

**Light, Nitrogen and Phosphorus Effects on Growth, Allocation of Biomass and
Nutrients, Reproduction and Fitness in Wild Rice (*Zizania palustris* L.)**

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Chapter 1. Implications of Allocation Patterns for the Development, Physiology, Ecology, and Evolution of Plants

Allocation patterns are an essential characteristic of the life history and function of all organisms. Allocation patterns describe the organization of elements, also called stoichiometry, and/ or biomass among tissues within organisms. Because internal and external resources are finite, organizational trade-offs guide an individual's allocation of internal resources to specific tissues. These trade-offs include allocating resources within an individual to specific tissues such shifts among roots, stems and leaves within a growing season and the production of seeds for the next generation. Each organism therefore responds to their external and internal resource availabilities to exhibit allocation patterns which may enable them to progress from one developmental stage to the next in order to complete their life history and reproduce as optimally as possible. An organism's allocation pattern is, however, constrained and influenced by its genetics and stoichiometric requirements as it progresses through development causing its tissues' elemental needs to change.

Plants and animals differ in their abilities to change their allocation patterns. Animals are generally mobile organisms with the ability to seek out limiting resources in their environment, thus changes in their allocation patterns are generally determined by developmental processes. Plants are generally immobile, especially terrestrial plants, and therefore depend on the resource environment in which they are rooted. Although allocation patterns of plants and animals change as they progress developmentally, plants plastically alter their allocation patterns in response to varying resources availabilities. Allocation patterns may vary within a plant species because individuals may experience

varying resource availabilities, but variation in allocation patterns within a species is generally less than the variation between species. Because variation in plant allocation patterns exists within and between species, plants may experience intra- and interspecific competition in response to limiting resource availabilities.

Allocation patterns in plants have generally been described by ratios that divide plants into functional parts. Root: shoot ratios and tissue: total biomass ratios are currently used to conceptually separate plant biomass into water and mineral nutrient absorption and carbon acquisition functions. Because of stoichiometric differences between shoots and roots which are primarily responsible for obtaining above and below ground resources, respectively, and because resources are more easily acquired when they are in greater abundances, there are cost to benefit ratios of changing allocation patterns to obtain limiting resources. Typically, shoots have higher nutrient concentrations whereas roots have higher carbon concentrations; it therefore costs more carbon to gain nutrients than to gain more carbon and it costs more nutrients to gain carbon than to gain more nutrients. Cost to benefit ratios of acquiring new resources are influenced not only by resource availability but also by a plant's current and future developmental needs.

Functional equilibrium and optimal allocation theories describe the reasons for adjustments in allocation patterns. Functional equilibrium theory suggests that a plant responds to changes in its ability to obtain nutrient or carbon resources in order to maintain a balance between acquired nutrient and carbon resources (Brouwer 1983). In order for a plant to complete its life cycle, it requires specific ratios of resources such as

carbon to other elements like nitrogen, phosphorus and potassium. According to this theory, if a plant is limited by carbon resources, the plant will allocate biomass or change photosynthetic abilities of tissues that acquire sunlight or CO₂ to enhance carbon fixation (Poorter and Nagel 2000). Likewise, if a plant is limited by nutrient resources, the plant will allocate biomass or change nutrient absorption abilities of roots to acquire limited nutrients and maintain the plant's functional equilibrium. Optimality theory expands the predictions of functional equilibrium theory to state that a plant will allocate biomass or nutrient resources toward tissues responsible for acquiring the most limiting resources (Bloom et al. 1985). Optimal allocation patterns therefore equally limit multiple resources which lead to maximum or optimal growth.

Developmental influences on allocation patterns are driven by ontogeny which entails the progression through developmental stages to maturity (Evans 1972). As plants progress through their life history their allocation patterns change to meet the resource demands of their current life stage and to prepare for later stages. The allocation patterns of current life stages provide tissues to acquire resources needed to progress through that life stage, but these patterns also influence the acquisition of resources needed for later life stages. Therefore, allocation of resources to particular tissues during early life stages may not be the best allocation pattern for acquiring external resources needed to meet different resource demands in later life stages. In general, plants preferentially allocate resources to roots early in development after germination and in seedling stages in order obtain nutrients for the growing plant after seed nutrient stores are exhausted. As plants progress through development, acquiring carbon for structural and reproductive efforts

becomes priority and allocation patterns shift toward above-ground vegetative growth. As plants mature and enter reproductive stages, nutrients and carbohydrates stores are translocated from storage tissues to reproductive tissue, which causes vegetative tissues to senesce allocation patterns to change.

