ECOLOGICAL STUDIES OF WILLOW (*SALIX CAROLINIANA*): MONTHLY STATUS REPORT #9

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Ecological Studies of Willow (*Salix caroliniana*): Monthly Status Report #9
Covering the time period from October 1-31, 2009

This status report summarizes progress made on the Ecological Studies of Willow project through October 31, 2009, with reference to the tasks and timeline outlined in the Scope of Work and presented in Table 1 below.

Table 1. Timeline of tasks to be accomplished in Years 1 & 2. Tasks initiated and underway in this reporting month are highlighted in blue, completed tasks in red.

YEAR 1

Quarter	Months	Tasks accomplished
1st	Oct – Dec, 2008	Initiate and complete Task 1 (*Finalize research plan*)
2nd	Jan – Mar, 2009	Initiate Task 2.1 (*Germination & early survival and growth experiments*) Initiate Task 2.4 (*Life history*)
3rd	Apr – Jun, 2009	Continue Task 2.1 (*Germination experiment*) Initiate Task 2.2 (*Willow transplantation*) Initiate Task 2.3 (*Fire response*) Continue Task 2.4 (*Life history*)
4th	Jul – Sep, 2009	Continue Task 2.4 (*Life history*) Complete Tasks 2.1 & 2.2 (*Germination experiment & Willow transplantation*) Complete Task 3.1 (*Data analysis and final report, Year1*)

YEAR 2

Quarter	Months	Tasks accomplished
1st	Oct – Dec, 2009	Continue Task 2.3 (*Fire response*) Continue Task 2.4 (*Life history*)
2nd	Jan – Mar, 2010	Continue Task 2.3 (*Fire response*) Continue Task 2.4 (*Life history*) Initiate Task 2.5 (*Spatial analysis of willow distribution*)
3rd	Apr – Jun, 2010	Initiate Task 2.2 (2nd iteration, *Willow transplantation*) Continue Task 2.3 (*Fire response*) Continue Task 2.4 (*Life history*) Continue Task 2.5 (*Spatial analysis of willow distribution*)
4th	Jul – Sep, 2010	Complete Task 2.2 (2nd iteration, *Willow transplantation*) Continue Task 2.3 (*Fire response*) Continue Task 2.4 (*Life history*) Continue Task 2.5 (*Spatial analysis of willow distribution*) Complete Task 3.2 (*Data analysis and final report, Year2*)

Progress on Task 2.1 – Germination and Early Survival and Growth Experiments

The UCF team completed the greenhouse experiments on willow seedlings and cuttings in early October. All data were entered and proofread, including dry mass of the above-ground tissue versus roots. Below we present our initial analyses of the seedling experiment, which is the more challenging of the two because of high mortality in certain watering treatments (Table 2). This led to an unbalanced design, which required a careful analysis. Once this challenging analysis is complete, we expect analysis of the cutting data will be very straightforward because few plants died and the design is nicely balanced, with no missing cells.

Table 2. Number of individuals per treatment combination that survived the whole experiment. Soil types are Blue Cypress (BC), River Lakes (RL), St. Johns (SJ) and their 50:50 mixtures. Each plant was subjected for a month to four watering regimes (ambient water, mild drought, standing water and episodic flooding) in random order.

Nutrients	Soil type					
	BC	BC/RL	BC/SJ	RL	SJ	RL/SJ
Control	3	2	4	0	2	1
micronutrients	4	2	2	4	2	2
N	3	4	3	1	3	0
N P	4	3	3	1	3	0
N P micronutrients	4	5	5	2	0	1
P	4	1	3	4	3	2

Plants on substrate combinations that contained BC soil had at least one survivor per treatment combination, so we analyzed a subset that including only those plants on BC soils and its combinations. We analyzed the variation in growth in both height and crown diameter:

$$growth = \log\left(\frac{final\_measurement}{initial\_measurement}\right)$$

This transformation accounted for individual variation in size and reduced the temporal correlation between consecutive evaluations (Table 3).

Table 3. Correlation of willow seedling height and crown growth between consecutive monthly intervals defined by the time when plants switched watering regimes.

Height growth				
Comparison	1 vs 2	2 vs 3	3 vs 4	4 vs 5
R square	0.133	0.388	0.148	0.005
Probability	0.127	0.000	0.156	0.962
N of plants	133	107	93	90
Crown growth				
Comparison	1 vs 2	2 vs 3	3 vs 4	4 vs 5
R square	-0.260	-0.304	-0.133	-0.003
Probability	0.003	0.002	0.205	0.981
N of plants	131	106	93	90

We controlled additional non-treatment variation by using size at the beginning of each interval as a covariate. This allometric function accounted for significant variation in both variables (Fig. 1, Tables 4-6). One seedling plant that lost all its leaves between treatments but did not die had exceptional decrease in crown diameters. We therefore analyzed the data with and without this outlier.

