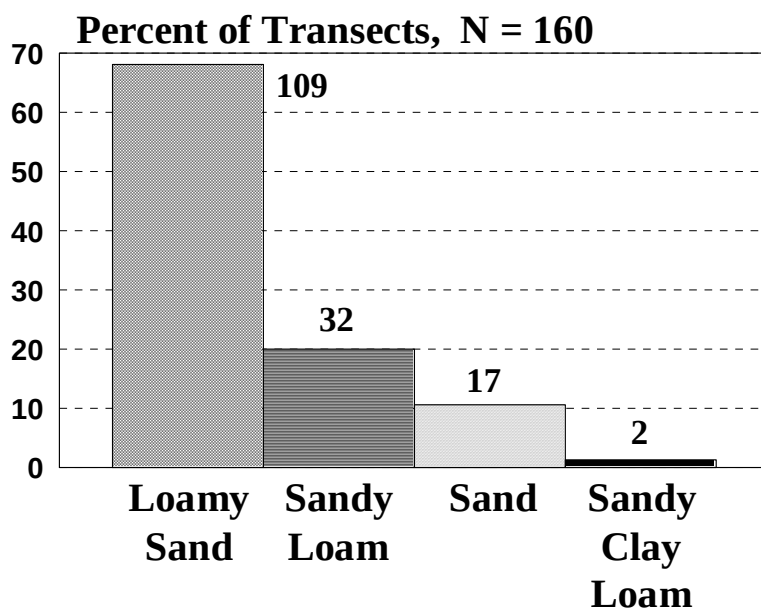
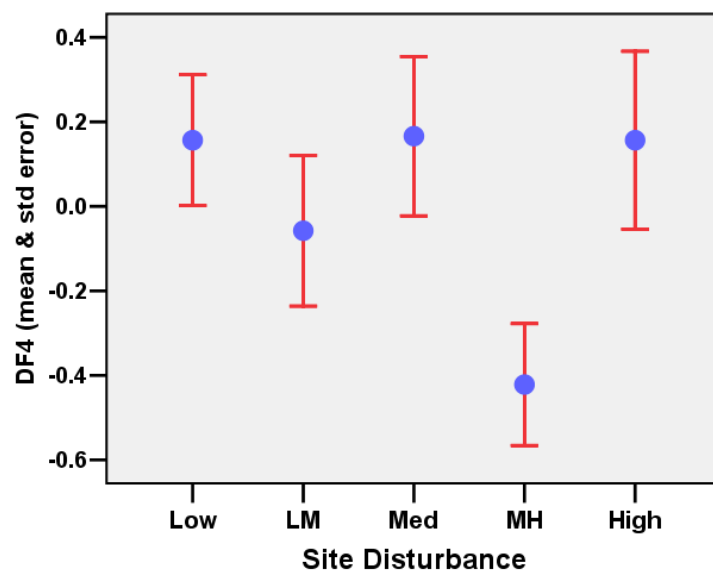
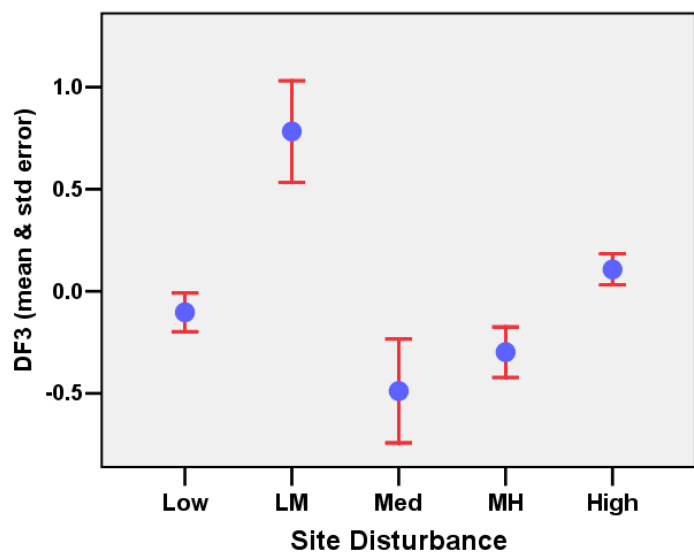