Plants also change allocation patterns based on their architectural form and how certain tissues function. Nutrient rich environments typically lead to larger plants with large quantities of leaves to capture limited sunlight; thus requiring a sufficient support structure (Tilman 1988). Plants therefore allocate biomass to stems to hold leaves in a competitive position in the canopy. In contrast, nutrient poor environments typically lead to smaller plants which require less above ground structural support, and plants subsequently increase allocation to roots to seek out limited nutrients. In addition, increasing nutrient concentrations in specific tissues by allocating nutrients to those tissues may enhance a plant's ability to acquire resources; for example, increased allocation of nitrogen to leaves may enhance photosynthetic abilities.

Although plants plastically respond to varied resource availabilities through changes in allocation patterns, creating multiple possible phenotypes per genotype, plant genotypes still can limit plastic responses. For example, genetic pathways control the production of hormones which regulate the growth and senescence of plant tissues. Growth of new tissue occurs in meristems and is primarily regulated by the hormones auxin, gibberellin and cytokinin. Growth inhibitors include the hormones ethylene and abscisic acid which lead to senescence of old tissue and force reproductive structures into dormancy, respectively. The production of growth promoting and inhibiting hormones is

plastic and is regulated by a plant's genetics. The degree of plasticity in allocation patterns is, therefore, constrained by a plant's genotype.

Allocation patterns have direct implications for the evolution of plants and thus the transfer of genetic information between generations. Natural selection favors allocation patterns that lead to the greatest growth rates (Tilman 1988). Because plant biomass is generally fifty percent carbon, investment in photosynthetic tissue is directly correlated to a plant's relative growth rate. Plants with greater relative growth rates generally have a competitive advantage over their neighbors whether they are of the same or different species. Differences in relative growth rates are most pronounced between annual and perennial plants. Annual plants allocate little resources to roots and storage structures compared to perennial plants in order to grow quickly and complete all life stages in one growing season; however, annuals only experience large growth rates when their resource needs are met. Although perennials grow more slowly than annuals, perennials can store nutrients in roots and stems for use in future growth and reproductive potential, and because they are higher in the canopy they can shade shorter plants. Additionally, storing resources could be of great selective advantage to survive resource poor years and reproduce later. A perennial's allocation pattern toward root and stem growth over leaf growth also limits the resources available to neighboring annuals through long-term nutrient storage and shading. It is therefore not enough for a plant species or individual to have an allocation pattern arranged for the greatest growth rates because that pattern assumes access to resources that may be limited by other plants. A

particular allocation pattern may therefore not only enable a plant to increase its own fitness but also decrease the fitness of its competitors (Harper 1977).

Plants pass their genes to offspring which transfers the genetic code for those particular parents' allocation pattern, but plastic allocation can disguise genetic influences on allocation patterns and thus limit the process of selection on genotypes. Selection occurs at the individual level, but differential reproduction results in a change in allele frequency in a population. Because an individual's genotype is only partly responsible for an individual's phenotype, and plastic responses to resource availability are responsible for the remainder, it is unclear how much influence the genotype or plastic responses account for the selection for or against an individual.

Variation in patterns of allocating biomass and nutrients to different tissues also influence a plant's physiological processes by altering the function of tissues acquiring above and below-ground resources. For example, allocating nitrogen to leaves of shade-intolerant plant species increases the photosynthetic capacity of those leaves (Gulmon and Chu 1981). Likewise, allocating nitrogen to leaves higher in the canopy that are exposed to more sunlight also increases the photosynthetic abilities of the plant (Field 1983). Allocating nitrogen to leaves of shade-tolerant plant species, however, will not result in increased photosynthetic abilities because of the high costs of maintaining nitrogen compounds (Chapin et al. 1987). Therefore, allocation patterns influence not only tissue biomass for resource acquisition but also the physiology of tissues.