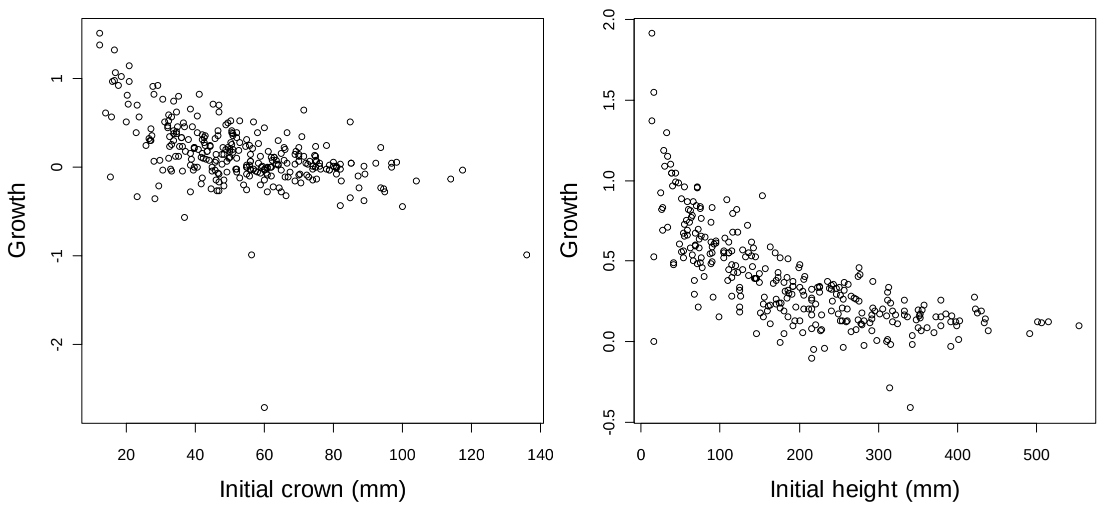

Fig. 1. Allometric relationship between initial size and growth in willow height and crown diameter. Notice the outlier in crown diameter, at 60 mm initial crown.

Table 4. Analysis of covariance of crown diameter growth as a function of soil, nutrients and watering regime. Log of initial size was used as covariate and the outlier seedling number 171 was eliminated from the analysis (r^2=0.50). Table entries are sources of variation, degrees of freedom (df), sums of squares (SS), mean squares (MS), F-ratios (F) and their associated probabilities (P).

Source of variation	df	SS	MS	F	P
log(initial)	1	12.799	12.799	220.6559	<0.0001
Soil	2	1.8725	0.9362	16.1407	<0.0001
Nutrients	5	0.4341	0.0868	1.4968	0.191
Watering regime	3	0.5767	0.1922	3.3139	0.020
log(initial) x Soil	2	0.3527	0.1763	3.0401	0.049
Residuals	276	16.0092	0.058		

Table 5. Analysis of covariance of growth in willow seedling crown diameter as a function of soil, nutrients and watering regime. Log of initial size was used as covariate. All plants on BC soil or its combinations were included (r^2=0.44). Table entries are sources of variation, degrees of freedom (df), sums of squares (SS), mean squares (MS), F-ratios (F) and their associated probabilities (P).

	df	SS	MS	F	P
log(initial)	1	13.3638	13.3638	165.4458	<0.0001
Soil	2	2.6211	1.3105	16.2245	<0.0001
Nutrients	5	0.437	0.0874	1.082	0.370
Watering	3	0.8845	0.2948	3.6501	0.013
log(initial) x Soil	2	0.2792	0.1396	1.7282	0.179
Residuals	281	22.6977	0.0808		

Table 6. Analysis of covariance of growth in willow seedling height as a function of soil, nutrients and watering regime and interactions. Log of initial size was used as covariate. All plants on BC soil or its combinations where included (r^2=0.748). Table entries are sources of variation, degrees of freedom (df), sums of squares (SS), mean squares (MS), F-ratios (F) and their associated probabilities (P).

	df	SS	MS	F	P
log(initial)	1	18.1312	18.1312	718.346	<0.0001
Soil	2	0.7215	0.3608	14.2936	<0.0001
Nutrients	5	0.4268	0.0854	3.3818	0.006
Watering regime	3	0.2098	0.0699	2.7703	0.042
log(initial) x Soil	2	0.6441	0.3221	12.7603	<0.0001
log(initial) x Nutrients	5	0.5595	0.1119	4.4336	<0.0007
Residuals	276	6.9663	0.0252		

The most informative model for growth in height included the main effect and interactions between soil and the covariable initial interval size and, between nutrients and the same covariable (Table 7).

