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An Experimental Approach to Teaching the Concept of Functional Diversity.

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An Experimental Approach to Teaching the Concept of Functional Diversity

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ABSTRACT

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This study tested an experimental approach to use in teaching the concept of functional diversity. The project culminated in a laboratory exercise for use in high schools.

Experimental design consisted of representatives of 3 functional groups of plants, (legumes, grasses, and forbs), planted singly, and in 2, or 3 species combinations. Legumes were represented by *Trifolium repens* and *Medicago lupulina*, grasses were represented by *Cynodon dactylon* and *Festuca rubra*, and forbs were represented by *Helianthus annus* and *Raphanus sativa*.

Plants were grown inside a controlled growth chamber. During the growth phase, measurements were taken to highlight temporal differences in development. After 2 months, wet and dry weights of aboveground and belowground portions were measured as indicators of productivity.

Research showed unique developmental patterns of functional groups. Secondly, functional combination, not functional group number, produced a significant difference in biomass.

Laboratory use involves group discussion, active-learning, and higher understanding of conservation.

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CHAPTER 1

INTRODUCTION

The "Big" Question

Recent controversy in the field of conservation biology stems from the question: Is the number of species (biological diversity) more important to maintaining ecosystem stability and productivity than the number of representatives or combinations of different functional groups (functional diversity/functional composition)? Biological diversity can be defined as "the variety of living organisms considered at all levels of organization, including the genetic, species, and higher taxonomic levels, and the variety of habitats and ecosystems, as well as the processes occurring therein" (Meffe 1997).

Functional diversity can be defined as the variety and/or combination of phenotypic characters contributing to an organism's or a community's survival and productivity. Functional group delineation consists of placing organisms with similar functions into the same groups. The questions and controversies behind the biological and functional diversity argument highlight differences in the environmental and emotional values of biodiversity. The goal of maintaining biological diversity is inherently attractive scientifically because extinction of a species results in the loss of unique genotypes. Similarly, biological diversity appeals to emotional values held by many people, because as human beings we tend to strive to protect other organisms from harm or extinction, rather than to destroy things around us (Wilson 1984).

Restoration Techniques and Functional Diversity

The ultimate goals of a pragmatic conservation program are to protect and restore ecosystems while maintaining productivity and sustainability of local and global communities. In the search for innovative restoration techniques, several groups of scientists have studied the effects of functional diversity on ecosystem processes. Categories for defining functional diversity include: morpho-physiological similarity, functional roles, functional combinations, and functional groupings. The morpho-physiological basis of different organisms characterizes their effects on surrounding organisms based on physical, biochemical, or genetic factors. The effects on the environment may be positive, negative, or nil. The functional role of any organism is its overall effect on an individual level, a community level, or even on a global scale. Functional combinations are the mixtures of different functional roles in a community. These varied combinations are at the heart of both this study and the future of conservation biology. Tilman et al. (1997) stated that certain aspects of plant growth and productivity including, percent nitrogen, plant total nitrogen, and light penetration were affected by functional diversity and functional composition. With this in mind, major environmental changes can occur through the alteration of functional diversity and functional composition.

Recent Studies

Recent research efforts in conservation biology have been directed toward measuring and comparing biological diversity because selected species may gain additional security from local extinction by having more biomass through increased biological diversity (Naeem and Li 1997). A more recent study suggests that functional diversity is more important than species diversity in ecosystem processes (Tilman et al. 1997). Hector (1999) also found that there was a reduction in the amount of aboveground biomass as the number of functional groups decreased.

Most studies to assess the role of functional diversity were carried out in large field plots, manipulating the numbers of species, functional groups, and functional group combinations. Past studies showed that the number of functional groups as well as the unique functional group combinations produced a greater change in aboveground biomass production than did the number of species in the field plot (Tilman et al. 1997). Larger studies can last a number of years, including a 7-year experiment that contained plots of 16 species, which gained 2.7 times more biomass than a monoculture (Tilman et al. 2001).

Almost all-recent studies have used the similar methods of combining different functional groups in field plots to test effects on resultant biomass. Biomass production was the main, and sometimes only, measure of productivity recorded in these field studies.

Three functional groups (forbs, legumes, and grasses) represent the only functional groups used and identified for research purposes in past studies, and they are included in this study because of their near universal recognition. Legumes represent species harboring nitrogen-fixing bacteria, which bring fixed nitrogen to the plant roots. As these plants decay, fixed nitrogen is added to the soil. Grasses often contain smaller leaf blades than forbs, which allow more sunlight penetration through the canopy, as well as massive root structures that create crowded root growing mediums. Forbs, like many other functional groups, are somewhat lacking in concrete characteristics, other than the fact that they are very different from the other 2 functional groups. Two species representing the legume group were alfalfa (*Medicago lupulina*) and red clover *(Trifolium repens*). The 2 species from the grass group were bermuda (*Cynodon dactylon*) and red fescue (*Festuca rubra*). The forb group representatives were radish (*Raphanus* *sativa*) and sunflower (*Helianthus annuus*). Many other species, including forbs, require further research. Some of these same species and functional groups have been used in other studies. Examples include C4 (warm season) and C3 (cool season) grasses that present different growth processes and possibly different functions. Identification of other functional groups are at the forefront of research, and their identification will be a major step for the proper combining of functional groups to create more stable and productive ecosystems. Possible groups may arise concerning developmental time, root growth patterns, decay patterns, spore production, or plant color. Students should have the opportunity to develop their own ideas.

Global Effects

Determining the value of functional diversity is an important focus of conservation biology research, mainly because of declining ecosystem stability and the increasing human population. Human expansion causes not only a loss of biodiversity (Naeem et al. 1994), but in recent studies it has even produced predictable functional shifts as new traits are replacing old ones (Loreau et al. 2001). This loss of diversity is a loss of life, as well as the loss of rare and perhaps functionally important species. Many times there have been alien species introduced into an ecosystem, and the invader would completely take over the system and drastically decrease both species diversity and functional diversity. Functional groups are not completely defined. For example, unnamed functions of rare species may be important to ecosystem function as evidenced by the observation that removing rare species from certain communities has been shown to cause an invasion of exotics (Lyons and Swarts 2001).

Conservation biology is also in dire need of more research directed toward understanding varying types of functional roles that may define an ecosystem, and how the roles may or may not be combined. China's serious, yet undefined problem with rice production was partially answered by simply growing two different strains of rice together, one standard strain that does not succumb to rice blast fungus, and another more economically valuable strain that does (Yoon 2000). The 2 strains filled functional roles that, in combination with one another, assisted in boosting total growth and productivity. Functional roles of these 2 rice plants were straight-forward. The more economically valuable sticky rice plant was tall and enjoyed sunny, warm, and dry conditions. The standard disease-resistant rice plants were shorter, allowing the sticky rice plants to have optimal conditions and become less prone to disease because the spores were not traveling between rows or fields. This is a simple functional combination, involving plant height characteristics, a struggle for resources, and blocking of airborne spores while in a heterogeneous mixture instead of a homogeneous mixture. In theory, optimal combinations of other functional roles could help agricultural systems to increase stability and productivity of food producing species (Yoon 2000).

Increased productivity could assist in filling needs for resources to support the increasing human population, which is causing a degradation of our biosphere, and an extinction of organisms and the habitats in which they live (National Research Council 1992). Economics may be linked to functional diversity research, because as the rice project has shown, functional combinations can increase biomass production in crop species. Therefore, farmers and corporations can be expected to fund related research projects.

Goals

The goal of this study was to design and analyze an experiment that could be adapted for use in a classroom setting in order to educate secondary education students about the concept of functional diversity. Activities related to preparation of experiments and gathering different types of data had the beginner student in mind. Three observable plant growth measurements (number of leaves, length of largest leaf blade, and shoot height) that were based on easily recognizable characteristics were used throughout the entire growth phase. Having students measure the plants during the growth phase would allow the students to stay actively involved in a weekly task while observing and comparing growth characteristics.

Research is commonly seen as an overwhelming task, but when the task is broken into many smaller parts and a class is divided into groups, a more positive feeling toward research may be achieved. As the experiment is broken down into smaller components (planting, growing, and final data collection) the components can be used in a cooperative classroom where students assist each other in reaching a common goal (Henton 1996). In addition to benefits associated with a cooperative classroom, students learn at a higher rate when the information is correlated to some type of hands-on activity, something that they can see in action. “Students do not just absorb content or learn by taking copious notes and studying for exams, but by critically analyzing, discussing, and using content in meaningful ways” (Meyers and Jones 1993).

Conservation biology is a subject that should be important in school curricula because students need to be aware of degrading environmental quality. Past topics concerning conservation biology have been directed more toward the overuse of resources and ways to be efficient in daily life. Research presented in this paper does not show how to recycle paper or use less water, rather it teaches basic principles of ecosystem processes and allows the student to visualize a “real life” experiment to help a “real life” problem. There is an importance to preserving all species because of their (perhaps) unknown function in the environment. This project will show students how scientists use an experimental approach to understand the potential value of a species from components of biodiversity.