In addition to altering photosynthetic abilities by modulating leaf tissue chemistry, plants may also change their nutrient absorption abilities by altering root

architecture and ion exchange abilities. Plants in nutrient limited environments may allocate resources toward different root size classes to increase nutrient and water absorption (Ryser and Eek 2000). This enables plants to enhance their absorption abilities without having to acquire additional biomass or nutrients because the adjustment in allocation occurs within the root tissue. Roots are also flexible in their permeability to nutrients in the sediments, such as ammonium, nitrate, and potassium, by changing the number of ion transporters or their functions, therefore enabling roots to take up more nutrients in nutrient poor environments (Glass 1983).

Plants may also exhibit variation in leaf and root tissue longevity. In resource rich environments, tissues are turned over quickly because of the abundance of resources; therefore, a plant's allocation pattern in these conditions may fluctuate rapidly as tissues are gained and lost. In resource poor environments, tissues are turned over slowly which enables plants to translocate internal resources from senescing tissues more thoroughly (Shaver and Melillo 1984); thus a plant's allocation pattern in these conditions may fluctuate slowly because of the high costs of producing new tissues.

Species composition within a plant community is dependent on resource availability which drives selection for specific allocation patterns. That is, species with favored allocation patterns for a particular environment can competitively displace species with less favorable allocation patterns (Tilman 1988). Interspecific competition can result in the competitively superior species to be represented in future community compositions, and because variation in allocation patterns exists within species,

intraspecific competition will lead to superior individuals within a species that contribute offspring to future generations.

Nutrient cycling within ecosystems may also depend on allocation patterns as they can determine the plant species present and the quantities and qualities of their litter returned to the ecosystem after senescence or plant death (Minor 2005). Plant litter is decomposed by microbes, returning many nutrients to the pools of available sediment resources for plants, but the relative nutrient requirements of those microbes is different than the quantity of nutrients contained in different plant tissues (Berg and McClaugherty 2008). In addition, roots, stems and leaves have different elemental chemistries and structural carbon compounds which determine litter quality. Higher quality litter is characterized by low C: element ratios and highly soluble organic compounds, making the litter easily decomposed by microbes. Leaves contain the greatest concentration of nitrogen and other macronutrients, as well as easily decomposable structural components, and are therefore more quickly decomposed than the more structural, less nutrient concentrated stems and roots of plants. Plants develop allocation patterns by responding to ontogenetic and resource availability cues, but when plants die, their allocation patterns affect the litter contributions to litter pools.

In conclusion, as a plant develops from seed germination to maturity and reproduction, it undergoes allocation pattern changes due not only to ontogeny but also to the resources available in its environment. Although plants exhibit plastic allocation patterns of biomass and nutrients, they also exhibit physiological plasticity by changing their photosynthetic and nutrient absorption rates, all of which enable plants to seek out

limiting resources. A plant's response to ontogeny and resource availability affects their survival and fitness by competing with other species and individuals of the same species who may have different allocation patterns. The influence of allocation patterns, however, goes beyond the survival and fitness of individuals because they also affect nutrient cycling dynamics in ecosystems by altering the litter quantity and quality available for microbial decomposition.

Wild rice (*Zizania palustris* L.), an annual macrophyte, is an excellent model plant to study how allocation patterns affect developmental, physiological, ecological and evolutionary phenomena. Because wild rice is an annual plant, it responds quickly to manipulations of environmental conditions, resulting in data collection within one growing season. Wild rice undergoes several distinct developmental stages which make following the plant through ontogeny feasible, and measurements on numerous plants are also easy to conduct because plants do not grow much larger than thirty grams. Many allocation studies have been conducted on terrestrial annuals, but few if any studies have examined allocation patterns in macrophytes.