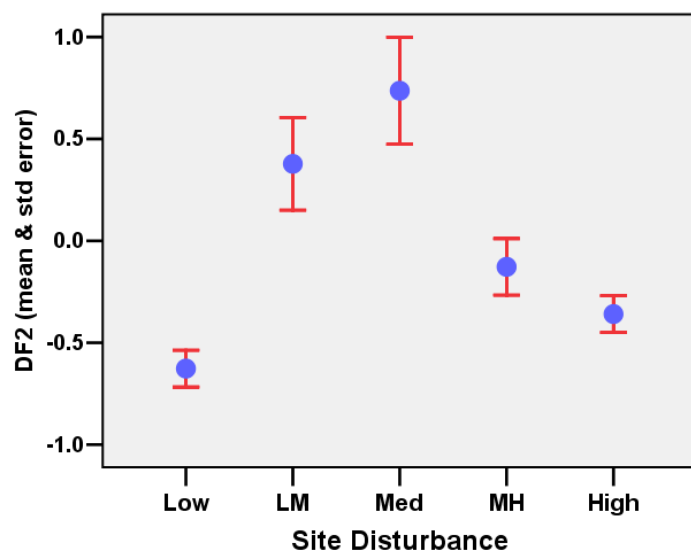
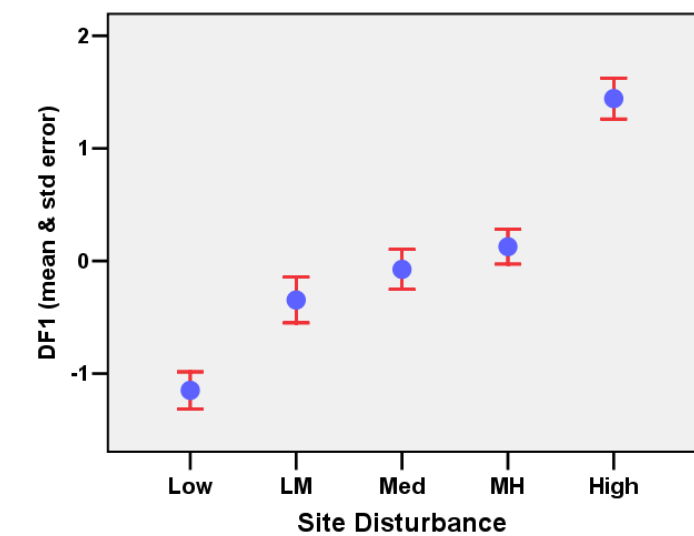
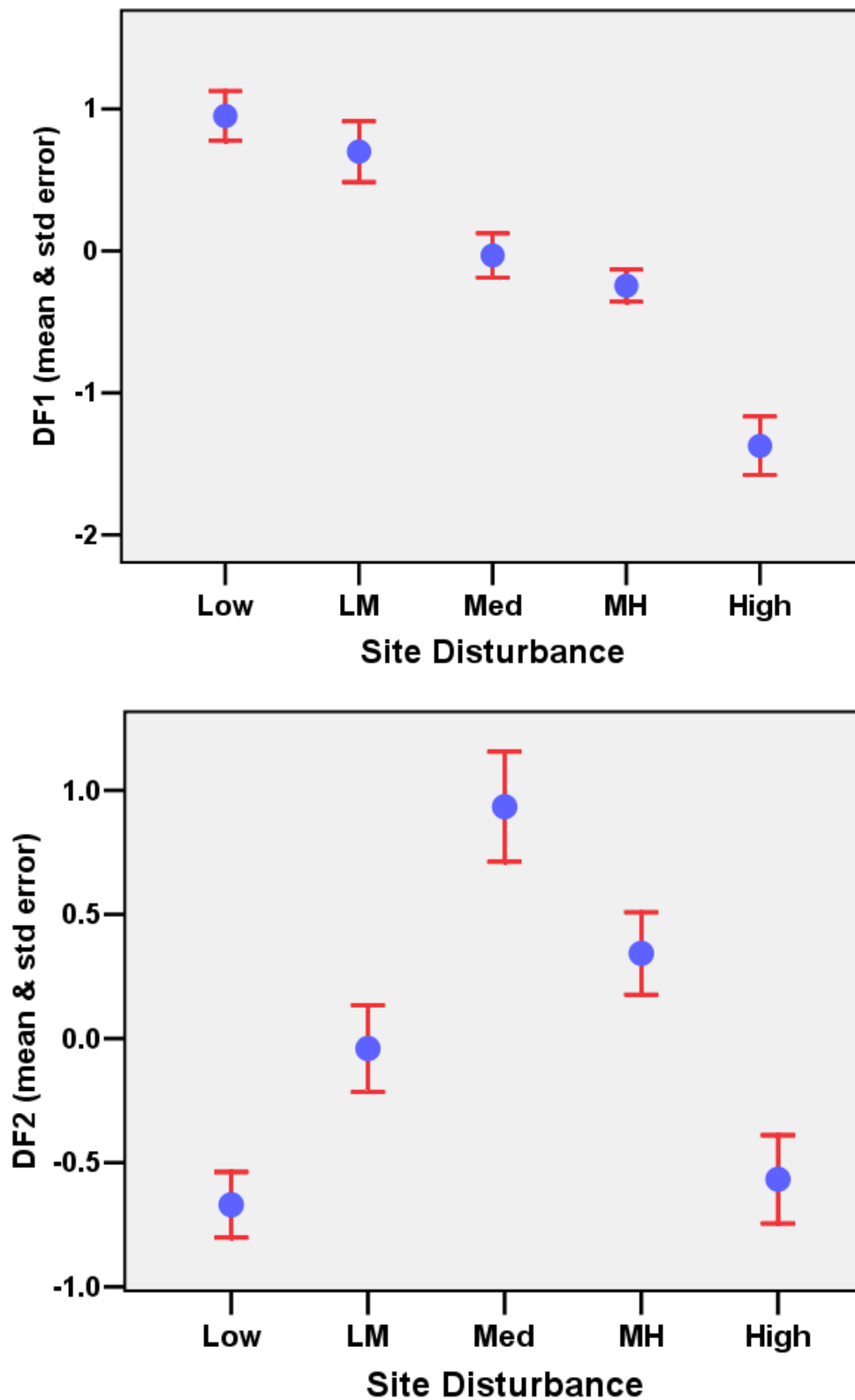