Table 7. Akaike Information Criterion (AIC) and AIC weights for models of growth in willow seedling height. Sources of variation: S, Soil; N Nutrients; W, Watering regime.

	Models for height growth	df	AIC	Akaike weight
1	ln(init) + S + N + W + ln(init):S + ln(init):N	20	-227.86	0.998
2	ln(init) + S + N + W + ln(init):S	15	-215.07	0.002
3	ln(init) + S + ln(init):S	7	-211.25	<0.0001
4	ln(init) + N + ln(init):N	13	-196.76	<0.0001
5	ln(init) + S + N + W	13	-194.85	<0.0001
6	ln(init) + S	5	-188.71	<0.0001
7	ln(init) + N	8	-176.21	<0.0001
8	ln(init) + W	6	-170.32	<0.0001
9	ln(init)	3	-169.48	<0.0001

The most informative models for growth in seedling crown diameter included the main effects and interaction between soil and the covariable initial interval size, plus the other main effects (Table 8). Residuals of the best models (Fig. 2) were randomly distributed, normal and independent, illustrating the effectiveness of the allometric covariate.

Table 8. Akaike Information Criterion (AIC) and AIC weights for models of growth in willow crown diameter.

	All data		Crown (all data)		Crown (-171)	
	Model	df	AIC	Akaike weight	AIC	Akaike weight
5	ln(init) + S + N + W	13	110.19	0.316	15.26	0.164
2	ln(init) + S + N + W + ln(init):S	15	110.58	0.260	12.94	0.524
6	ln(init) + S	5	110.69	0.247	16.64	0.082
3	ln(init) + S + ln(init):S	7	111.43	0.170	14.82	0.204
1	ln(init) + S + N + W + ln(init):S + ln(init):N	20	118.00	0.006	18.93	0.026
8	ln(init) + W	6	131.57	0.000	38.18	<0.0001
9	ln(init)	3	136.91	0.000	42.32	<0.0001
7	ln(init) + N	8	141.91	0.000	43.91	<0.0001
4	ln(init) + N + ln(init):N	13	148.06	0.000	48.42	<0.0001

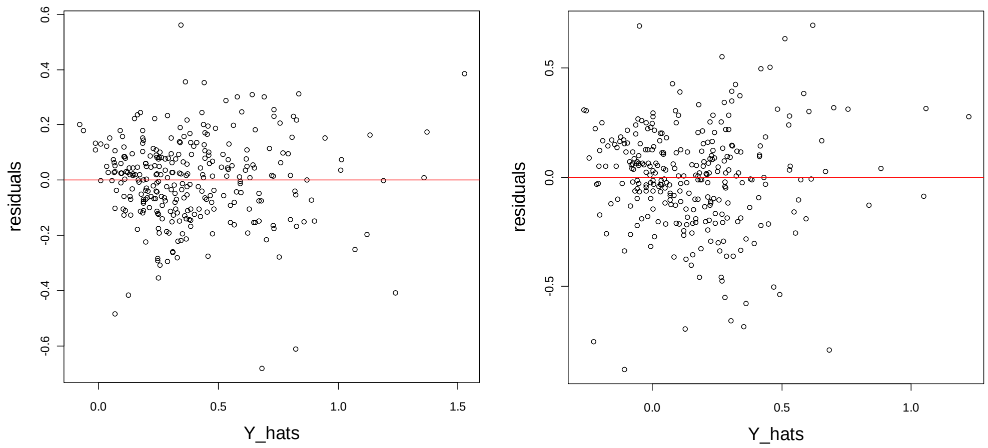

Fig. 2. Distribution of residuals for the best models: (left) height growth model #1; (right) crown growth model #5.