The initial indoor trial, conducted in a growth chamber, tested the procedures for measuring 3 physical characteristics that could be followed during the entire life of the plants. There were 25 separate pots (plots), an amount that is close to the number of students in a classroom. Plant species were chosen for the functional groups based on familiarity, availability of seeds, and easily observable traits. For example, athletes may know Bermuda grass for its wide use on athletic fields. Agriculture students may recognize radish plants, or alfalfa for its use in combination with hay in feeding livestock. Sunflowers are bright and vibrant plants that are easily identifiably by students. Any and all possible methods that grab the interests of students should be used. The design that was used may be replicated using windowsills if a growth chamber is not available.

Helping students understand the concept of functional roles can be accomplished with discussions concerning societies as well as personalities. Working communities are based on the presence of different work trades. Without a mechanic, a community would have too many non-functional cars, but with too many mechanics, there would not be enough teachers for education. This very simple example shows the work trades can be assigned to functional roles. Personalities can represent competition between species, and individuals can be assigned varying personalities, then the personalities combined in different ways to see consequences similar to those in an ecosystem. Many different lessons can stem from this experiment, but the main goal is to let students get hands on experience with the importance of functional diversity and the need for conservation, because the curricula of many secondary and other higher education biology classes do not contain sections on functional diversity. A brief overview of current laboratory manuals shows that biodiversity is still stressed more than functional diversity.

Sections on diversity stick to the idea that many species are good, but they do not present reasons. A need for conservation is growing and details behind the topic will need to be stressed. Studying functional diversity is the next step in saving our ecosystem and having more productive communities, because “to the degree that we come to understand other organisms, we will place greater value on them, and ourselves” (Wilson 1984).

CHAPTER 2

MATERIALS AND METHODS

Experimental Approach

Initial Trial (Field)

The experimental approach of the project was to grow plants in plots that differed in the number of functional groups, as well as in combinations of functional groups. The species chosen were assigned to 1 of 3 functional groups: legumes, grasses, and forbs. Two species representatives from each functional group were used in order to determine if outcomes differ between species within the same functional group. Similar outcomes would imply that functional group identity is more important than species identity. The 2 species from the legume group were alfalfa (*Medicago lupulina*) and red clover *(Trifolium repens*); from the grass group, bermuda (*Cynodon dactylon*) and red fescue (*Festuca rubra*); and from the forb group, radish (*Raphanus sativa*) and sunflower (*Helianthus annuus*).

The field design consisted of different species grown in different functional combinations. The combinations included 1, 2, or 3 functional groups per plot, represented by 7 different types of unique functional group mixtures. In the field, the study unit was a 15 cm^2 plot. Eighteen plots contained only 1 species each, consisting of 3 replicates of each of the 6 species. Another 36 plots were used for the 2 functional group combinations consisting of 12 combinations each replicated 3 times, and the final 21 plots were used for the 3 functional group combinations consisting of 7 different combinations, each replicated 3 times (Table 1).

The 1st set of trials was conducted in a field plot south of the outdoor track on the East Tennessee State University campus. The area was enclosed in a fence to prevent herbivore damage. Each of the 15 cm^2 plots was set apart by plywood in a checkerboard fashion. Plywood was used to minimize unwanted weeds surrounding the test plot. Plots were weeded by hand weekly. Water supply was partially controlled; during times in which the rain was scarce, water was added to field capacity.

Seedlings were propagated in a growth chamber at East Tennessee State University in order to stock the field plots. Seeds were germinated on filter paper that was folded into 2.5 cm x 2.5 cm x 7.5 cm hollow devices known as phytometers. Phytometers were placed side by side in a flat filled with potting mix (Fafard 3B) and a seed was added to each. The flat was filled with water, never letting the level in the flat drop to zero, a method that kept the soil water at field capacity. Light and temperature variations in the growth chamber simulated the changing outside environment (Table 2). Seeds were allowed to germinate and grow for approximately 5 weeks, and then the seedlings were transplanted into the field plots. The transplantation process involved digging a small hole to hold the phytometer. The phytometer along with the seedling was then inserted into the soil and watered. There were many problems associated with the transplantation process, such as phytometers sticking together and breaking, outside soil being much harder than Fafard 3B, and excess weeds. These problems led to further research indoors. Conducting the project indoors eliminated both the need to transplant and the problem

Table 1. Trial 1 Field Design

	1	2	3	4	5	6	7	8	9
A	26A	5C	18A	4B	7A	1C	21A	15B	11A
B	13B	5A	14C	15A	ctrl	11B	2A	10C	19B
C	12C	8B	22B	24A	10A	25B	21C	26B	6B
D	14A	7C	20A	25C	1B	4C	16A	19C	4A
E	20B	17C	16B	1A	23C	18B	9A	ctrl	10B
F	5B	6A	8C	12B	20C	23A	6C	13C	22A
G	9B	25A	ctrl	2B	17A	24C	3A	24B	16C
H	19A	2C	13A	11C	22C	9C	21B	26C	7B
I	17B	23B	15C	14B	8A	18C	3B	3C	12A

Table 2. Time, Temperature, and Light Settings for the Growth Chamber.

Time	Temperature (0C)	Lights flourescent	Lights Incandescent	
700	18	0	2	
800	19	2	2	Dawn
900	20	3	3	
1000	22	4	4	
1100	22	4	4	
1200	22	4	4	
1300	22	4	4	
1400	22	4	4	
1500	22	2	2	
1600	22	2	2	
1700	20	2	2	
1800	19	0	2	Dusk
1900	19	0	2	
2000	18	0	0	

*2000-0700 (nighttime)

of weed invasion. Indoors also provided a more controlled environment.

Second Trial (Growth Chamber)

A 2nd experiment was conducted in a growth chamber. 15 cm^3 pots were used as the individual test plots, and 6 were placed in each flat inside the growth chamber. Soil was kept at field capacity by preventing the water level in the flats from reaching zero. Temperature and light simulated daily rhythms. The experiment ran for 9 weeks during which growth phase measurements were taken every 2 weeks. Vegetative characters assayed were height of the above ground plant, length of largest leaf blade, and the number of leaves.

Final Trial (Growth Chamber)

The final phase of the project used design modifications that incorporated improvements gained from the first 2 trials. Seeds were planted at a depth of 2 to 3 centimeters in the 15 cm^3 pots and given the maximum amount of distance apart from each other in the test pot (Table 3). Individuals were arranged in pots of mixed species to maximize distances between conspecific neighbors (Table 4). Only 2 pots were placed in each flat in order to spread the plants and reduce potential crowding and/or shading effects that may have been caused by plants outside a plant's own pot. Watering in the early stages was only as needed to prevent over-saturation. Watering after a 3-week period was from the top of the pot with a small stream. Flats were filled to the top in order to give the plants plentiful water, but not an amount that would simulate

Table 3. Trial 3 Design.

Pot 1		**Pot 6**		**Pot 11**		**Pot 16**		**Pot 21**	
1a	1d	6a	6d	11a	11d	16a	16d	21a	21d
1b	1e	6b	6e	11b	11e	16b	16e	21b	21e
1c	1f	6c	6f	11c	11f	16c	16f	21c	21f

Pot 2		**Pot 7**		**Pot 12**		**Pot 17**		**Pot 22**	
2a	2d	7a	7d	12a	12d	17a	17d	22a	22d
2b	2e	7b	7e	12b	12e	17b	17e	22b	22e
2c	2f	7c	7f	12c	12f	17c	17f	22c	22f

Pot 3		**Pot 8**		**Pot 13**		**Pot 18**		**Pot 23**	
3a	3d	8a	8d	13a	13d	18a	18d	23a	23d
3b	3e	8b	8e	13b	13e	18b	18e	23b	23e
3c	3f	8c	8f	13c	13f	18c	18f	23c	23f

Pot 4		**Pot 9**		**Pot 14**		**Pot 19**		**Pot 24**	
4a	4d	9a	9d	14a	14d	19a	19d	24a	24d
4b	4e	9b	9e	14b	14e	19b	19e	24b	24e
4c	4f	9c	9f	14c	14f	19c	19f	24c	24f

Pot 5		**Pot 10**		**Pot 15**		**Pot 20**		**Pot 25**	
5a	5d	10a	10d	15a	15d	20a	20d	25a	25d
5b	5e	10b	10e	15b	15e	20b	20e	25b	25e
5c	5f	10c	10f	15c	15f	20c	20f	25c	25f

Table 4. Spacing for Pot Growth Conditions.

One Species

1	1
1	1
1	1

Two Species

1	2
2	1
1	2

Three Species

1	3
2	1
3	2

1= Functional group representative #1
2= Functional group representative #2
3= Functional group representative #3

flooding. The plants were then allowed to grow for 9 weeks. During the 9-week growth phase, bi-weekly assays included measuring the number of leaves per plants. Other optional measurements include length of largest leaf blade, and possibly shoot height. Measurements were taken with a simple ruler.

Biomass Assays

Plants were harvested following the 9-week growth phase. Plants were weighed to determine the above and belowground biomass. First, the plant was severed at ground level and the aboveground biomass was placed in labeled plastic bags. Capturing the belowground biomass was a task, for the roots were easy to break while attempting to separate the soil from them. The best method was to remove the entire soil mass from the pot, manually massage the soil, and then soak it in a tub of water. After the soil was removed, the root mass was transferred to a sink and running water was used for final soil removal. The resultant belowground biomass was then placed in a labeled plastic bag. Wet weight was determined by weighing on a balance.