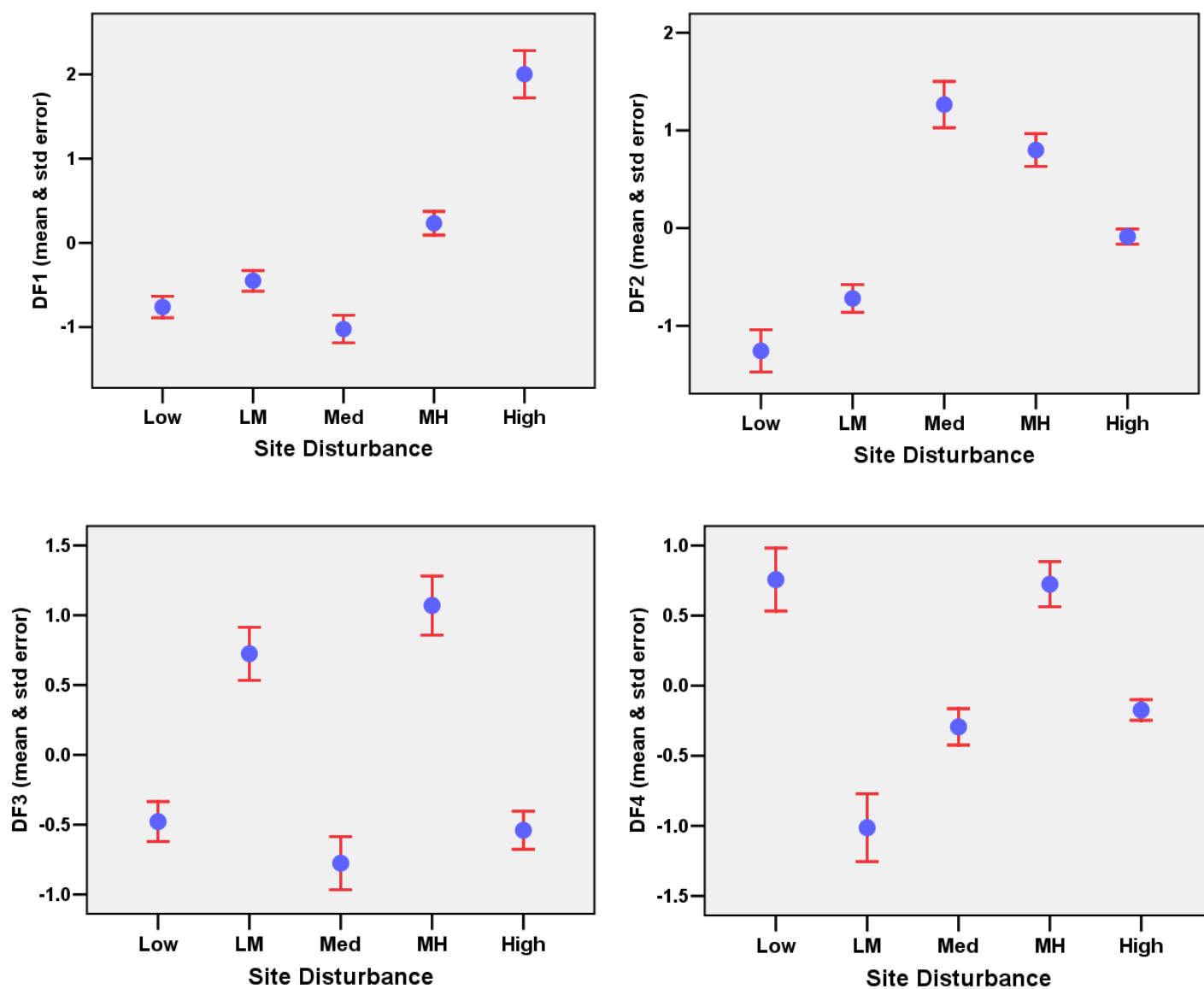
Figure 43. Soil texture classification for the 160 transects at the 40 Phase II research sites. The numerical values in the figure are number of transects.