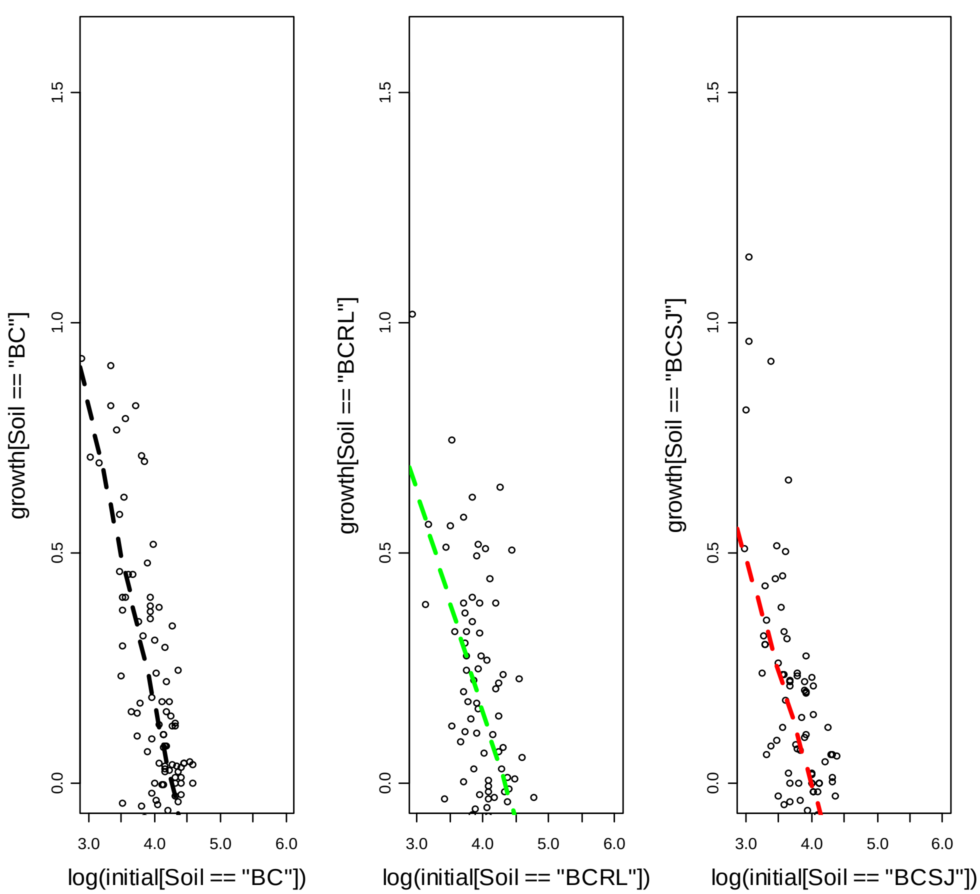

Fig. 3. Growth in willow crown diameter as a function of initial size and soil treatment. Notice the higher growth of seedlings on BC soil.

Growth in crown diameter and height varied with initial diameter entering the treatment period in the crossover design. Willow seedlings established on BC soil had the largest growth in both crown diameter and height (Figs. 3-4). Although we found weaker evidence for crown diameter.

Compared to the control, only the one-half watering treatment significantly affected willow height growth (Table 6, Fig. 5). One-half and submerged treatments affected crown diameter growth. Seedlings in these treatments grew slowly, if they survived. These results are consistent with the field competition and island experiments, where seedlings died during the dry season and at the highest elevations above marsh level, and did not survived when submerged.

Crown diameter varied among nutrients. There was weak evidence that plants under NP treatment had higher growth than those in the control. Growth in height of willow seedlings varied significantly among nutrient treatments (Tables 5 & 9). The most striking result was lower mean height growth in the nitrogen-addition treatments relative to the controls (Figs. 7-8). This result can arise if nitrogen addition promotes growth of microbes (soil algae, bacteria and fungi) that may interfere or compete with willow seedlings. We will test this idea in the field competition experiment, by adding nitrogen addition as a factor in the experimental design.