Dry weight was assayed by pressing and drying the plants, similar to the process used to prepare herbarium specimens. Each plant was placed between sheets of newspaper, then the plants were pressed in a plant press and placed in a drying cabinet. The drying process lasted 17 days at 51.7^{o} C. The lengthy process was needed to fully dry the thick sunflowers and the radish roots. Following drying, the dry-weights were obtained in the same manner as the wet-weights. Results were plotted on a bar graph based on the average plant biomass per pot and showing results based on functional group number.

Statistical methods that were used included one-way analysis of variance (ANOVA). The analysis consisted of 2 treatment types: number of functional groups and type of functional combinations. The ANOVAs compared 3 dependent variables: total biomass, aboveground biomass, and belowground biomass. Finally, a Tukey's test (a posteriori test) pointed out where the significant differences between the means of functional combinations were located. This basically compared the means of all combinations and monocultures to determine if a significant difference did exist.

These types of analyses (gathering data, graphical presentation, statistical analysis, and written communication) are all tools for students to use in answering the experiment's dynamic goal: "What is the effect of functional diversity on biomass production in a test plot?"

CHAPTER 3

RESULTS

Temporal Development

Results from bi-weekly measurements are highlighted in 6 graphs (Figures 1-6) that indicate the number of leaves produced per control plant in comparison with combinations that contain the same species. These graphs can be used to see trends in temporal development. For example, Figure 1 compares alfalfa and combinations that contain alfalfa. Alfalfa and forbs are not productive functional combinations, because alfalfa only yielded an average of 13 leaves when combined with a forb, far from the average 45 to 50 leaves in other groupings (Figure 1). The alfalfa/bermuda and the alfalfa/radish/fescue combinations diverged initially, but they ended the 5-week growth period with similar measurements. There was a leveling off of clover leaf-number at the 3-week time period (Figure 2). Only 3 of the groups grew continually, and they were the control, clover/sunflower, and the clover/sunflower/fescue combinations.

The number of leaves that grew in the combinations that contained bermuda showed trends associated with the bermuda such as an extra week for germination and a leveling-off in growth after week 4 (Figure 3). Fescue had a low rate of survival. The combinations that did survive were combinations that contained alfalfa, sunflower, or both (Figure 4).

