Strategic Environmental Research and Development Program (SERDP)

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SERDP-EI Research Team

This annual report represents a summary of research team progress in the SERDP Research project CS-1114B "Development of Ecological Indicator Guilds for Land Management" for the fiscal year 2001. Research activities were conducted at nine sites in the Sand Hills of Fort Benning, Georgia. The nine sites represented three disturbance classes: Low, Medium, and High, each with three replicates.

More details on specific research efforts are available in individual researcher's quarterly reports. The mailing and e-mail addresses, and telephone/FAX numbers of the Principle Investigators are found in Appendix A.

Ten Ecological Indicator (EI) systems are being researched to develop and integrate a "Guild System" for assessing disturbance gradients, ecological changes, and thresholds relevant to landuse management decisions, primarily addressing military training environmental impacts. Nine EI systems are applicable to different ecological systems and scales, while the tenth represents the synthesis of the first nine (Table 1).

Table 1. The 10 Ecological Indicator Systems Used in this Research

- **Developmental Instability**
- **Microbial Functional Diversity and Soil Nutrients**
- **Nutrient Flux and Leakage**
- **Plant Physiology and Stress Metrics**
- **Community Interactions and Integrity**
- **Ecological MultiScale Metrics**
- **GeoIndicators**
- **EcoFunction Groups**
- **Indicator Taxa and Communities**
- **Integration of EI Systems 1 to 9**

Quantification of Habitat Parameters

The collection of field data for this component of the project took place between 30 April and 7 June, 2001. The data were collected at the nine research sites in the Bonham Creek and Sally Branch watersheds. The field data represented the collection of a wide variety of selected habitat parameters at all nine research sites. The motivation was to characterize and quantify the nine sites both within, but particularly between, the three disturbance classes – LOW, MEDIUM, HIGH. There are three replicate sites in each disturbance class: L1, L2, M1, M2, H1, H2 (Bonham Creek) and L3, M3, H3 (Sally Branch). A great deal of exploratory data analysis was conducted on the field data to assess robustness and analysis stability. Four analysis methods were used: Discriminant Analysis, Principal Components Analysis, Nonmetric Multidimensional Scaling, and One-Way Analysis of Variance with Bonferroni adjusted post-hoc multiple comparisons.

Habitat Parameters (N = 78)

Ground Cover Habitat Variables (N = 7):

Ground cover habitat variables were: percent cover of legumes, grass, forbs, litter, and bare ground; mean and standard deviation of the number of taxa per sample transect (taxa = woody species + ferns + *Yucca* + *Opuntia*).

General Habitat Variables N = 8)

General habitat variables were: mean and standard deviation of canopy cover (%), A-horizon soil depth (cm), soil compaction (Land Penetrometer), and Bitterlich basal area (ft^2/acre).

Tree Habitat Variables (N = 63)

Tree habitat variables were:

1) Density, mean DBH, and standard deviation of DBH for 15 tree species (N = 45); (one of these was a class for 4 combined uncommon species)
2) Density, mean DBH, standard deviation of DBH, and basal area for all trees; and for four general classes of trees: pines, oaks, and 8 combined species (N = 16)
3) Standard deviation of tree density and total basal area in quarter sample plots (N = 2)

Results and Discussion

One-Way Analysis of Variance

The Low and Medium disturbance sites were similar to one another and differed from High disturbance sites not only in their habitat characteristics, but also in their responses to virtually all of the ecological indicator systems that have been examined. The only habitat variable of significance ($P<0.001$) that separated Low from Medium sites was "soil compaction", although Longleaf Pine DBH ($P<0.001$), pine (all species combined) DBH ($P<0.001$), and all tree species DBH ($P<0.001$) were also statistically greater at Medium disturbance sites. Eight habitat variables differed statistically between Medium and High disturbance sites: A-horizon soil depth ($P<0.001$), canopy cover ($P<0.001$), Bitterlich basal area ($P<0.001$), tree basal area ($P=0.001$), tree DBH ($P<0.001$), litter ($P<0.001$), bare ground ($P=0.002$), and number of woody taxa in ground cover ($P=0.038$).