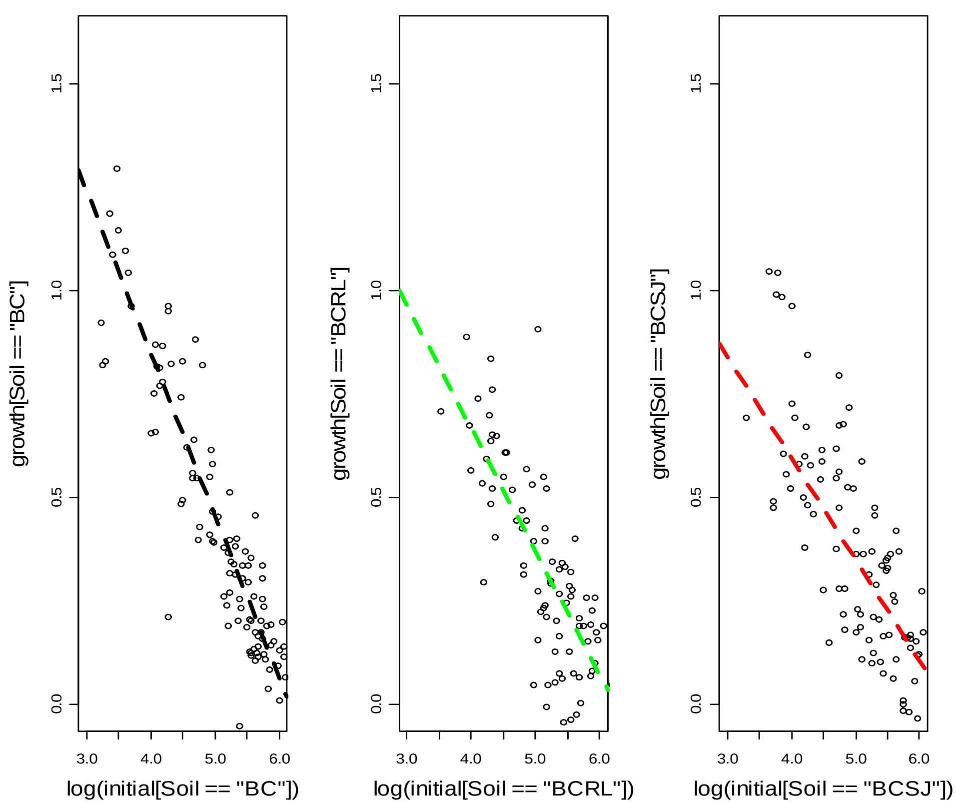

Fig. 4. Growth in willow seedling height as a function of initial seedling size and soil treatment. Notice the higher growth of seedlings on BC soil.

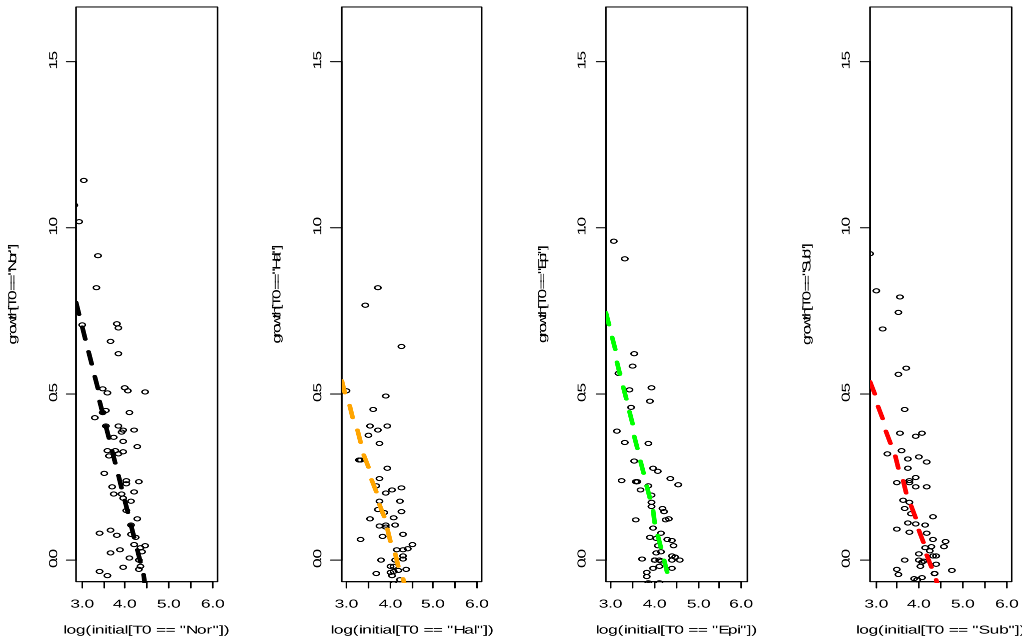

Fig. 5. Growth in willow crown diameter as a function of initial seedling size and watering treatments. Notice the lower growth of seedlings in the half and submerged n treatments. ambient water: Nor; mild drought: Hal; standing water: Sub; and episodic flooding: Epi)