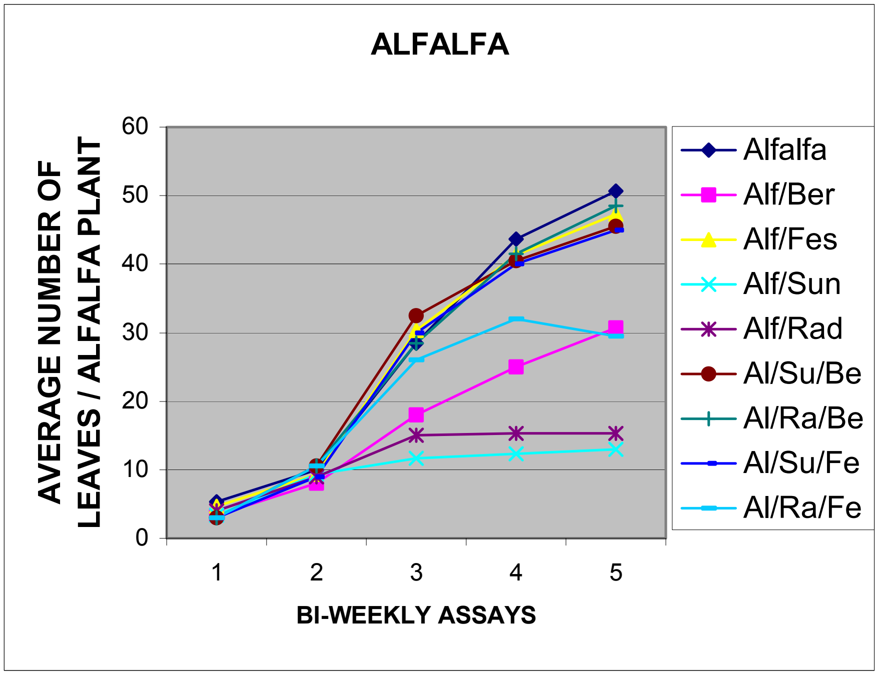

Figure 1. Alfalfa: Number of Leaves in Pure and Mixed Culture

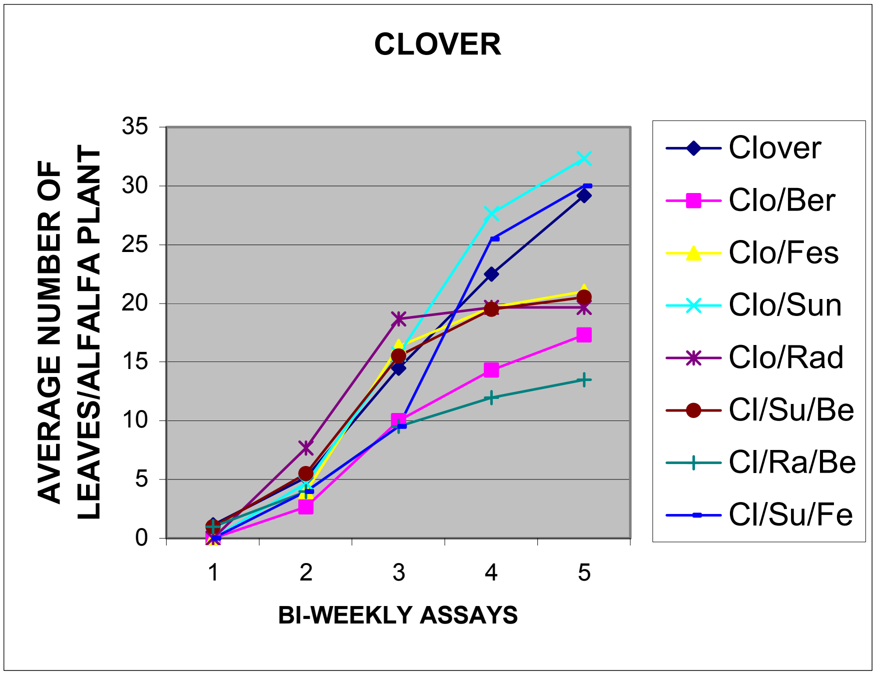

Figure 2. Clover: Number of Leaves in Pure and Mixed Culture

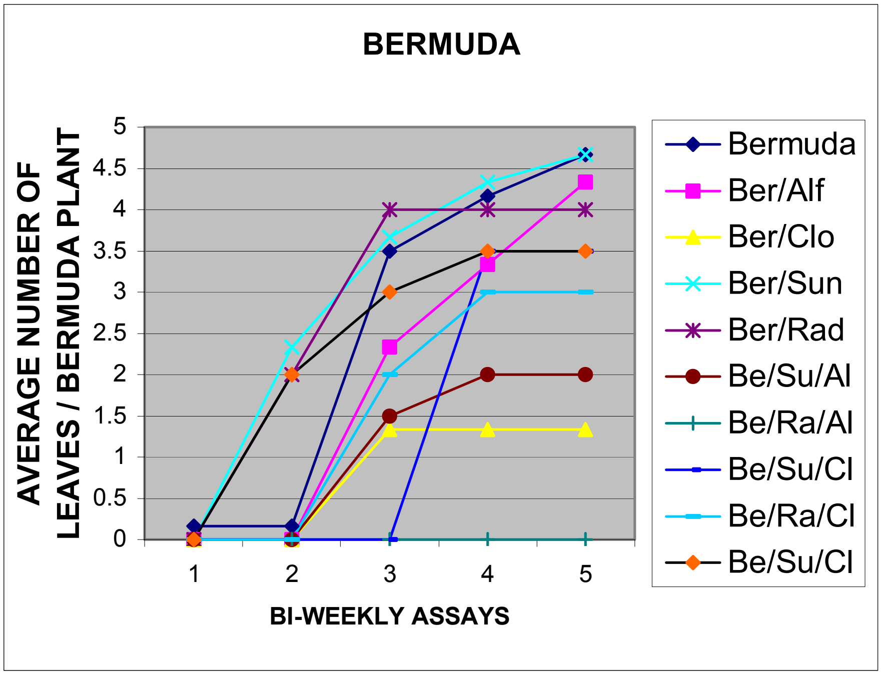

Figure 3. Bermuda: Number of Leaves in Pure and Mixed Culture

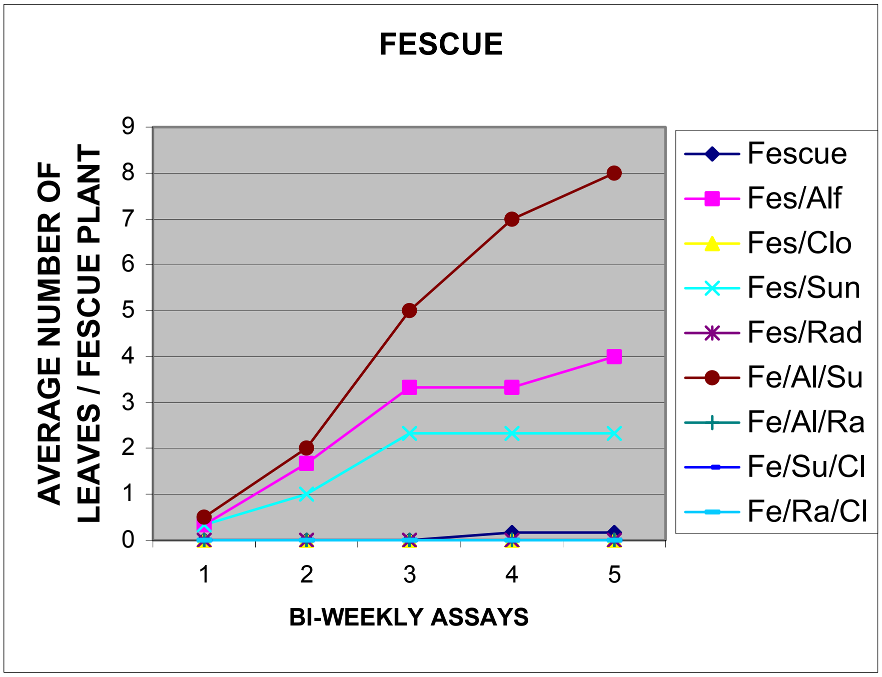

Figure 4. Fescue: Number of Leaves in Pure and Mixed Culture

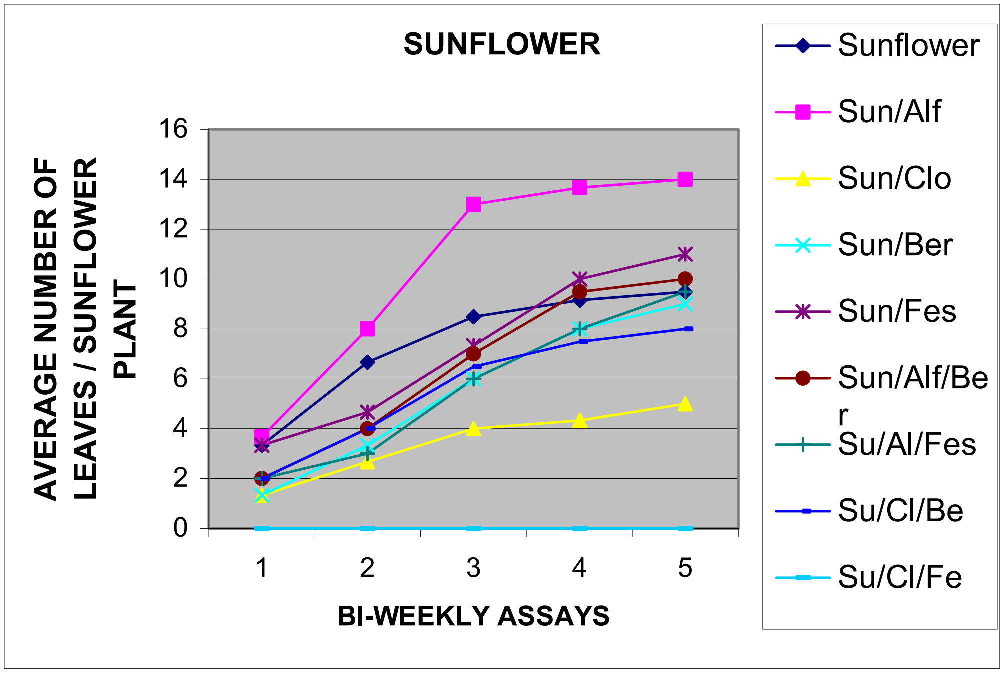

Figure 5. Sunflower: Number of Leaves in Pure and Mixed Culture

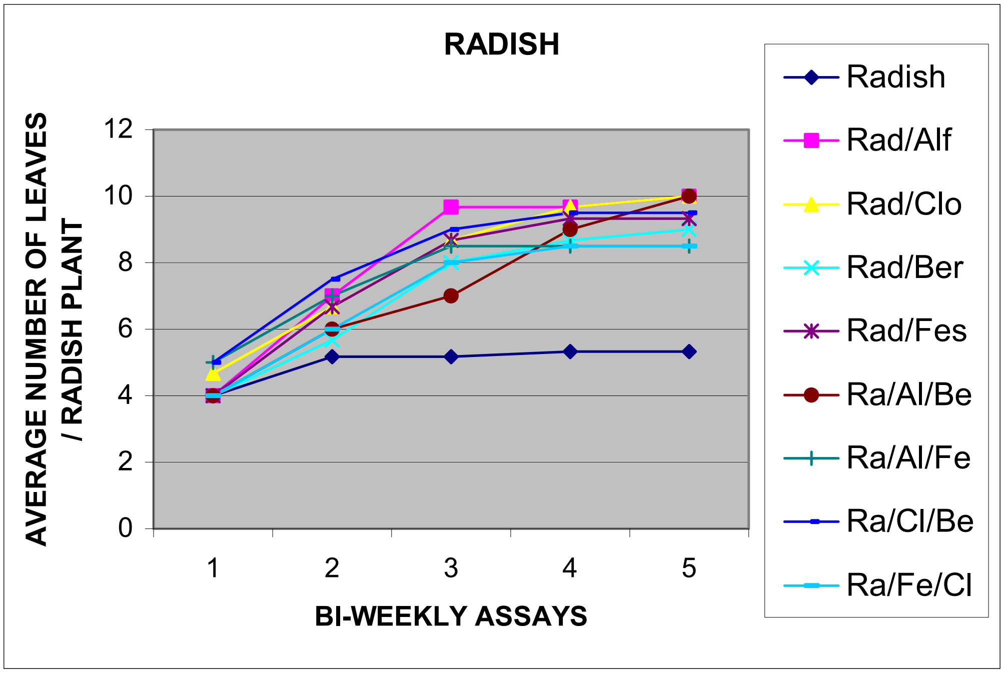

Figure 6. Radish: Number of Leaves in Pure and Mixed Culture

The sunflower combinations showed the similar trends as the control group, except for the combination with alfalfa that showed an increase and the combination with clover that showed a decrease (Figure 5). Finally, the radish leaf number changed little between the 2nd and 5th weeks in the control group, and between the 3rd and 5th weeks with many of the other groups (Figure 6).

Plant Biomass

Neither the number of functional groups ($p = .349$) nor the type of functional group combination ($p = .148$) caused significant differences in aboveground biomass (Tables 5 and 6). Belowground biomass was similarly unaffected by the number of functional groups ($p = .506$) (Table 7). However, belowground biomass was marginally influenced by the type of functional group combination ($p = .066$) (Table 8).

Similar amounts of plant biomass were found for total plant biomass (Figure 9) and aboveground biomass (Figure 7). The significant variations in biomass were found in the radish plot as a control group. Two other areas with related biomass reduction were the alfalfa/radish/fescue and the clover/radish/fescue combinations (Figure 8). The radish plant is a plant in which the total plant biomass is overwhelmingly controlled by belowground components. Differences in biomass result from the ratio of above and belowground biomass for every combination that contains the radish (Figure 9). Total plant biomass, aboveground biomass, and belowground biomass of both individual functional group plots and of the combined functional group plots are shown in Figures 7, 8, and 9. A presentation of the unique functional group combinations by plot numbers is presented in Table 9.

The number of functional groups ($p = .318$) caused no significant difference in the total plant biomass (Table 10). However, the type of functional combination ($p = .002$) resulted in a

Table 5. Results of One-way ANOVA to Test the Effect of Number of Functional Groups on Aboveground Dry-weight Biomass.

Source	**DF**	**SS**	**MS**	**F**	**P**
Number of Functional Groups	2	271	136	1.11	0.349
Error	22	2698	123		
Total	24	2969			

Table 6. Results of One-way ANOVA to Test the Effect of Type of Functional Combination on Aboveground Dry-weight Biomass.

Source	**DF**	**SS**	**MS**	**F**	**P**
Type of Functional Combination	6	1128	188	1.84	0.148
Error	18	1841	102		
Total	24	2969			

Table 7. Results of One-way ANOVA to Test the Effect of Number of Functional Groups on Belowground Dry-weight Biomass.

Source	**DF**	**SS**	**MS**	**F**	**P**
Number of Functional Groups	2	23.4	11.7	0.70	0.506
Error	22	366.7	16.7		
Total	24	390.1			

Table 8. Results of One-way ANOVA to Test the Effect of Type of Functional Combination on Belowground Dry-weight Biomass.

Source	**DF**	**SS**	**MS**	**F**	**P**
Type of Functional Combination	6	175.4	29.2	2.45	0.066
Error	18	214.7	11.9		
Total	24	390.1			

Table 9. Growth Pot Combinations.