Discriminant Analysis and Principle Components Analysis

Over 80 Discriminant Analyses and 13 Principal Components Analyses were performed. These multivariate techniques were effective in identifying subsets of habitat variables that were important in characterizing the three disturbance classes (Low, Medium, High) and nine research sites. Discriminant Function 1 (DF1) of **Analysis 12** characterized the major disturbance gradient, effectively separating the three disturbance classes, with Medium sites positioned closer to High sites (Figure 1). Parameter variability as measured by standard deviation (SD) was more important than mean parameter value in discrimination. The habitat parameters of

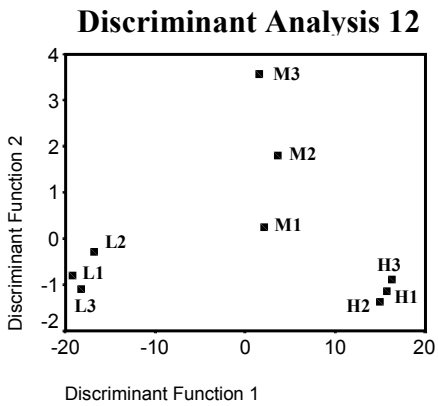
Figure 1. Discriminant Analysis 12

Input variables:

cansd soilsd langsd bittsd
legume grass forb litter bare

Standardized Canonical Discriminant Function Coefficients

	Function 1	2
CANSD	12.758	-.534
SOILSD	-1.242	1.799
LANGSD	11.739	.361
BITTSD	-15.596	-2.039
LEGUME	.131	.741
GRASS	-6.262	-.081



canopy cover SD, soil compaction SD, and a measure of tree basal area SD were the primary variables associated with DF1. The three Medium sites were highly separated on DF2, relating to high variability in A-horizon soil depth. DF1 of **Analysis 8** strongly discriminated Low disturbance sites from Medium and High sites, while DF2 effectively separated High and Medium sites (Figure 2). Along DF1, Low sites were characterized by the density of oaks and pines and forb cover, while the High and Medium sites were characterized by the density of "other" tree species and grass cover. Along DF2, Medium sites were associated with pine density and legume cover, while High sites were associated with forb cover and "other" tree density.

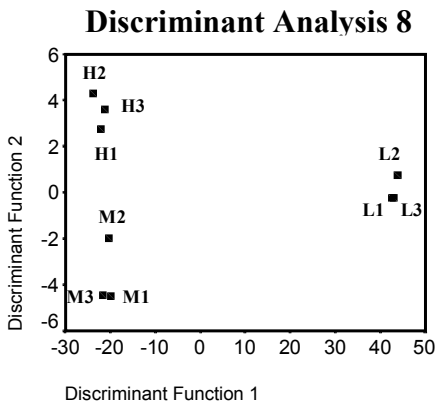
Figure 2. Discriminant Analysis 8

Input variables:

pinen oakn misc8n
legume grass forb litter bare ntaxam ntaxasd

Standardized Canonical Discriminant Function Coefficients

	Function 1	2
PINEN	13.409	-4.531
OAKN	25.031	.308
MISC8N	-21.673	3.982
LEGUME	3.989	-4.140
GRASS	-13.263	-1.035
FORB	13.113	6.766



Additionally, it was imperative to assess the performance, reliability, and robustness of these habitat parameters to separate specific PAIRS of disturbance classes in discriminant space.

Four pairs of contrast were of interest:

Low + Medium	versus	High
Low	versus	Medium + High
Low	versus	Medium
Medium	versus	High

DA analyses that were effective in discriminating in these four pairs of contrasts were as follows:

Contrast	Best DA (Number)	Next Best DA	Other DA
L-M vs H	12	none	----
L vs M-H	8	4	12
L vs M	3, 11, 12 (equal)	----	----
M vs H	8	5	3, 4, 12 (equal)

Note that Analyses 8 and 12 consistently show up as having high discriminating power in both three class and paired contrasts. Analyses 3 and 4, as in the case of three class analysis, also possessed discriminating power. Analyses 7 and 10 were important for the three class contrasts, while 5 and 11 were important for paired contrasts.

Principal Components Analysis 1 was informative and produced a good sites ordination (Figure 3). The first principal component (PC1) effectively separated Low and Medium sites form the High sites. PC1 (the VARIMAX rotated solution) was positively correlated with A-horizon soil depth (0.97), tree basal area (0.95), Bitterlich basal area (0.91), and canopy cover (0.90). PC2 separated Low sites from the Medium and High sites, where this component was positively correlated with tree density (0.89) and negatively correlated with with soil compaction (-0.87). Principal Components Analysis 9 also produced a reasonable ordination with similar habitat variable interpretation (Figure 4). In these principal components ordinations, general habitat variables again proved to be dependable quantifiers of habitat disturbance. Nevertheless, additional Principal Components Analysis and other ordinations, such as Nonmetric Multidimensional Scaling and Canonical Correspondence Analysis are in order.