Figures 44 and 45 (upper), 46 and 47 (lower). General Ground Cover Guild Discriminant Functions: DF1, DF2, DF3, DF4. The values are mean and standard error.



Figures 48 (upper) and 49 (lower). Floristics Ground Cover Guild Discriminant Functions DF1 and DF2. The values are mean and standard error.



Figures 50 and 51 (upper), 52 and 53 (lower). Ground/Litter Ant Community Guild Discriminant Functions DF1, DF2, DF3, DF4. The values are mean and standard error.

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Appendix A

Outlined Site Selection Criteria:

- 1) Upland Forests or potential Upland Forests (i.e., highly disturbed landscapes) ONLY
- 2) Reasonably large forest patches, where a 4-hectare study plot could be centrally located
- 3) Widest range of military training disturbance available

Sites selected that represented highly disturbed and “barren” military training areas:

D15-1, D15-3, D15-6, D6-1, J4;

SREL sites possessed low to moderate training disturbance;

Many sites selected in D15 and D16, because of:

- 1) extensive mechanized and foot traffic infantry training activities, and
- 2) a very broad range of landscape disturbance intensity could be found in these training compartments

4) The extensive use of available GIS data themes was the central focal point in our site selection process (see list below).

5) The use of existing SEMP research sites

SREL research sites possessed many desirable characteristics directly relevant to our sampling design:

Large spatial scales

Upland pine forests

Known and available environmental characteristics

sandy vs clayey soils

2 yr vs 4 yr fire frequencies

light vs moderate military training activities

Based on our GIS data themes, visual field assessments, and SREL collected data:

14 SREL sites were selected

6) Fort Benning identified “Unique Ecological Areas”

Eight upland forest areas were identified by Fort Benning as Unique Ecological Areas (see below). Two of these in the northeastern portion of the installation were Xeric Pine Savanna Sandhills, “Hastings Relict Sandhills” and “Longleaf Pine Sandhills”. We examined both of these, and although they appeared similar, Hastings Relict Sandhills vegetation and soils better represented a xeric Sandhills community. The “Longleaf Pine Sandhills” site was very small in area (K13-K8 boundary), while the Hastings UEA was extensive and extended over several training compartments. We considered these as “similar communities” and selected the “Hastings Relict Sandhills” (K13) as our UEA representing Xeric Sandhills. Longleaf Pine was the only pine species at our K13 site. Our site J6 was spatially very close to and adjacent to the

Appendix A (cont.)