Pot #	Combination	Functional type
1	ALFALFA	Legume
2	CLOVER	Legume
3	BERMUDA	Grass
4	FESCUE	Grass
5	SUNFLOWER	Forb
6	RADISH	Forb
7	ALFALFA/BERMUDA	Leg/Gra
8	ALFALFA/FESCUE	Leg/Gra
9	CLOVER/BERMUDA	Leg/Gra
10	CLOVER/FESCUE	Leg/Gra
11	ALFALFA/SUNFLOWER	Leg/For
12	ALFALFA/RADISH	Leg/For
13	CLOVER/SUNFLOWER	Leg/For
14	CLOVER/RADISH	Leg/For
15	BERMUDA/SUNFLOWER	Gra/For
16	BERMUDA/RADISH	Gra/For
17	FESCUE/RADISH	Gra/For
18	FESCUE/SUNFLOWER	Gra/For
19	ALFALFA/RADISH/BERMUDA	Leg/For/Gra
20	SUNFLOWER/ALFALFA/FESCUE	Leg/For/Gra
21	ALFALFA/RADISH/FESCUE	Leg/For/Gra
22	CLOVER/RADISH/BERMUDA	Leg/For/Gra
23	CLOVER/SUNFLOWER/BERMUDA	Leg/For/Gra
24	CLOVER/FESCUE/SUNFLOWER	Leg/For/Gra
25	CLOVER/RADISH/FESCUE	Leg/For/Gra

Table 10. Results of One-way ANOVA to Test the Effect of Number of Functional Groups on Total Dry-weight Biomass.

Source	DF	SS	MS	F	P
Number of Functional Groups	2	326	163	1.21	0.318
Error	22	2965	135		
Total	24	3291			

significant difference in the total plant biomass (Table 11a). Of the 7 possible functional combinations, the Legume-only type (L) had significantly lower biomass than either the Legume/Grass (L/G) or Forb (F) types (Table 11b). Obviously, genetically larger plants can acquire more biomass than genetically smaller plants, but a closer examination of Figures 7,8, and 9 highlights the differences of particular functional combinations. Some combinations are merely an average between the biomass of the 3 or 3 component species in the mixture. In some cases, the largest species appeared unimpacted by neighbors as exemplified by the fact that the clover and sunflower, bermuda and sunflower, and the fescue and sunflower combinations' total aboveground biomass is at the same height as the sunflower alone. In contrast, both the bermuda and fescue showed a large increase over their control plots when combined with the radish (Figure 7).

Functional Group Variance

Final analyses of variance linked to functional group number indicated that stability was gained through increasing the number of functional groups. A comparison between 1, 2, and 3 functional groups showed a significant difference ($p< 0.03$), and the variances associated with increased functional group number declined (Table 12). The variance of 3 groups was significantly lower than the variance of either 1 group ($p< 0.01$) or 2 groups ($p< 0.01$), but there was no significant difference between 1 and 2 functional groups ($p= 0.99$). In addition, figure 10 shows individual variances and standard errors to relate stability to increased functional group number.

Table 11a. Results of One-way ANOVA to Test the Effects of Type of Functional Combination on Total Dry-weight Biomass.

Source	**DF**	**SS**	**MS**	**F**	**P**
Type of Functional Combination	6	2116.4	352.7	5.41	0.002
Error	18	1174.5	65.3		
Total	24	3290.9			

11b. A Posteriori (Tukey's) Test to Show the Location of Differences Associated with the One-way ANOVA.

TYPE OF COMBINATION:	**L/F**	**F**	**G/F**	**L/G/F**	**L/G**	**G**	**L**
MEAN:	29^{a}	27^{a}	19^{ab}	12^{ab}	11^{ab}	11^{ab}	0.9^{b}

- L(legume), G(grass), F(forb).
- Means followed by different superscripts are significantly different.