I investigated the effect of nitrogen, phosphorus and light - the three resources most likely limiting to wild rice – on relative growth rates and allocation to roots, shoots and stems. In Chapter 2, I present results from an investigation of wild rice growth responses by examining the individual and cumulative effects of the morphological, physiological and allocation components of relative growth rates and find wild rice responds differently to each resource for each growth rate component. I also investigated changes in allocation patterns of wild rice by isolating growth limiting, ontogenetic and

plastic responses when grown under different resource levels. I found wild rice growth is primarily limited by nitrogen availability, plants change allocation patterns throughout ontogeny, and plants respond plastically to differences in nitrogen and light resource availability. In Chapter 3, I present results from an investigation of the reproduction of wild rice by examining the tillering, seed head production, seed number production, and individual seed weight characteristics of plants grown under different nitrogen, phosphorus and light levels. In addition, I investigated the germination rates of seeds produced by those parent plants grown under different resource availabilities and demonstrate that seedlings produced the following year per parent plant is a better estimate of plant fitness than total seed biomass or seed number per plant. I found greater nitrogen and light availability results in parent plants producing more seedlings the following year. These two chapters combined present results of the life history of wild rice grown under different nitrogen, phosphorus and light availabilities as well as the parent plant's offspring contributions to future generations.

Chapter 2. Light, Nitrogen and Phosphorus Effects on Growth and Allocation of Biomass and Nutrients in Wild Rice

Overview

Growth and allocation processes are often difficult to understand because several mechanisms determine a plant's response to varying resource availabilities. For example, leaf and root allocation as well as morphological and physiological responses determine a growth response to an environmental resource, but allocation to different tissues includes ontogenetic, plastic and growth limitation responses. These can be a challenge to separate because they often occur simultaneously. Wild rice (*Zizania palustris* L.) is an annual plant that must complete all growth and allocation processes in the same season as its germination. Wild rice was grown under different nitrogen, phosphorus and light levels to examine growth limitation and allocation responses. Wild rice growth was primarily limited by nitrogen. Phosphorus and light each had no effect on growth but for different reasons: phosphorus did not affect the components which underlie relative growth rate whereas light affected all the components but in compensatory directions. Plants responded ontogenetically to nitrogen additions and full sunlight by accelerating their developmental rate and plastically by decreasing and increasing their root: shoot ratios, respectively. Phosphorus additions had no effect on any allocation response. Differential decomposition of roots and shoots from each year's growth cause fluctuations in the availability of nutrients to future generations. The next year's generation therefore faces altered nutrient availability caused by the production patterns of new root and shoot litter from the previous year. Growth and allocation patterns one year therefore cause the next

year's generation to face different allocation challenges because of altered nutrient availability.

Introduction

External and internal resource availabilities partly determine plant growth and allocation patterns. Water and nutrients in sediment or soil as well as sunlight constitute external resources; stores of carbohydrates and nutrients in different tissues constitute internal resources (Bloom et al. 1985). Leaves and green parts of stems provide the entire plant with its carbon supply, whereas roots take up nitrogen and phosphorus which are then translocated to the rest of the plant. Because carbon and nutrients are absorbed by different tissues, the plant faces a tradeoff in allocation of its growth to these different parts when either carbon or nutrients are limiting.

It is generally thought that plants plastically allocate growth to those parts which absorb the resource in most limiting supply (Bloom et al. 1985; Tilman 1988). Plasticity entails changes in allocation to improve capture of a limiting resource whose supply varies during the growing season; for example, growing more roots to absorb limited nutrients at the sacrifice of shoot growth or growing more shoots to capture more sunlight under low light conditions at the sacrifice of root growth (Chapin 1980). But the tradeoffs of allocating carbohydrates and nutrients to either new shoots or new roots are not one-to-one because of the characteristically low nutrient concentrations in roots and high concentrations in shoots (Bloom et al. 1985): it takes a greater amount of nutrients to make a gram of shoots than to make a gram of roots. Besides altering their biomass allocation patterns, plants can also plastically change tissue morphology, such as surface

area per unit mass (Ryser and Eek 2000), and physiology, such as photosynthetic and nutrient absorption rates.

In addition, each stage of a plant's life cycle has its own nutrient and environmental requirements; therefore, plants ontogenetically allocate growth based both on its current life stage as well as available nutrients and light. Ontogeny entails changes in allocation determined by developmental stage; for example increased root growth early in the growing season or translocating internal resources toward seeds during reproduction (Larcher 1995). Ontogeny is also determined by physical and chemical demands of plant form and function; for example, an increase in stem growth to support increased leaf tissue, or leaves being sloughed off as their carbohydrates and nutrients are translocated to new leaves or developing seeds (Evans 1972). Ontogenetic allocation to different tissues at one stage in the life cycle influences the surface areas for resource acquisition at later stages (Gedroc et al. 1996; McConnaughay and Coleman 1998).