Figure 3. Principal Components Analysis 1

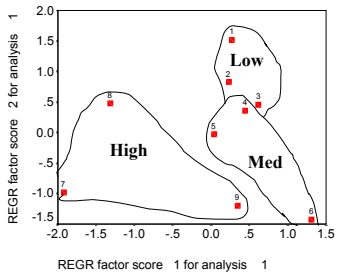
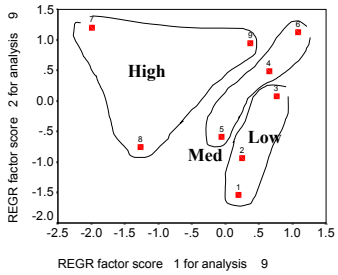


Figure 4. Principal Components Analysis 9

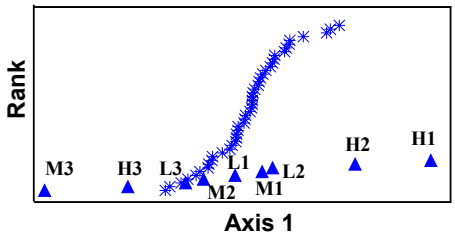


Ten habitat variables, from the original pool of 78 variables, appear to represent excellent ecological indicators of habitat condition on the basis of their discriminating power in DA analyses. Principal Components ordinations also strongly indicated that general habitat variables were important in distinguishing disturbance classes. These are: four variables expressing variability in general habitat features (canopy cover SD, A-horizon soil depth SD, soil compaction SD, and Bitterlich basal area SD), three ground cover variables (forbs, grasses, legumes), and three general tree density variables (oak density, pine density, and "other species" tree density. These variables in different, but similar, combinations were able to reliable and consistently discriminant among not only the three disturbance classes Low, Medium, and High; but also different combinations of paired comparisons among these three classes.
The current analysis was instrumental in the planning of our collection of habitat data during the spring of 2002. Additional data analyses on these data sets will continue. Important analyses to follow include ground cover and community ordinations, the calculation of similarity coefficients for each site, and cluster analysis. After characterizing the nine research sites in terms of their habitat parameters, the sites and their habitat variables will be associated with the rest of the Ecological Indicator Data Sets collected by our research team. The integration will be carried out with multivariate methods and Structured Equation Modeling.

Nonmetric Multidimensional Scaling

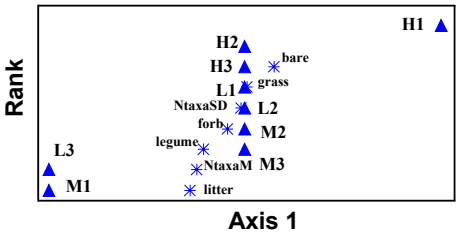
Nonmetric Multidimensional Scaling ordination was effective in quantifying the relationships among the nine research sites on the basis of tree floristics (Figure 5) and ground cover (Figure 6). The value of tree floristics was that the ordination clearly separated the two watersheds of this research study, Bonham Creek and Sally Branch (sites L3, M3, and H3), independent of habitat disturbance class. Additionally, two visually very similar sites L2 and M1 were very close in ordination space. The High disturbance sites H1 and H2 were well separated from the other sites.

Figure 5. NMS Sites by Trees



All Tree Species: density, mean DBH, Std. Dev. DBH

Figure 6. NMS Sites by Ground Cover



The ground cover ordination based on the environment gradient of bare ground to high litter effectively separated H1 (the site with the greatest disturbance of ground attributes) from L3 an M1 (the sites with the densest and most complex ground cover). The value of the ground cover ordination was that it was effective at ranking the age of controlled burns. Note from Figure 6 the ordination gradient of L1 – L2 – M2. This corresponded to gradient of most recent burn to the oldest burn in approximately annual intervals. M3 is next in the ordination, and it was scheduled to be burned while we were recording this data.