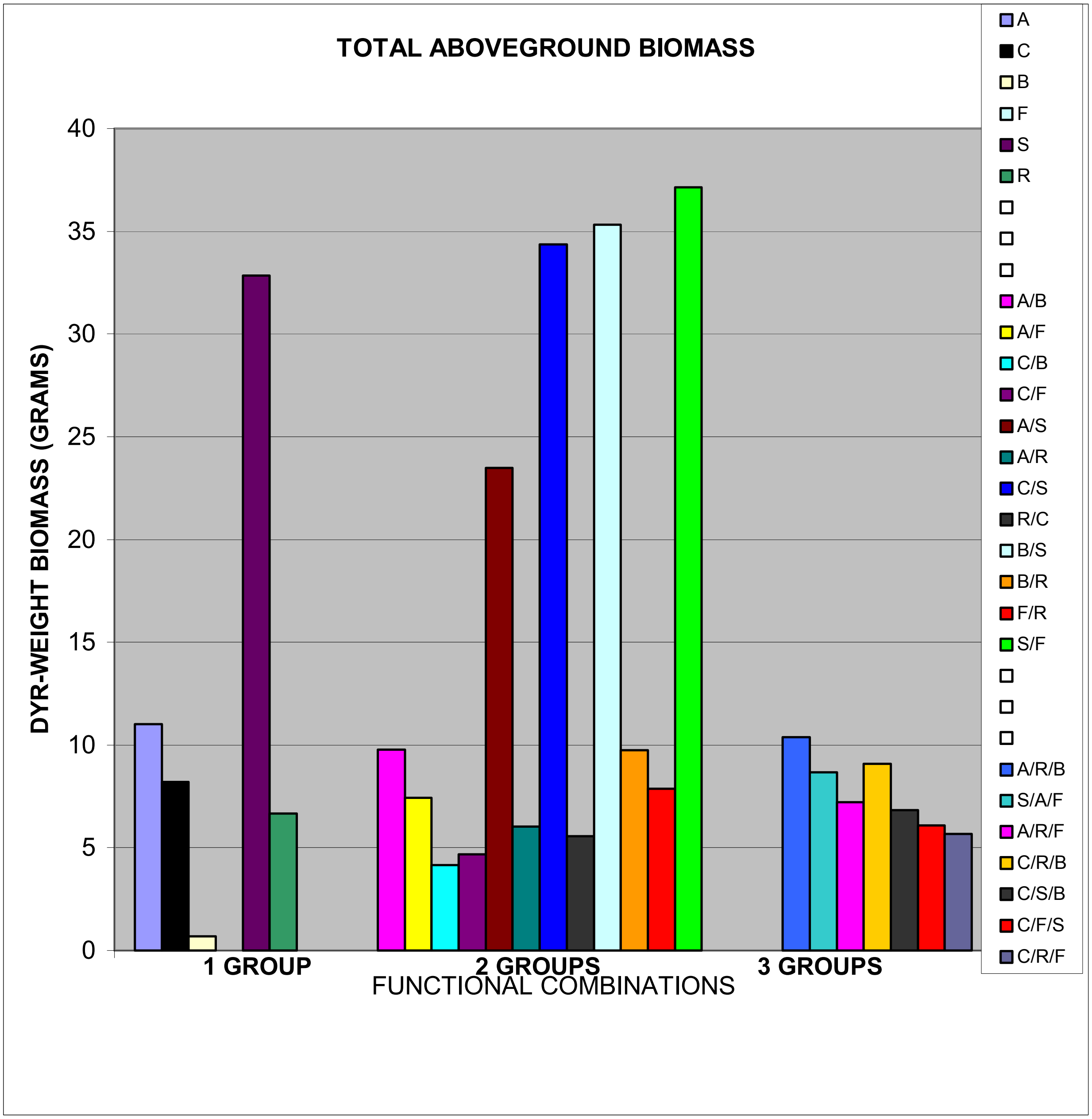

Figure 7. Total Aboveground Dry-weight Biomass per Number of Functional Groups

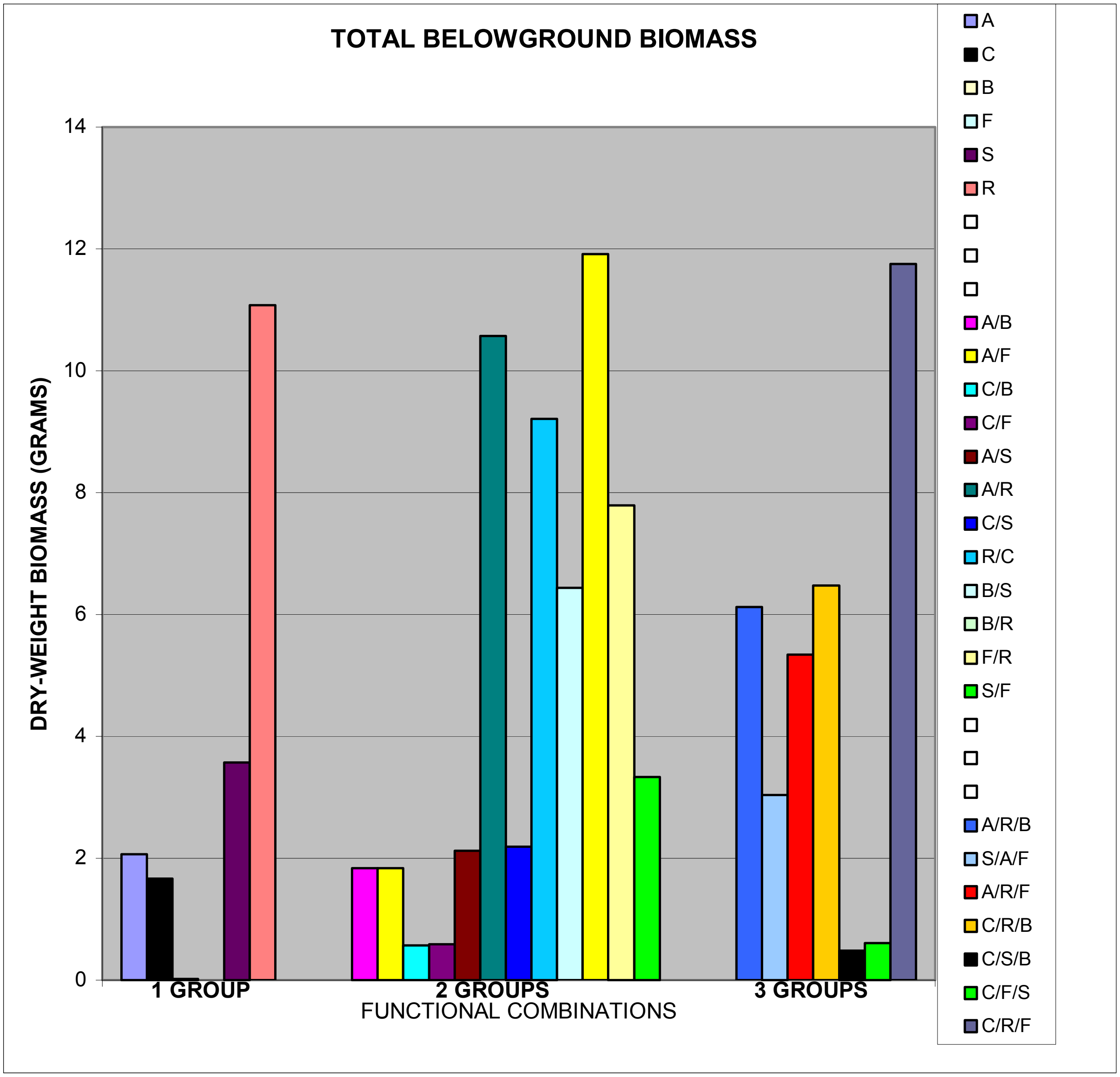

Figure 8. Total Belowground Dry-weight Biomass per Number of Functional Groups

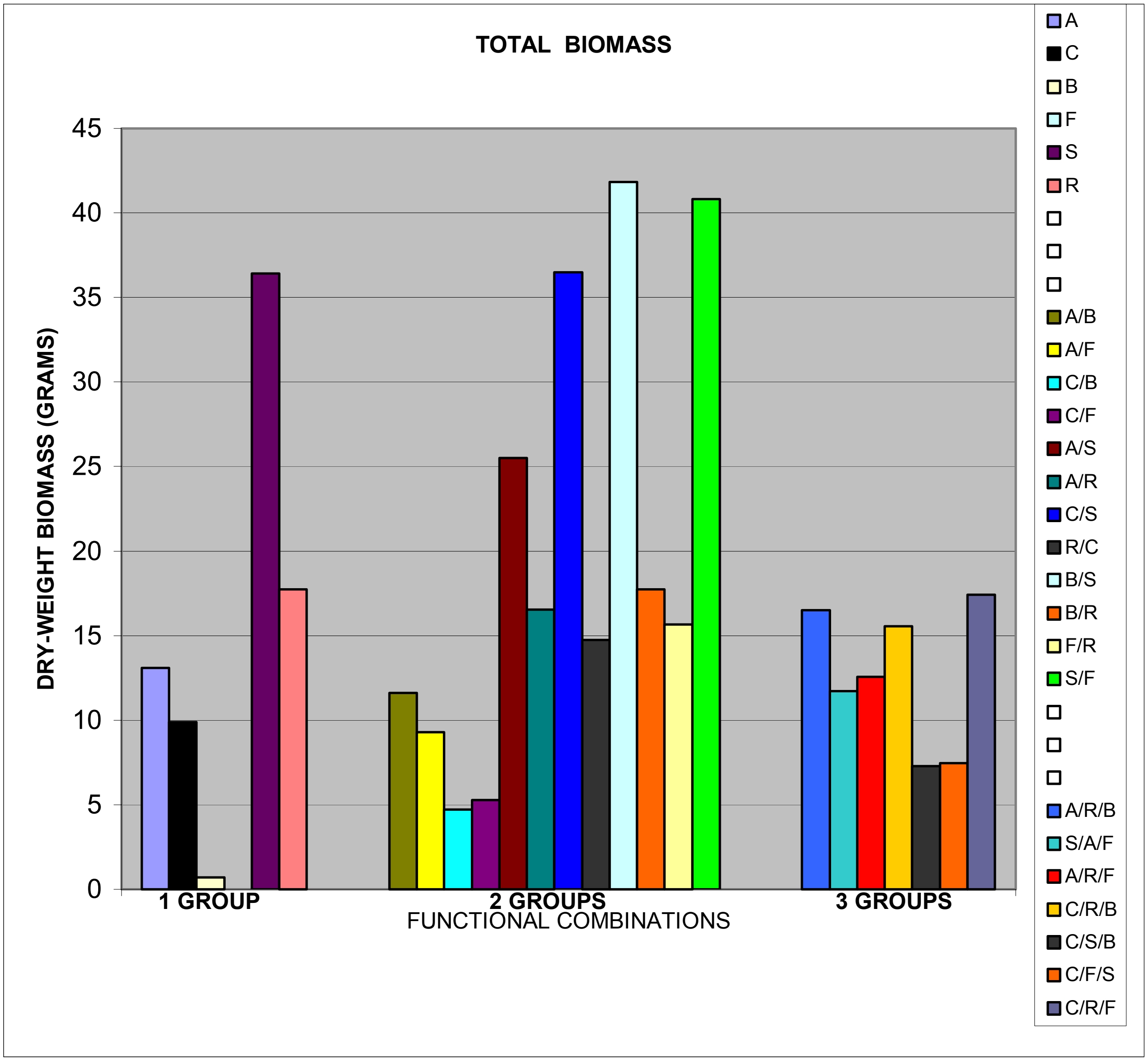

Figure 9. Total Dry-weight Biomass per Number of Functional Groups

Table 12. Results of the Test of Homogeneity of Variance Between 1, 2, and 3 Functional Groups

Group	Degrees of freedom	s_i^2	ln s_i^2
1	5	179.48	5.19
2	11	174.19	5.16
3	6	17.03	2.83

$X^2 = 7.55$
$P < 0.03$

-Significant difference between functional group number variances

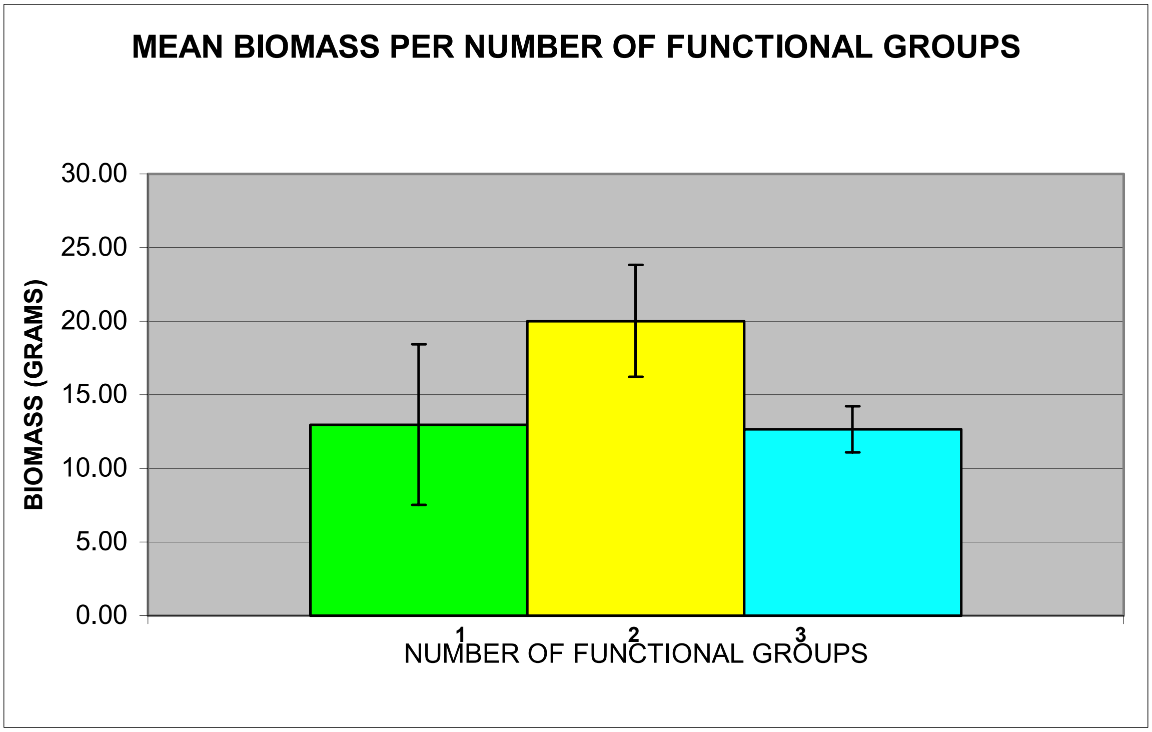

Figure 10. Mean Biomass per Number of Functional Groups (+/- SEM)

CHAPTER 4

DISCUSSION

Classroom Adventures

An effective biology classroom is one in which students learn by participating in different activities. The best classroom adventures would include many subtopics that build on a larger common educational goal. As the shifts in subtopics proceed, students will be able to gain the experience of a researcher not only through learning biology but also by applying mathematics, statistics, data collection, and writing. Biological research and the scientific method are based on answering a question, and for the beginning student peripheral knowledge and experience is gained during the research project. Research projects are able to give students a discovery process because the experience is theirs (Henton 1996).