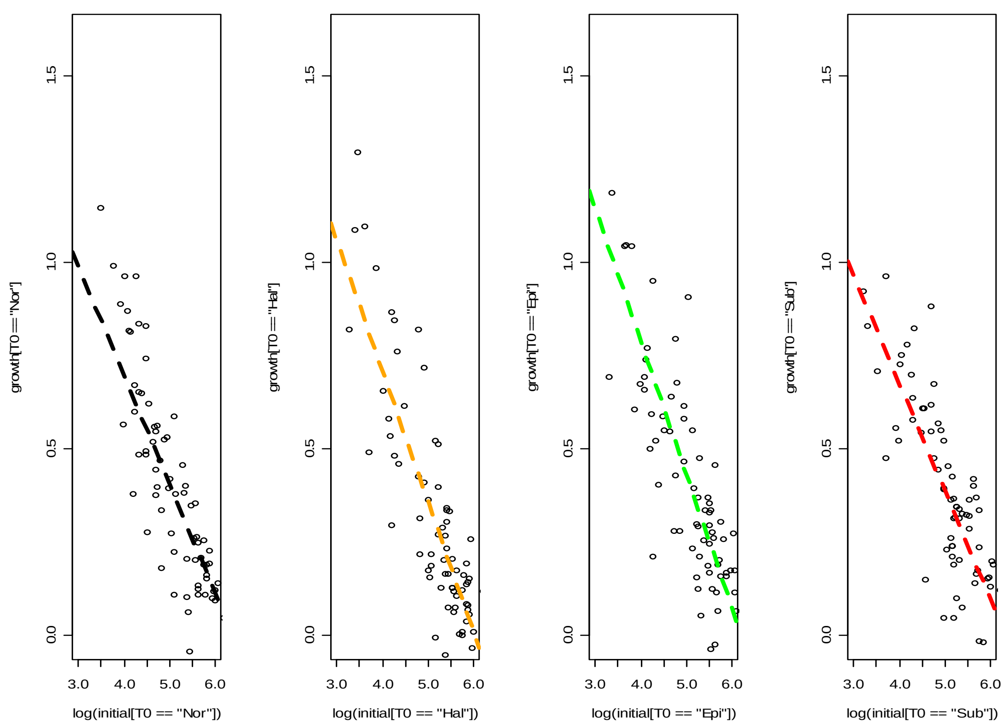

Fig. 6. Growth in willow height as a function of initial seedling size and watering treatments. Notice the lower growth of seedlings in the half and submerged n treatments.

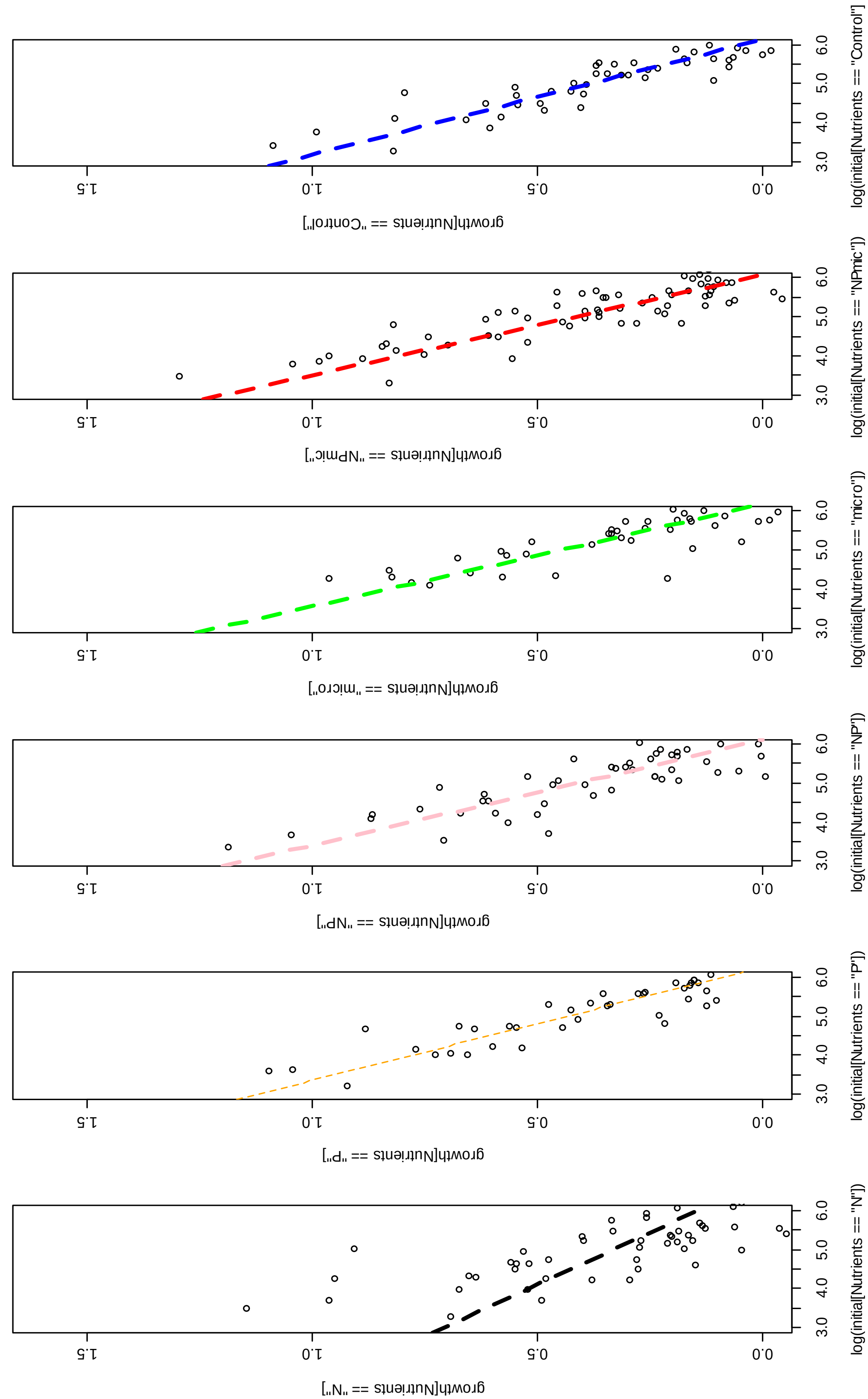

Fig. 7. Growth in willow seedling height as a function of initial seedling size and nutrient treatments. Notice the lower growth of seedlings in the nitrogen treatment.