Soil Microbial Diversity and Related Soil Parameters

Soil chemistry was assessed by One-Way ANOVA between May (a dry period) and November (a wet year) in both upland and bottomland habitats (Figure 7, 2000 data). Ammonia and microbial carbon biomass did not differ among the contrasts. The pH did not differ in the upland sites, but High disturbance sites had a higher pH in the bottomlands. Soil organic matter and nitrate was higher in the less disturbed sites. Preliminary results indicate that soil organic matter levels have not changed between years for any site along the watershed. The highest soil organic matter levels do occur in the low disturbance sites.

pH has not changed significantly between years for any site along the watershed and between upland and bottom land sites. pH has averaged 5.2 for all sites in both 2000 and 2001.

Nitrate values do show significant changes across years for all sites, both upland and bottom lands. The highest levels of nitrate were detected in May 2001. In that sampling period, nitrate values were highest in the high disturbance sites.

Figure 7. Soil Chemistry (2000)
(One-Way ANOVA)

	May (dry)		November (wet)	
Parameter	**Upland**	**Bottomland**	**Upland**	**Bottomland**
NO_3	L>H, M>H	L>H, M>H	H>L, H>M	M>L, H>L
NH_4	NS	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)
Soil Organic	L>H, M>H	L>M	L>H, M>H	NS (ANOVA)
Microbial C Biomass	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)	NS (ANOVA)
pH	NS (ANOVA)	H>L, H>M	NS (ANOVA)	H>L, H>M

The soil microbial data only showed significant contrasts in the uplands during a drought period (Figure 8, 2000 data). At this time fungal and bacterial substrate functional richness, and bacteria activity were greater in the less disturbed landscapes.

The low disturbed upland sites have not changed substantially in either bacterial or fungal functional diversity over the past year. These data suggest that low impacted sites are characterized by low seasonal and yearly variation in microbial functional diversity. The major impact of disturbance within this landscape results in an increase in the seasonal heterogeneity in the ability of the soil microbes to process carbon. It is hypothesized that the high seasonal variation in microbial functional diversity, that is a result of disturbance will result in ecosystem instability and increased susceptibility to climatic stress, such as drought.

Figure 8. Soil Microbial (2000)
(One-Way ANOVA)

	May (dry)		November (wet)	
Parameter	**Upland**	**Bottomland**	**Upland**	**Bottomland**
Fungal Tot Activity	NS(ANOVA)	NS(ANOVA)	NS(ANOVA)	NS(ANOVA)
Fungal Subs Rich	L > H M > H	NS(ANOVA)	NS(ANOVA)	NS(ANOVA)
Bacteria Tot Activity	M > H	NS(ANOVA)	NS(ANOVA)	NS(ANOVA)
Bacteria Subs Rich	L > H M > H	NS(ANOVA)	NS(ANOVA)	NS(ANOVA)

Bacterial and fungal functional diversity and microbial carbon biomass have not been impacted by disturbance at most of the bottomland sites. The exception is the H1 site where the destruction of the trees at this location has reduced microbial functional diversity and microbial carbon biomass when compared to the other bottomland sites.

Microbial Biomass carbon (µg/g dry wt of soil) was not significantly different between the low and medium disturbance upland sites. The high disturbance upland sites had significantly less microbial biomass than either the low or medium disturbed sites. When compared to values obtained for May 2000, the microbial biomass values were not significantly different between years for the low disturbance upland sites. Yearly differences in microbial biomass carbon were substantial in the high disturbance site. These data also support the hypothesis that disturbance in this system increases yearly and seasonal variations in microbial activities.

Lysimeter Data and Nutrient Leakage

Table 2 provides the Lysimeter data for cation and anion leakage. The data is from Bonham Creek watershed, 2000. Each of the six research sites has six lysimeters, three in the uplands and three in the bottomlands. The High disturbance sites leached more magnesium, calcium, nitrate and sulfate than the Medium disturbance sites. There was no significance difference in leaching with the following ions: sodium, potassium, chlorine, and fluorine. The lack of significance with Low disturbance sites can be attributed to small sample sizes, because of the drought, vandalism, and lysimeter damage from land management activities (controlled burns and tree removal by heavy equipment).