Along with the individual quest for knowledge and experience, students need to be able to work in a group and understand that there are many different types of opinions and unique ideas. In a society full of star athletes and a need to be the best, we sometimes forget that one idea is not the best, rather a combination of parts of many different ideas may create the most accurate answer. Similarly, scientific knowledge results from an accumulation and synthesis of diverse ideas and experiences. Group involvement may also assist students with skills that will help them mature into more outgoing and confident people. Small groups are able to strengthen skills related to active learning such as talking, listening, reading, writing, and reflecting (Meyers and Jones 1993). From this type of research approach, active learning will be achieved through the experiments and the interaction with peers.

Methods

The planning behind this experiment on functional diversity included active learning and group participation. Biology is a subject that can easily be used in an active learning classroom. This research project was designed to excite the student with a topic that is both current and a subject of considerable debate between opposing views among conservation biologists. The concept of functional diversity is easily understood and can be related to many aspects of a student's life and thought processes. Some students have difficulty relating some subjects and/or topics to their present or future lives, but functional diversity relates to 1 aspect of all lives: eating. Students will be able to relate to the need for food as well as the impact that the increasing world population will have on the requirement for better farming methods. Mixtures encompassing functional diversity have already been used to double rice yields and nearly eliminate devastating rice diseases in China, remarkably without using chemical treatments or extra money (Yoon, 2000). Active learning is used as the students gain knowledge through actively setting the experiment up, collecting data, analyzing data, and presenting results.

The proposed classroom project places students into research teams that interact over a period of months. There will be 3 teams (Team 1: 1 functional group; Team 2: 2 functional groups; and Team 3: 3 functional groups). Using teams will give the opportunity to enhance organization, generate a wider range of ideas, and build teamwork. There are many different kinds of "intelligence": some students are good at writing; some are good at mathematics; while others are good with computers or organization. A research project that involves the scientific method allows a group of students to bring their better qualities together, and helps other students to learn from others whose strengths differ. The scientific method involves answering questions and allows the use of other skills including; organization, writing, mathematics, computer, library, and other important skills.

At the end of the growing process, each group will be able to present information in it's unique way. This could be a first encounter with the scientific method for many students, and the teacher would need to assist the students with creating testable ideas and answerable questions. Questions have to come from some observation gained through nature or past studies. 1 question that relates to many recent studies is; does the number of species or the number of functional groups produce more biomass? Another question could cover functional composition, and concern the number of functional groups or the type of functional combination in regards to biomass. Most classrooms do not have the option of visiting research sites or setting up large or lengthy experiments, so current papers will be a good start. Finally, the ideas and hypotheses created from the questions need to be testable within the bounds of a classroom. As we move into experimental designs, students need to be accustomed to basic design features, which include experimental groups, control groups, replication, and the experimental variable being tested. Examples used with the functional diversity experiment are simple and easy to use.

Growth Area

An outdoor growth area, used initially for the development of this experiment, is an option, depending on the soil conditions, confidence in transplantation, availability for weed removal, and nutrient attention. The area that was used for the 2^{nd} testing phase of the experiment was a large growth chamber with automatic settings for light and temperature (Table 2). In a classroom without a growth chamber, a large table and a windowsill will work for the growth area. The use of items that are available to students at home could lead to a discussion about early researchers who had only limited and simple research tools. Extra credit could be given to students who would be willing to grow replicates at home under similar environmental conditions as those in the classroom.

Data Collection

The data collection process has been designed in a manner that allows students to collect and observe plants during the growth phase and also at the end of the experiment. Having collection times during the growth phase helps keep students engaged throughout the experiment. Observations can be conducted weekly. Plant vigor indicators such as leaf number, length of the largest leaf blade, and shoot height can be plotted over time to show developmental trends. Tracking trends may help students appreciate significant differences that arise from further analysis. In addition to the importance that the timed observations give to uncovering trends, they also keep the student actively participating in the project. Watering will have to been done every 2 to 3 days and it is a routine task that does not require much mental involvement yet does incorporate the responsibilities behind research methods. Weekly measurements will take about 10 to 20 minutes per student, which gives students a closer identity to their individual plants and to the project as a whole.

The final data collection process requires more precision and patience from the students than the weekly measurements. The teacher will need to show the students in a detailed manner the process behind removing aboveground biomass, paying careful attention to the removal of soil from belowground biomass. This final data collection of the experiment should have been discussed throughout, and the younger students should know that careful attention is needed. The aboveground removal is an easy step that only requires the cutting of the aboveground biomass at the soil level. The belowground removal requires more attention and patience because the roots will break easily. Containment of the plants in plastic bags is simple and cheap. Bag number correlates to the group number obtained early in the project, lessening confusion.

Aboveground and belowground wet weight measurements can be taken to assess the drastic differences between the wet and dry weight of plants, giving students an idea of how much water is inside a plant. Weighing of the wet plants is also good practice for the final dry weighing of the plants. Wet weighing does not require removal of the plant from the plastic bag, as the bag is included when taring the balance prior to the weighing procedure. Dry weighing is more time consuming because plants must be dried in an oven. If a drying oven is not available, then drying can be accomplished by leaving the plants in a press for a more extended amount of time or a kitchen oven.

Showing students the process of pressing and drying plants allows the teacher to present another activity of importance to biological research. Some students may become interested in plant collection and choose to make it an ongoing activity. During the drying process, the plants will have been labeled with a number on the newspaper beside the plant. Weighing the dry plants can be done with the same balances as before. The plants will be out of the plastic bags from the drying process, and the placement of the plants back into the bags would be a good idea so the plants may be used for any future analyses.

Data Presentation

Data presentation is the final aspect of the scientific method, and there are many ways to analyze and present data. Students who have just started high school may be unfamiliar with statistical methods. Therefore, a statistical analysis is optional, depending on the age group and grade level of the class. The teacher should present some basic forms of statistical analysis such as comparing means, standard deviations, standard errors of the mean, and speak of their importance. ANOVAS should be avoided for classes that have not learned about them. Graphical analysis is an important aspect to science investigations, and a graphical presentation should be included in all student reports because they efficiently convey results.

Data can be presented in a variety of different ways depending upon grade level and student skills. Graphic, verbal and statistical analyses can be tailored to the students' level. Computers may also assist the student. Graphs of weekly growth measurements, wet aboveground/belowground/total biomass, and dry aboveground/belowground/total biomass all need to be included in the analyses to observe trends related to the functional number or the functional combination.

Functional Group Number

The 1st factor considered in the results was; Does the number of functional groups impact total biomass? This question represents a modern perspective on species diversity. The role of functional diversity, is 1 of the 2 aspects of biodiversity that distinguishes opposing sides of the current controversy in conservation biology. The data produced from this experiment showed the number of functional groups in a sample plot does not lead to differences on ecosystem productivity or sustainability. There were no significant differences between the aboveground, belowground, or the combined plant biomass when comparing the numbers of functional groups.

Functional Group Combinations

The 2nd factor considered relates to varying types of functional group combinations in a biological community. This factor is also a leading part of the argument for maintaining functional diversity. Particular functional combinations may have evolved to work together, while other possibly positive combinations that are set apart by genetic, physical, or environmental barriers may never combine without human intervention. Many future research projects await defining and identifying functional groups and experimentation with different combinations of the functional groups.

Is there a difference in biomass from unique combinations of functional groups? In this experiment there were significant differences in the type of functional group combinations. The total plant biomass showed higher significant differences in the Grass/Forb, Legume/Grass/Forb, Legume/Grass, and Grass combinations. The belowground biomass showed a marginally significant difference, but the aboveground did not. Special attention needs to be paid to the fact that both the aboveground and belowground were not significantly impacted by functional combinations, but the total biomass was. A likely reason underlying this difference is that the ratios of aboveground and belowground biomass are different between the plant species used. Radish plants have a huge belowground biomass in comparison to their aboveground biomass, while sunflowers are the opposite. Difference between the radish and the sunflower present differences between the forb group's species. Students will need to understand that these are early-stages in the defining of functional groups and that their ideas can be used in the search for more defining characteristics. This adds to the inquiry-based process behind the experiment.

Less Variance, More Stability

In addition to the biomass assays, a final analysis was directed toward the expectation that there will be less plot-to-plot variance in more stable communities. Therefore, variation can be used as an indicator of stability. A simple Bartlett's test comparing the variance between 1, 2, and 3 functional groups revealed that there was more stability with increasing numbers of functional groups. We may expect communities to maintain a more constant level of productivity as functional groups are added (Figure 10).