“Lakeland Sandhills” (J7-D14) UEA. The vegetation and soils appeared very similar. The only apparent difference was that the J6 site was exposed to some military maneuvers, although only light impacts confined to several trails were evident. We considered J6 to be the equivalent of “Lakeland Sandhills”.

The UEA “Piedmont Interface” consists of several training compartments along the northern boundary of the installation, with a portion running south into O10. Although our sites O10 and M8 do not spatially fall into this UEA, they had vegetation that characterizes the Piedmont (e.g., Loblolly Pine was the only pine species present).

Three of our additional sites were selected in URAs: “Prosperity Church Oak-Hickory Forest” (E5), “Arkansas Oak Rock Hills” (F4), and “Longleaf Pine Loamhills” (A15). Seven of our selected sites represented Fort Benning UEAs (see below).

7) Limitations to site selection:

We continually consulted with Hugh Westbury (SEMP Site Coordinator) and Pete Swiderek (Wildlife Manager) on potential site access problems for GIS selected sites. We were informed that many potentially excellent research sites based on spatial size and forest type, including mature forest stands, were either “off-limits” as research sites, or would have difficult, inconsistent, or unreliable access, because of live-fire, unexploded ordnance, or safety fan restrictions. These sites were dropped for additional consideration.

8) Despite the continuous cooperation of Range Control, and the excellent scheduling efforts of Hugh Westbury, under current U.S. Army scheduling policy, it was physically impossible to schedule and investigate more than 40 sites. On the basis of experience at our former 9 sites, the range of upland forest types and their condition available at Fort Benning, and the range of environmental heterogeneity that we required for indicator validation, 40 sites were considered optimal.

9) The final site selection process was our continual iteration of GIS Themes (Number 4) with ground truthing and how it related to Numbers 1, 2, 3, 5, 6, 7, under the practical constraints of Number 8.

10) Soil A-Horizon Depth was identified in Phase I as an important Guild component to characterize site condition or habitat disturbance. This parameter is closely related to important ecological processes such as nutrient and microbial dynamics, critical environmental parameters such as soil compaction and soil carbon, and the relative condition of a habitat patch. In order to successfully evaluate the potential of selected Ecological Indicator Guilds it was a priori considered important and very desirable that this parameter exhibited a continuous distribution among the 40 selected heterogeneous Phase II sites. Figure 42 verifies *a posteriori* the validity of our site selection process with respect to validating our extracted Ecological Indicator Guilds.

Appendix A (cont.)

Each of the 40 research sites was represented by 40 A-Horizon Depth measures taken in a systematic-random design on 4-hectare plots. Note that the three lowest ranked sites had a complete lack of A-Horizon layer. The two sites with the deepest A-Horizons were relatively

different from the rest of the sites. However, note that the other 38 sites exhibited a uniform continuous distribution. This is strong evidence that we selected sites with a broad and continuous range of important environmental characteristics.

GIS files used for research site selection

File Names: forest_inventory and forstand

Polygon feature used to determine forest type and forest stand age during site selection.

Potential upland forest sites were initially categorized by stand age. Then all other GIS themes (digital orthophotos, road access, training compartment, etc...) were investigated to judge site suitability.

File Name: Ft._Benning.sid

Compilation of digital orthophoto quadrangles covering Fort Benning, Georgia.

Used to approximate site disturbance, forest structure, and canopy cover.

File Name: roads2

Line feature that depicts transportation network within Fort Benning.

Data was sorted by ROAD_TYP (path, unpaved_rd, paved_rd, 2_ln_hwy), and labeled by name to facilitate entry into a selected site.

File Name: Training_comps

Polygon file used to map military training compartments.

Feature was labeled to ensure correct determination of military training compartments and to aid SEMP Host Site Coordinator in gaining access for researchers.

File Name: ecmiunit

Polygon feature used to depict watersheds within Fort Benning.

Aided in site selection by ensuring an addition of sites in watersheds previously not sampled.