Table 2. Lysimeter Anions and Cations (2000)
(One-Way ANOVA)

			H>M	H>M-L
Na	NS	Mg	(0.015)	(0.006)
K	NS	Ca	(0.008)	(0.005)
Cl	NS	NO_3	(0.034)	(0.011)
F	NS	SO_4	(0.003)	(0.002)
		ALL	(0.006)	(0.004)

Invertebrates and Fish as Indicators of Ecological Condition

The high disturbance site H1 had more individuals of ants (dominated by *Dorymyrmex pyramicus*), but possessed a lower species diversity than sites L1 and L2 in 2000 (Table 3). A few individuals of *Crematogaster ashmeadi* were collected, an arboreal species of high forage value to the endangered Red-cockaded Woodpecker. Additional sampling efforts will be made in 2002 to assess the relative abundance patterns of this species among the disturbance classes.

Table 3. Fort Benning Ants (2000)
sampled at: L1 L2 H1

Species	L1	L2	H1
Dorymyrmex pyramicus	125	53	7647
Pheidole sp1	104	151	13
Aphaenogaster floridana	5	13	0
Forelius pruinosus	12	2	0
Aphaenogaster ashmeadi	7	2	0
Crematogaster ashmeadi (an important arboreal species)	1	2	1

Sixty-seven percent of the ants collected in 2001 have been counted and identified -- a total of 9,500 ants, comprising 26 species. Preliminary analysis indicates that species diversity of ants is

significantly less (Student-Neuman-Keuls, P< 0.05) in the High disturbance sites than in the Medium and Low disturbance sites. Similar results were observed in 2000.

There were no significant differences in total numbers of ants among disturbance classes in 2001. There were, however, significant differences in total numbers of ants among sites within disturbance classes. Site H3 (Sally Branch), for example, had very high densities of ants (primarily *Dorymyrmex pyramicus*), much higher than any site. High disturbance sites were much more variable than Low and Medium disturbed sites.

There does not appear to be a great difference in species richness between Low and High disturbance wit he data that we have collected thus far (Table 4). However, a greater number of individuals were collected at site L1 than site L2 or H1. Interestingly, each of the three sites examined possessed different species.

Table 4. Fort Benning Spiders (2000)
sampled at: L1 L2 H1

Total Species: 26
Number of Families: 8
In general, each Site had different species

	L1	L2	H1
Number of Individuals	64	14	12
Number of Species	10	8	11

Developmental instability and Plant Physiological Responses

Fluctuating Asymmetry, a measure of Developmental Instability (DI), for Winged Sumac is summarized in Table 5, and for Morning Glory (*Ipomoea*) in Table 6. Although there was a great deal of variability in the data, higher disturbance sites displayed greater DI.

Table 5. Fluctuating Asymmetry of Leaflet Main Vein in Sumac
(One-Way ANOVA)

Parameter	1999 Comparison	2000 Comparison
1st Pair	H > L 0.028	NS (ANOVA)
2nd Pair	NS (ANOVA)	L > M 0.003
3rd Pair	NS (ANOVA)	------
4th Pair	H > L 0.036	------

Table 6. Fluctuating Asymmetry in Ipomoea (2000)
(One-Way ANOVA)

Parameter	Comparison	Significance
FA in Leaf Width	H > M	0.008
FA in Leaf Lobe Depth	L > M H > M	0.027 <0.001
Size Ratio: main vein length / leaf width	L > M H > M	<0.001 <0.001

Plant physiological responses for 2000 are summarized in Table 7. Transpiration rates, stomatal conductance, and variance in stomatal conductance significantly increased as disturbance level increased.

Net photosynthesis, transpiration and stomatal conductance were measured for sumac at four sites using the CID model 5100 portable gas analyzer equipped with its own light source. Machine malfunction, caused by smoke from the controlled fires, restricted the number of sites that we could examine. Twenty plants were examined per site, three leaves were examined per plant and two leaflets were examined per leaf. The data were analyzed using a nested model analysis of variance. The plant term was nested within site, and leaf term was nested within plant the plant term. For each plant sampled, we also centered a $1M^2$ quadrat around the individual. Using Daubenmire cover classes we estimated the percent of ground covered by all plant, the

target species, legumes and litter. We also estimated the percentage of bare ground. These latter terms were treated as covariates in the initial analysis. As none of the proved to be significant, they were dropped in the final analysis.