Besides plastic and ontogenetic allocation responses, plants can simply increase growth rates when resources become less limiting without any proportional changes in tissue allocation. Ontogenetic and plastic changes in allocation as well as increased growth might all occur simultaneously or in any combination in response to alleviation of the same resource limitation. Therefore, to determine how resource availabilities affect growth and allocation patterns, the effects of ontogeny, plasticity and growth limitation must be separated (McConnaughay and Coleman 1999).

Wild rice (*Zizania palustris* L.) is an annual plant which must go through all allocation processes required to complete its life cycle in the same year as its germination

(Aiken et al. 1988). More specifically, the plant allocates nutrients and carbohydrates between roots, stems, leaves, and seeds in response to changing priorities throughout the growing season to produce seeds for next year's generation (Mooney 1972). Because all tissues except seeds die at the end of the growing season, plants are unable to store nutrients in their roots for next year's growth and reproductive efforts. The decomposing roots and shoots from each year's growth, however, cause fluctuations in the availability of sediment nutrients to future generations (Walker et al. 2006; Walker et al. 2010). The next year's generation therefore faces altered nutrient availability caused by the production of new root and shoot litter from the previous year. Growth and allocation one year therefore causes the next year's generation to face different allocation challenges because of altered nutrient availability.

Wild rice morphology varied between natural stands and years across Saskatchewan, but when seed from these different stands were grown in similar conditions, morphological variation decreased considerably, suggesting wild rice responds plastically to environmental conditions (Archibold et al. 1989). Wild rice growth appears to be limited by the supply of nitrogen (Keenan and Lee 1988, Day and Lee 1989, Walker et al. 2010). Little is known about ontogenetic changes in wild rice allocation during the annual life cycle, and there have been no studies on ontogenetic, plastic and growth limitation responses of allocation in wild rice and in few other plant species (but see McConnaughay and Coleman 1999 and Weiner 2004). Because it goes through its entire life cycle in one year, wild rice is therefore an excellent model to

examine questions of how ontogeny, plasticity, and limiting resources determine growth responses to changes in resource availabilities.

Theoretical Growth and Allocation Framework

Plastic allocation, morphological and physiological responses to resource availability can be explored using plant growth analysis (Evans 1972; Hunt 1990; Poorter and Nagel 2000). Relative growth rate (*RGR*) is the quantity of biomass produced per unit of biomass existing at time (t_1) per unit of time and is calculated as $RGR = (\ln W_2 - \ln W_1) / \Delta t$ where W_1 and W_2 are plant dry weights at times t_1 and t_2 , respectively. *RGR* measures the whole plant production rate of new biomass per unit of existing biomass; however, *RGR* can be decomposed into leaf and root components (Eq. 2.1) which are responsible for carbon and nutrient acquisition, respectively,

where *ULR* is unit leaf rate, *SLA* is specific leaf area and *LWR* is leaf weight ratio and *URR* is unit root rate, *SRA* is specific root area and *RWR* is root weight ratio (Evans 1972). *ULR* is the new biomass produced between harvest dates per unit of average leaf area between harvest dates per unit of time during the production of new biomass.

Unlike *RGR* which measures the production rate of existing biomass, *ULR* measures the production rate of existing leaf surface area. *SLA*, a measure of morphology, is the leaf area produced per unit of leaf weight. *LWR* is the ratio of a plant's leaf weight per unit of total biomass produced. *ULR*, *SLA* and *LWR* are representative of the return on photosynthetic efforts, leaf morphology and leaf biomass allocation respectively that can help explain the *RGR* (Poorter and Nagel 2000). *URR*, *SRA* and *RWR* are calculated

similarly except using root area instead of leaf area and represent the return on nutrient absorption efforts, root morphology and root biomass allocation respectively.

Either light or nutrients may affect each of these components in the same direction or unequal magnitudes in opposite directions, producing a strong effect on *RGR*, or in similar magnitudes in opposite and therefore compensating directions, yielding a weak or even no effect on *RGR*. Therefore, how *ULR*, *SLA* and *LWR* or *URR*, *SRA* and *RWR* respond to light and nutrients could explain how *RGR* responds to light and nutrients. The null hypothesis of no effect of a resource on *RGR* would be supported if either the resources had no effect on *RGR*'s components or alternatively if the responses of these components compensated for one another. Therefore, understanding the null response of *RGR* to changes in resource availability requires an examination of the responses of each of *RGR*'s underlying components.