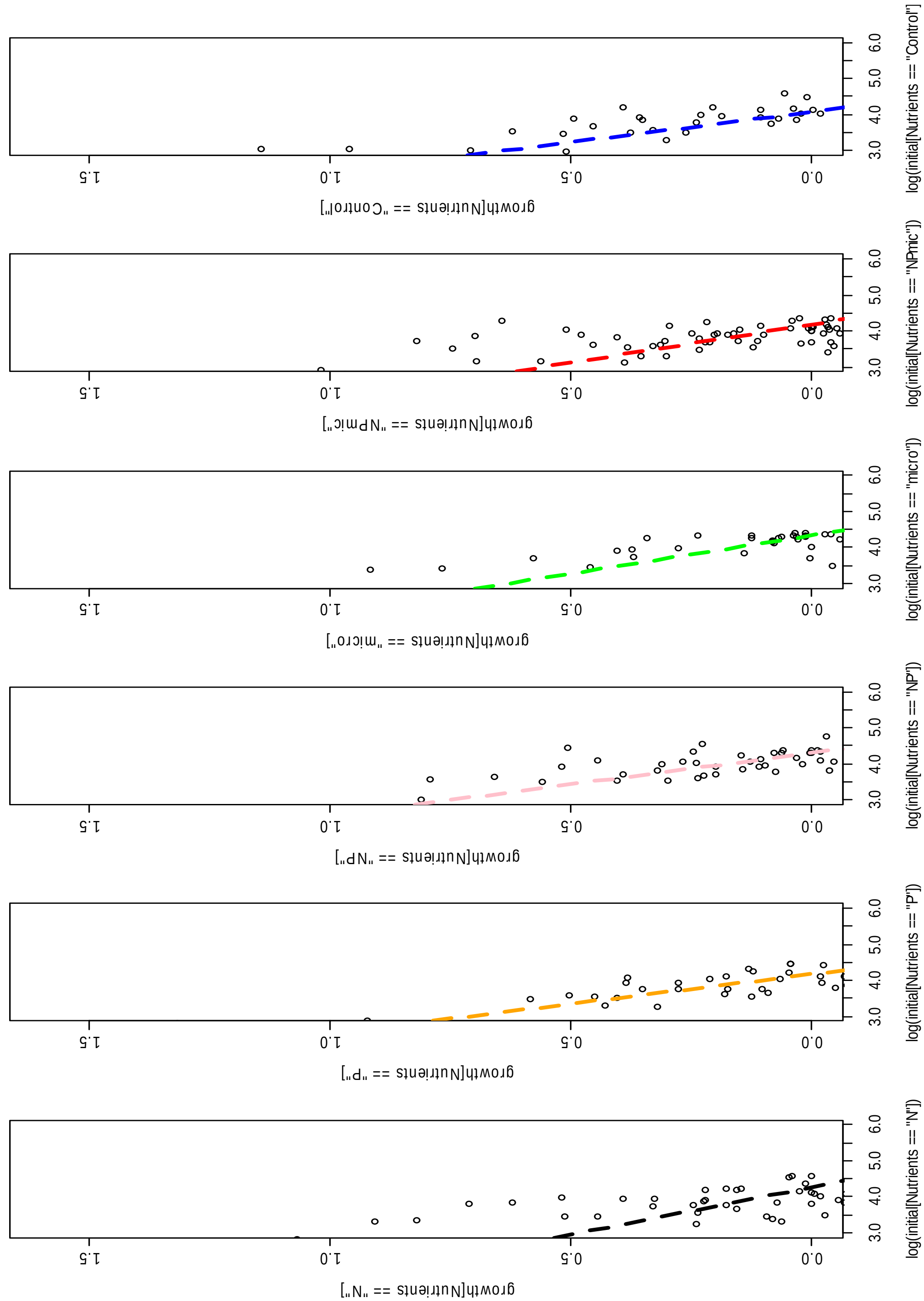

Fig. 8. Growth in willow seedling crown diameter as a function of initial seedling size and nutrient treatments. Notice the lower growth of seedlings in the nitrogen treatment.

Table 9. Simple contrasts among treatments levels for growth in willow seedling height. The reference treatments (BC for soils, ambient nutrient levels, and normal water) do not appear in the list. Statistically significant contrasts with reference treatments are in bold. Because contrasts were not independent we used Bonferroni $(\alpha' = \alpha / k)$ to obtain an experimentwise error rate of 0.017 for nutrient comparisons and 0.01 for watering treatments and soils.