File Name: river

Line feature created to depict rivers within Fort Benning.

This feature was used in conjunction DEM to eliminate floodplain forests as potential research sites.

File Name: landformtopdem

A generated Digital Elevation Model (DEM) depicting the topographic landscape within Fort Benning. Aided in site selection by eliminating possible floodplain forests.

Appendix A (cont.)

File Name: semp_siteinfo_shp

Point feature depicting location of all SEMP research sites at Fort Benning as of February, 2002.

Upland Forest Unique Ecological Areas	Research Sites
Hastings Relict Sandhills	K13
Longleaf Pine Sandhills	Comparable to K13
Lakeland Sandhills	Comparable to J6
Piedmont Interface	Comparable to O10 and M8
Prosperity Church Oak-Hickory Forest	E5
Arkansas Oak Rock Hills	F4
Longleaf Pine Loamhills	A15
Slopes of Northern Affinities	None

The relationship among Fort Benning's Unique Ecological Areas (UEAs) and sites selected to represent them.

Research Team Publications Plan for SERDP-TAC

Research Project CS-1114B

Development of Ecological Indicator Guilds for Land Management

Anthony J. Krzysik, Prescott College, Prescott, AZ

15 December 2005

Ecological Society of America Presentations: 10 presentations

2002, Tucson, AZ: Graham, J.H., Krzysik, A.J.

2003, Savannah, GA: Graham, J.H., Kovacic, D.A., Krzysik, A.J.

2004, Portland, OR: Krzysik, A.J.

2005, Montreal, Canada: Graham, J.H., Kovacic, D.A., Krzysik, A.J. (2)

Published

Estimating disturbance effects from military training using developmental instability and physiological measures of plant stress.

Duda, J.J., D.C. Freeman, M.L. Brown, J.H. Graham, A.J. Krzysik, J.M. Emlen, J.C. Zak, and D.A. Kovacic. 2003.

Ecological Indicators 3:251-262.

The soil FungiLog procedure: method and analytical approaches towards understanding fungal functional diversity.

Sobek, E.A., and J.C. Zak. 2003.

Mycologia 95:590-602.

Habitat disturbance and the diversity and abundance of ants (Formicidae) in the Southeastern Fall-Line Sandhills.

Graham, J.H., H.H. Hughie, S. Jones, K. Wrinn, A.J. Krzysik, J.J. Duda, D.C. Freeman, J.M. Emlen, J.C. Zak, D.A. Kovacic, C. Chamberlin-Graham, and H.E. Balbach. 2004.

Journal of Insect Science 4:30, 15pp. Available online: insectscience.org/4.30.

Developmental instability in *Rhus copallinum* L.: Multiple stressors, years, and responses.

Freeman, D.C., M.L. Brown, J.J. Duda, J.H. Graham, J.M. Emlen, A.J. Krzysik, H.E. Balbach, D.A. Kovacic, and J.C. Zak. 2004.

International Journal of Plant Sciences 165:53-63.

Photosynthesis and fluctuating asymmetry as indicators of plant response to soil disturbance in the Fall-Line Sandhills of Georgia: A case study using *Rhus copallinum* and *Ipomoea pandurata*.

Freeman, D.C., M.L. Brown, J.J. Duda, J.H. Graham, J.M. Emlen, A.J. Krzysik, H.E. Balbach, D.A. Kovacic, and J.C. Zak. 2004.

International Journal of Plant Sciences 165:805-816.

Leaf fluctuating asymmetry, soil disturbance and plant stress: a multiple year comparison using two herbs, *Ipomoea pandurata* and *Cnidoscolus stimulosus*.

Freeman, D.C., M.L. Brown, J.J. Duda, J.H. Graham, J.M. Emlen, A.J. Krzysik, H.E. Balbach, D.A. Kovacic, and J.C. Zak. 2005.
Ecological Indicators 5:85-95.

Technical Reports

Development of a Site Condition Index: Southeast Upland Forests.
Krzysik, A.J., and H.E. Balbach.
Draft Technical Report, 2 September 2004, 24pp.