Although ontogenetic, plastic, and growth limitation responses often occur simultaneously, these can each be isolated from one another by plotting a parameter which measures allocation patterns, such as root:shoot ratio, against total biomass. Ontogenetic, plastic and growth limitation responses affect the slopes, intercepts, and trajectories of these responses in different ways (Weiner 2004; Fig. 1). Different resource availabilities may cause an ontogenetic response by changing the plant's developmental rate, thereby causing plants to be at different life stages on the same dates (Fig. 1b, 2). Different resource availabilities may elicit a plastic response by changing the slope and/or intercept of a trajectory (Fig. 1c, 2). However, a growth limitation response to limiting resource availability changes the growth rate which can move a plant's size slower along

the same trajectory, thereby causing plants to be smaller on the same date (Fig. 1d, 2). Different resource availabilities may also cause any combination of any two (Fig. 1e – g) or all three (Fig. 1h).

Materials and Methods

Light, nitrogen and phosphorus availabilities to wild rice (*Zizania palustris* L.) were altered to determine their affect on growth and allocation responses within the framework of Figure 1. This experiment was conducted at the University of Minnesota Duluth (UMD) Research and Field Studies Station in Duluth, MN. Wild rice was grown in 25 x 20 x 61cm gusseted 3ml plastic bags from U.S. Plastics Corp (Lima, OH), and each bag was clipped into a 20 x 15 cm rectangle as part of a 12 x 5 rectangle, cattle fence grid. Each grid was suspended in a galvanized steel stock tank of dimensions 3.05 x 0.91 x 0.61 m. Each bag contained two liters of washed sand below three liters of sediment, and then watered to 28 cm above the sediment. Space in tanks around bags was also filled with water to support bags and decrease diurnal temperature fluctuations. The sediments were collected from Rice Portage Lake, a natural wild rice lake on the Fond du Lac Chippewa Reservation in Carlton County, Minnesota. Before distributing to plastic bags, the sediment was mixed in large stock tanks. To determine initial sediment chemistries, three sediment grab samples were taken after mixing; the sediments contain total C% = 10.410 ± 0.231 SD, total N% = 0.980 ± 0.016 SD, and organic P% = 0.058 ± 0.003 SD (Harmon and Lajtha 1999). Seeds were gathered from a wild rice pond on the UMD campus in fall of 2008 and were cold stratified until spring 2009 at 4°C to fulfill

dormancy requirements before sowing on 5 June 2009. Two seeds were planted in each bag to help ensure that at least one seed germinated.

Nutrient availabilities and light levels were manipulated using fertilizer additions and shade cloths, respectively. Eight treatments of two light, two nitrogen and two phosphorus levels were arranged in a split-plot design. Each of two light levels was applied to three tanks as the whole plots, and each of four nutrient treatments was randomly assigned to twelve bags within each tank as subplots.

Cumulative nitrogen and phosphorus additions of 300mg and 110mg respectively per plant were added to fertilized treatment bags over the growing season, based on quantities contained in a 30g plant (Grava and Raisanen 1978; Table 1). Control bags were grown with ambient sediment chemistries. Also, 50mg of potassium was added to every bag two times throughout the growing season to minimize possible, although unlikely, potassium limitation effects. Nutrients were added weekly over a sixteen week fertilizer schedule, and the elemental weights of nutrients given to fertilized treatments changed each week to follow the phenology of wild rice nutrient uptake (Grava and Raisanen 1978; Table 1, 2). Fertilizer solutions were prepared using NH_4Cl , $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and KCl . Using a syringe and for all fertilizer solutions and concentrations, which changed weekly, 10ml of solution were injected into designated bags at the sediment/ sand interface.