Table 7. Plant Physiological Responses (2000)
(One-Way ANOVA)

Parameter	Comparison	Significance
Variable Fluorescence	M > L M > H	<0.001 <0.001
Stomatal Conductance	H > L H > M M > L	<0.001 <0.001 0.032
Variance in Stomatal Conduct.	H > L H > M	<0.001 <0.001
Transpiration Rate	H > L H > M M > L	<0.001 <0.001 0.023
Photosynthesis	NS (ANOVA)	------

Net photosynthesis, transpiration and stomatal conductance varied significantly among the site ($F_{3,74}$= 71.97, 22.59 and 14.60 respectively, $P << 0.001$ in all cases). Net photosynthesis declined as disturbance increased, while transpiration and stomatal conductance were significantly higher at the heavily disturbed site than at the other sites.

We earlier noted that we conducted an analysis of covariance and that when all sites were considered the variables describing the vegetation around the target individual of sumac showed no significant relationship with the values of the mean physiological variables. We have further explored this issue by conducting pair-wise correlation analysis for sites individually. The results indicate that net photosynthesis is significantly negatively correlated with the total plant cover but only at the moderate and highly disturbed Sally Branch sites. Net photosynthesis was also significantly negatively correlated with the cover of legumes only at the highly disturbed site. This pattern was also held for the cover of litter. The percentage of bare ground, on the other hand, was significantly positively correlated with net photosynthesis at the highly disturbed site, but not at the other sites. Transpiration and stomatal conductance showed similar patterns to one another. Both of these parameters were significantly positively correlated with the total plant cover at both the moderate and highly disturbed sites, but not at the low disturbance site. Both values were **positively** related to bare ground at the moderately disturbed, as one might expect, but surprisingly significantly **negatively** correlated with bare ground at the highly disturbed site. The cover by legumes was negatively correlated with both transpiration and stomatal conductance rates at the moderately disturbed sites and positively related to these parameters at the high disturbance sites, though this pattern was significant only for stomatal conductance. A similar flip-flop in the sign of the relationship also occurred for total plant cover. We tentatively interpret this changing of sign as follows:

Normally, one would expect that net photosynthesis would decline under stress and that water loss would increase. Hence it is comforting to see that photosynthesis does decline with increasing total plant cover particularly at the moderately and highly disturbed site. Water loss (transpiration and stomatal conductance) can be expected to increase as temperature increases. Consequently, the light that reflects off of the bare ground should increase water loss. This pattern is indeed observed at the moderately disturbed site. What is surprising is that at the highly disturbed site bare ground is significantly negatively correlated with water loss. One would expect the reverse pattern. Apparently at the highly disturbed site, the competition from other plants, for nutrients, causes sumac plants to keep their stomata open and to thus loose more water than plants than would occur if the plant were surrounded by more bare ground. This explanation is consistent with the results of other team members and the fact that cover of the target species is positively correlated with water loss at the moderately disturbed site but strongly negatively correlated with water loss at the highly disturbed. Because sumac is clonal, the cover of target species is simply apt to reflect other ramets of the same clone.

We also examined the fluorescence from sumac leaves. The ratio of (maximum-minimum)/maximum is often used as a measure of stress. We examined twenty plants per site, three leaves per plant, and two leaflets per leaf. The data were again analyzed using a nested model analysis of variance with leaf nested within plant, and plant nested within site. The minimum, maximum and ratio differed significantly among the sites ($F_{8, 171} = 6.57$, 7.37 and 4.07 respectively, $P < 0.001$ in all cases). The minimum fluorescence tended to decline with disturbance, though this was not the case with Sally Branch High. Similarly the maximum fluorescence also tended to decline with increasing disturbance. However, the percent deviation from the maximum fluorescence exhibited a more complex pattern being lowest at intermediate disturbance sites and Sally Branch High, and highest at the highly disturbed Rowan Hill site.

Once again, the relationship between a stress parameter, in this case, the relative deviation from the maximum fluorescence, is differentially influenced by surrounding plant cover. Cover by legumes was positively correlated with stress at the low disturbance site, but significantly negatively correlated with stress at the moderately disturbed site. This implies that the legumes may be providing a net increase in soil nitrogen at the moderately disturbed site.

We have decomposed the mean squares from the analysis of variance tables. In the case of net photosynthesis, transpiration and stomatal conductance, the bulk of the variance is due to site, with the plant term accounting for most of the remaining variance. The variation among leaves never accounted for more than 13% of the variance and error never more than 16% of the error. The reverse situation occurs for the fluorescence measures with most of the variance being due to error. We are re-evaluating the use of fluorescence to indicate the condition of the plants.