Development of Ecological Indicator Guilds for Land Management.
Krzysik, A.J., H.E. Balbach, J.J. Duda, J.M. Emlen, D.C. Freeman, J.H. Graham, D.A. Kovacic, L.M. Smith*, and J.C. Zak. *deceased
Final SERDP Technical Report, 25 December 2005, 118pp.

Manuscripts Submitted or to be Submitted within the Month

A multiple year study of the influence of disturbance and prescribed fires on the growth and development instability of loblolly pine (*Pinus taeda*) in the Fall-Line Sandhills of Georgia.
Freeman, D.C. lead author
Oecologia
Submitted April 2005
Status Unknown

Intermediate disturbance and ant communities in a forested ecosystem.
Graham, J.H., A.J. Krzysik, D.A. Kovacic, J.J. Duda, D.C. Freeman, J.M. Emlen, J.C. Zak, W.R. Long, M.P. Wallace, C. Chamberlin-Graham, J. Nutter, and H.E. Balbach.
Diversity and Distributions
To be submitted by 30 December 2005

Nutrient leakage as an ecological indicator of landscape disturbance.
Kovacic, D.A. lead author
Ecological Indicators
To be submitted by 30 December 2005

Soil mineralization potential as an ecological indicator of forest disturbance.
Kovacic, D.A., A.J. Krzysik, M.P. Wallace, J.C. Zak, D.C. Freeman, J.H. Graham, H.E. Balbach, J.J. Duda, and J.M. Emlen
Environmental Management
To be submitted by 31 January 2006

Development of ecological indicator guilds for land management: I. Research design and analysis.
Krzysik, A.J. lead author
Ecological Indicators
To be submitted by 31 January 2006

Development of ecological indicator guilds for land management: II. Identifying ecological indicator metrics of landscape disturbance.

Krzysik, A.J. lead author

Ecological Indicators

To be submitted by 31 January 2006

Manuscripts Currently in Preparation (by lead author)

Tanks, training and the developmental instability of finger-rot (*Cnidoscolus stimulosus*).

Freeman, D.C. lead author

To be submitted to Oecologia

Status Unknown

Community and population responses of Orthoptera and Blattaria to habitat disturbance.

Graham, J.H., A.J. Krzysik, J. Dolbeer, W.R. Long, J.J. Duda, D.C. Freeman, J.M. Emlen, J.C. Zak, D.A. Kovacic, C.C. Graham, and H.E. Balbach.

Ecological Indicators

To be submitted by 28 February 2006

The “disturbance-availability” hypothesis.

Kovacic, D.A. lead author

Science or Nature

Status Unknown

Soil A-horizon depth as a reliable, integrative, and ecosystem relevant ecological indicator of landscape disturbance.

Krzysik, A.J. lead author

A SEMP-team manuscript with Univ. of Georgia / Savannah River Ecology Laboratory and Univ. of Florida

Environmental Management

To be submitted by 15 February 2006

Assessing the accuracy of estimating basal area in diverse forest stands in the Upper Coastal Plain of the Southeast.

Krzysik, A.J. lead author

Journal of Forestry

To be submitted by 28 February 2006

Development of ecological indicator guilds for land management: III. Validation and multivariate integration of ecological indicators in a complex regional ecotone.

Krzysik, A.J. lead author

Ecological Indicators

To be submitted by 31 March 2006

Ground cover as an ecological indicator for landscape disturbance: The relative importance of physiognomy, floristics, taxa hierarchies, and life form.

Krzysik, A.J. lead author

Ecological Applications

To be submitted by 17 April 2006

A site comparison index for the Southeast Fall-Line Sandhills.

Krzysik, A.J. lead author

Forest Ecology and Management

To be submitted by 1 May 2006

Landscape disturbance and biodiversity patterns in a complex regional ecotone.

Krzysik, A.J. lead author

Ecological Applications

To be submitted by 1 May 2006

Biodiversity in a hyper-rich transition zone, the Fall-Line Sandhills of southeastern United States.

Krzysik, A.J. lead author

Natural Areas Journal

To be submitted by 30 May 2006

Impacts of disturbance severity on forest soil microbial activity: Implications to management strategies.

Zak, J.C. lead author

Soil Biology and Biochemistry

Status Unknown

Microbial response to multiple stressors within a forest landscape.

Zak, J.C. lead author

Ecology

Status Unknown