Full sunlight and 25% of full sunlight represent light saturated net photosynthesis (A_{max}) and the half saturation point of wild rice A_{max} respectively (Zhoa et al. 2004; Sims and Hildebrandt unpubl.). Wild rice's A_{max} , between 15 to 35°C, occurs under

photosynthetically active radiation (PAR) levels ranging from 1000 to 1040 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Zhoa et al. 2004), and half saturation of net photosynthesis is around 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Sims and Hildebrandt unpubl.). The shaded environment was created by one wooden/rope frame over three adjacent stock tanks, and shade cloth from American Nettings and Fabric Inc. (Ferndale, WA) was draped over this frame. When averaged over different weather conditions, the shade structure let through $25.3\% \pm 1.6$ s.e. of full sunlight, and the air temperature was $0.343^{\circ}\text{C} \pm 0.033$ s.e. lower than outside the shade structure. At natural wild rice stands in Grand Rapids, MN and Superior, WI, 13.3% and $19.5\% \pm 0.7$ s.e. of full sunlight reached the water surface, respectively, and 63.3% and $37.9\% \pm 0.9$ s.e. of full sunlight penetrated halfway through the canopy, respectively (Clay and Oelke 1987; Sims observation). Therefore, the shade cloth transmitted similar amounts of light as do natural wild rice canopies, and plants grown under the shade cloth faced similar light regimes as small rice plants growing under a canopy of larger plants in natural stands.

Six harvests were conducted throughout the growing season to characterize plant growth and biomass allocation patterns at the different growth stages of wild rice (Weir and Dale 1960; Grava and Raisanen 1978; Oelke et al. 1982) (Table 1, 2). Growth stages for all plants were documented twice each week during the growing season. The first harvest at the floating leaf stage thinned every bag to one plant to be harvested at another date. If only one of the two sown seeds germinated, no plant was harvested from that bag. Interactions between the two plants at this stage were assumed to be negligible because the plants were small. The second harvest was at the second aerial leaf stage

which begins the vegetative production period. The third harvest at the jointing stage preceded the characteristic 10-14 day period when roughly 50% of final above-ground N and P is taken up (Grava and Raisanen, 1978). The fourth harvest at the early flowering stage represented a possible stall in nutrient uptake when panicles and subsequent florescence are beginning to develop. The fifth harvest at the mid flowering stage was just prior to seed filling. During the final harvest from September 30th to October 14th, a plant was harvested after all of its seeds had shattered and been collected. Plants lost their seeds at different times; hence the final harvest of all plants was spread over two weeks. Six plants for each treatment were harvested on sample dates two through five; this was done by randomly sampling two plants per fertilizer treatment in each of three tanks of the same light level. Twelve plants were sampled per treatment during the final harvest; this was done by randomly sampling four plants per fertilizer treatment in each of three tanks of the same light level.

Plants were harvested in the field and brought back to the laboratory for processing. Plants were carefully removed from their sediment in the field to minimize root loss and then placed in a plastic bag with cold water. These bags were transported to the laboratory on ice in coolers and stored in a cold room until processed. Leaf area was determined immediately using WinFOLIA PRO 2006 (Regent Instruments, Ottawa, ON) and the remaining plant was dissected into stems and roots; the seeds were collected prior to harvesting. Leaf area was not determined for the first harvest date. Seeds, leaves and stems were dried at 60°C for at least one week then weighed, but the roots were stored in water-saturated sediment in a cold room (4°C) until they could be washed and scanned

using WinRHIZO REG 2007 (Regent Instruments, Ottawa, ON), after which they too were dried and weighed. It took approximately two weeks to scan each harvest's set of roots, and root systems were processed in random order.

The experimental design was a completely random split-plot design as described above. All treatments were considered fixed in both repeated measures ANOVA and ANCOVA tests. In ANOVA, tanks were random effects nested within light, and there were no significant tank effects.

The responses of total biomass, *RGR* and *RGR*'s leaf and root components (Eq. 2.1) to light, nitrogen, and phosphorus were tested with repeated measures ANOVAs (RM-ANOVA). When necessary, data was natural log transformed to better fit normality and homoscedasticity assumptions. Tukey's HSD was used to determine differences between individual treatments. Responses of plant developmental rates through growth stages were tested using ANOVAs on each sample date. To test for plastic allocation patterns, ANCOVAs were done of $\ln(\text{root}:\text{shoot})$ using $\ln(\text{total vegetative biomass})$ as the covariate. Vegetative biomass included roots and shoots but not seeds. Data from all sample dates were used during ANCOVA tests. Significant effect*covariate interactions indicate different slopes for trajectories for that effect, and a significant effect indicates a difference in the intercepts for trajectories for that effect. Lack-of-fit test results indicated that a linear model is appropriate for these ANCOVA data.