Concluding Remarks

The need for new and innovative teaching methods grows constantly. The way that students learn is related to the changing times and their attitudes toward education. Educators need to focus on the minds and environments of students as well as involving them in active learning. Teachers may not be able to make students interested in every aspect of their educational goals, but we can attempt to introduce students to different types of learning techniques: writing, computers, experimenting, talking, mathematical analysis, etc. Along with new innovative techniques, there are past methods that may work for students such as memorization and extensive lecturing. A teacher is not just a presenter of information, but a psychologist, an inventor, and most importantly, a person who believes that the student can learn through various strategies. This project attempts to aid students in answering a current and ongoing argument in the field of conservation biology. Many students enjoy a good argument as much as they love challenging authority, so the topic suits teenage personalities. Beyond this there is an interactive system that allows the students to work in dynamic groups. This provides the students with skills that they will use in a working society, on an athletic team, or at home. Grouping also enables teachers to focus on smaller groups instead of 20 to 25 students at a time.

Using the scientific method is an integral aspect of the project. Students have the opportunity to use and understand how this method can be adapted in their coursework in biology and other classes. Reading recent studies will give the students some suggestions by which they may learn how others use the scientific method. Students can create their own hypothesis prior to their own attempt. The set up by which the hypotheses can be tested is also related to recent studies, and it is simple enough for beginner students to understand. Results from the experiment may vary for different classrooms, and the results will require methods for analysis.

If the students stick to using graphs for analysis, the weekly measurements will be a good start in finding trends in the data (Figures 4-10). Figure 10 represents the mean amount of biomass in relation to the number of functional groups combined and represents added stability or predictability through increased functional groups. The conclusion that stability and predictability of the system is strengthened is derived from a demonstration that the standard error is reduced. Recent studies have also found similar results (Lehman and Tilman 2000). The graphs on plant biomass are important for analysis because they represent biomass differences from unique functional combinations of all species together (Figures 1-3). Other combination differences are found in graphs, which represent all the different types of plant mixtures based on the comparison of one individual species (Figure 11). These graphs can be broken down into aboveground and belowground biomass (Figures 11-12). Other figures can be produced to represent other species' combinations. These 2 figures show trends related to radish growth with varying numbers of functional groups. Belowground biomass has better biomass production when in 3 functional groups (Figure 12). Aboveground biomass production follows similar trends, yet contains an increase with grass combinations (Figure 11). Students should accomplish a comparison of other combinations. If the classes are ready for statistics, then a statistical analysis discussion based on means, standard deviations, standard errors of the mean, t-tests, and ANOVAS will be appropriate for use. These steps to the scientific method are pertinent to know in the field of biology, as the scientific method is discussed in several high school biology classrooms and in all college level courses including ecology, genetics, biochemistry, and conservation biology. The above courses can be combined or used separately in an attempt to identify functional roles of plant species and combinational effects.

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APPENDICES

APPENDIX A

Lesson Plans for an Inexpensive Experiment on Functional Diversity

Day 1.

* Discuss the scientific method.
* Discuss Biodiversity (the variety of organisms on the planet).
* Ask students to explain Biodiversity in their own words (groups of 4).
* Talk about current events and studies surrounding Biodiversity.
* Present a movie concerning biodiversity to excite the students.
* (Homework) Have students bring in a picture and description of their favorite organism(s).

Day 2.

* Discuss homework.
* Introduce concept of functional diversity.
* Compare and contrast functional diversity and biological or species diversity.
* Describe possible functions of the students' chosen organisms.
* Explain different functions already identified (nitrogen-fixation, strong roots, allelopathy, etc.).
* Assign previously identified functions to students and have them combine their functions in a manner that could be positive for the environment.
* Ask students to explain why certain combinations would and would not be expected to work.
* Search for other plant characteristics that would be considered as different functions (current topic).
* Discuss the first and second parts of the scientific method (observation and asking questions).
* Ask the class what they observe about biological and/or functional diversity and have them think of questions from the observations.
* Explain the reasons for doing the experiment and the questions being asked.
* (Homework) Write in your own words a comparison/contrast of functional and biological diversity. Ask some questions that arise concerning the topic.

Day 3.

* Discuss homework.
* Have the class discuss their own experimental approach.
* Discuss the experimental variable, control group, experimental group, constants, and types of comparisons.
* Present the design used in this study.
* Prepare to plant the seeds on Day 4.
* Assign students their individual functional combination and combination number.
* Pass out and explain data sheets (attachment B).
* Explain the phenotypic characters to measure (shoot height, leaf number, and largest leaf blade).

* Demonstrate measurement techniques.
* Gather 25 pots (6in. by 6in. by 6in.).
* Label the pots with the number assigned to each student.

Day 4.

* Fill the pots to the top with soil (Fafard 3B).
* Use fingertips to make a 2cm hole in the soil.
* Insert seeds into the holes.
* Place the seeds in the following locations for optimal spacing.

one species			two species			three species		
1	1	1	1	2	1	1	2	3
1	1	1	2	1	2	3	1	2

1= functional group representative #1
2= functional group representative #2
3= functional group representative #3

* Place the plants into a flat. Cover the entire bottom of the pot with water (>4cm tall.)
* Keep the flat filled with water, and slowly increase the amount of water placed above the seed (start with 10 drops from a common dropper).
* Throughout the experiment keep the light, temperature, and water levels constant.
* Flats will need to have water stay above ground level ("Field Capacity").

Day 5.

* Review the scientific method (give examples).
* Have students form hypothesis.
* Point out the "control".
* What has to be kept constant.
* Discuss presentation and analysis of data (statistics, graphs, and words).

Final Measurements

* Plant growth will be measured until plants begin to bolt and/or flower (approximately two months in a growth chamber or window sill).
* Plants will be cut at ground level and placed in a labeled bag.
* Roots will be exposed by carefully washing off the soil and placed in a labeled bag.
* Aboveground and belowground biomass will be dried by pressing and then placing in a drying oven.
* Drying oven time will be 17 days and the temperature will be 125 ^{0}C (use a cooking oven if necessary.
* Biomass assays will be done with a scale, balance, and microscale.

APPENDIX B

Data Table for Bi-weekly Analyses

Plant ID #	ID	germination date	leaf number	shoot height	Length of largest blade	aboveground biomass	belowground biomass	
1a	A							
1b	A							
1c	A							
1d	A							
1e	A							
1f	A							
2a	C							
2b	C							
2c	C							
2d	C							
2e	C							
2f	C							
3a	B							
3b	B							
3c	B							
3d	B							
3e	B							
3f	B							
4a	F							
4b	F							
4c	F							
4d	F							
4e	F							
4f	F							
5a	S							
5b	S							
5c	S							
5d	S							
5e	S							
5f	S							
6a	R							
6b	R							

Data Table for Bi-Weekly Analyses

Plant ID #	ID	germination date	leaf number	shoot height	Length of largest blade	aboveground biomass	belowground biomass	
6c	R							
6d	R							
6e	R							
6f	R							
7a	A							
7b	B							
7c	A							
7d	B							
7e	A							
7f	B							
8a	A							
8b	F							
8c	A							
8d	F							
8e	A							
8f	F							
9a	C							
9b	B							
9c	C							
9d	B							
9e	C							
9f	B							
10a	C							
10b	F							
10c	C							
10d	F							
10e	C							
10f	F							
11a	A							
11b	A							
11c	S							
11d	A							
11e	S							
11f	S							
12a	A							
12b	R							
12c	A							

Data Table for Bi-weekly Analyses

Plant ID #	ID	germination date	leaf number	shoot height	length of largest blade	aboveground biomass	belowground biomass	
12d	R							
12e	A							
12f	R							
13a	C							
13b	S							
13c	C							
13d	S							
13e	C							
13f	S							
14a	C							
14b	R							
14c	C							
14d	R							
14e	C							
14f	R							
15a	B							
15b	S							
15c	B							
15d	S							
15e	B							
15f	S							
16a	F							
16b	S							
16c	F							
16d	S							
16e	F							
16f	S							
17a	B							
17b	R							
17c	B							
17d	R							
17e	B							
17f	R							
18a	F							
18b	R							
18c	F							
18d	R							

Data Table for Bi-weekly Analyses

Plant ID #	ID	germination date	leaf number	shoot height	length of largest blade	aboveground biomass	belowground biomass
18f	R						
19a	F						
19b	S						
19c	F						
19d	S						
19e	F						
19f	S						
20a	A						
20b	A						
20c	R						
20d	R						
20e	B						
20f	B						
21a	A						
21b	A						
21c	S						
21d	S						
21e	F						
21f	F						
22a	A						
22b	A						
22c	R						
22d	R						
22e	F						
22f	F						
23a	C						
23b	C						
23c	S						
23d	S						
23e	B						
23f	B						
24a	C						
24b	C						
24c	R						
24d	R						
24e	B						

Data Table for Bi-weekly Analyses

Plant ID #	ID	germination date	leaf number	shoot height	Length of largest blade	aboveground biomass	belowground biomass	
25a	C							
25b	C							
25c	S							
25d	S							
25e	B							
25f	B							
26a	C							
26b	C							
26c	S							
26d	S							
26e	F							
26f	F							
27a	C							
27b	C							
27c	F							
27d	F							
27e	R							
27f	R							

VITA

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