Coefficients:	Estimate	Std.Error	t	Pr(>|t|)
(Intercept)	2.381	0.187	12.728	<0.0001
log(initial)	-0.386	0.037	-10.418	**<0.0001**
Soil BCD	-0.539	0.180	-2.994	**0.003**
Soil BCU	-0.678	0.147	-4.605	**<0.0001**
Nutrients micro	0.190	0.254	0.747	0.456
Nutrients N	-0.573	0.225	-2.550	**0.011**
Nutrients NP	0.203	0.226	0.896	0.371
Nutrients NPmic	0.368	0.225	1.634	0.103
Nutrients P	-0.050	0.235	-0.212	0.832
Episodic	0.020	0.026	0.760	0.448
Half	-0.053	0.026	-2.052	0.041
Submerged	-0.024	0.026	-0.922	0.357
log(initial) x SoilBCD	0.091	0.035	2.578	**0.010**
log(initial) x SoilBCU	0.117	0.029	4.028	**<0.0001**
log(initial) x Nutrientsmicro	-0.027	0.049	-0.553	0.581
log(initial) x NutrientsN	0.108	0.045	2.404	**0.017**
log(initial) x NutrientsNP	-0.034	0.045	-0.763	0.446
log(initial) x NutrientsNPmic	-0.065	0.044	-1.472	0.142
log(initial) x NutrientsP	0.017	0.047	0.372	0.710

Fig. 9 John Fauth separating the aboveground part of a willow cutting

Table 10. Simple contrasts among treatments levels for growth in crown diameter. The reference treatment does not appear in the list. Statistically significant contrasts are in bold. Because contrasts were not independent we used Bonferroni $(\alpha' = \alpha / k)$ to obtain an experimentwise error rate of 0.017 for nutrient comparisons and 0.01 for watering treatments and soils.

Coefficients:	Estimate	Std.Error	t	Pr(>|t|)
(Intercept)	2.849	0.222	12.822	**<0.0001**
log(initial)	-0.665	0.056	-11.970	**<0.0001**
SoilBCD	-0.793	0.358	-2.216	0.028
SoilBCU	-0.807	0.322	-2.509	**0.013**
Nutrientsmicro	0.076	0.056	1.365	0.173
NutrientsN	0.026	0.051	0.514	0.608
NutrientsNP	0.105	0.050	2.085	0.038
NutrientsNPmic	0.017	0.047	0.372	0.710
NutrientsP	0.053	0.053	1.002	0.317
Episodic	-0.056	0.040	-1.402	0.162
Half	-0.123	0.040	-3.075	**0.002**
Submerged	-0.100	0.040	-2.499	**0.013**
log(initial):SoilBCD	0.196	0.089	2.205	0.028
log(initial):SoilBCU	0.162	0.083	1.952	0.052

Progress on Task 2.2 – Willow Transplantation

A. Competition Experiment – We monitored all blocks of this field experiment to evaluate responses of willow seedlings and cuttings to competition, and to verify that missing individuals indeed were dead. Indeed, no seedlings or cutting were alive in the northern and central blocks. Several cuttings - but no seedlings - were alive in the southernmost block. We will continue monitoring these plants to estimate growth and survivorship.

B. Hydrology Experiment – We performed our monthly monitoring of the willow island experiment, which tests the ability of seedlings and cuttings to survive at different elevations in the marsh. Islands were less than halfway submerged in early October and differences among elevations in survival remained apparent, especially for seedlings. Other marsh plants are colonizing the islands and we expect they are competing with the willows, a hypothesis that we can test by including neighboring plants into our model. We will continue monitoring these plants to estimate growth and survivorship but will remove them before they flower.

Initiate Task 2.3 - Fire response

We are in the process of finalizing our sampling strategy for this task. The main challenge is identifying accessible reference sites to pair with the burned treatments.

Progress on Task 2.4 - Life History

We are modifying our approach to use more deterministic sampling based on soil types, vegetation assemblage, and human influences (e.g., proximity to roads and levees vs. open marsh). Inability to access sites made our initial stratified random sampling method infeasible.

Progress on Task 2.5 – Spatial Analysis of Willow Distribution.

We concentrated on the greenhouse experiments during this period and did not modify our existing spatial model.

Summary of Activity
During this reporting period, the UCF team completed two greenhouse experiments and maintained two field experiments (Fig. 9). We dried and weighed both above- and below-ground biomass; the latter required washing roots free of soil, which was very time-consuming. We completed initial analyses of the greenhouse seedling experiment, which is the more challenging of the two due to high mortality in the one-half rainfall treatments and on the RL and SJ soils. Additional analyses will be included in the subsequent reports.