Scatterplots of element concentration versus element content in shoots and roots were used to further determine nutrient limitations beyond examining total biomass alone and to explain the mechanisms of significant interaction effects in total biomass (Timmer

and Stone 1978). Treatment trajectories in comparison to a control's (Fig. 3) were used to determine whether a plant's response to increased resource availability is caused by toxicity, luxury consumption, decreased limitation to growth, or dilution of the nutrient with increased carbon fixation (Fig. 3). See Timmer and Stone (1978) for more details about this graphical technique.

Results

Effects of N, P and Light Availability on Biomass. Wild rice biomass responded differently to nitrogen, phosphorus and sunlight (Fig. 4). Nitrogen additions strongly increased plant biomass over the growing season ($F_{N(RM-ANOVA)} = 282.348, p < 0.001$), but phosphorus additions did not (Fig. 4). Full sunlight increased plant biomass compared to shade ($F_{L(RM-ANOVA)} = 196.125, p < 0.001$), but the increase in biomass in response to light occurred only when plants also received added nitrogen ($F_{L*N(RM-ANOVA)} = 3.842, p = 0.057$); Fig. 4). By the final harvest, there was no difference in biomass between plants grown without added nitrogen in shade or full sunlight, but plants grown with added nitrogen in full sunlight had greater biomass than plants with added nitrogen in the shade (Fig. 4).

Additions of nitrogen significantly increased biomass over controls by sample date two ($F_{N(ANOVA \text{ on date } 2)} = 5.15, p = 0.029$), full sunlight significantly increased biomass over shade by sample date three ($F_{L(ANOVA \text{ on date } 3)} = 9.96, p = 0.004$), and phosphorus significantly increased biomass over controls by sample date five but only when nitrogen was also added ($F_{N*P(ANOVA \text{ on date } 5)} = 28.471, p < 0.001$; Fig. 4). Full sunlight increased biomass over shade only after nitrogen began increasing biomass during the vegetative

growth stages. At the final harvest, the largest plants grew when phosphorus was added with nitrogen, but phosphorus increased biomass only with added nitrogen ($F_{N*P(ANOVA\ on\ date\ 6)} = 47.102, p < 0.001$; Fig. 4).

Causes of Relative Growth Rate Responses or Lack Thereof. Only nitrogen additions significantly increased *RGR* ($F = 58.857, p < 0.001$; Table 3). Although nitrogen additions decreased *SLA*, this did not compensate completely for the stronger increases in *ULR* and *LWR* (Fig. 5). Nitrogen additions strongly increased *URR* but slightly decreased *SRA* and *RWR*, which also results in an overall increase in *RGR*.

Phosphorus had no detectable effect on *RGR* ($F = 2.249, p = 0.142$). Phosphorus weakly increased *ULR* and *URR*, weakly decreased *SRA* and had no effect on *SLA*, *LWR* and *RWR*; therefore, phosphorus had no effect on *RGR* because of minimal or no effect on its underlying parameters.

In contrast, light also had no detectable effect on *RGR* ($F = 2.126, p = 0.153$) but for different reasons (Table 3). Light strongly increased *ULR* but this increase was compensated by strong decreases in *SLA* and *LWR* (Fig. 5 & Table 3). The response of root parameters also compensated for one another because light weakly decreased *URR* but increased *RWR* and had no effect on *SRA* (Fig. 6 & Table 3).

Separation of Ontogenetic, Plastic and Growth Limiting Allocation Responses. Nitrogen additions weakly accelerated the developmental rate, decreased the root:shoot ratio trajectory, as shown by a lower intercept ($F = 3.840, p = 0.051$) but same slope ($F = 0.168, p = 0.682$) when root:shoot ratio was plotted against total vegetative biomass, and accelerated the rate by which plants traveled along their growth trajectory, indicating a

nitrogen limitation in ambient conditions (Fig. 7a). Although the growth limitation effect was minimal early in the growing season, it increased continuously through maturity (Fig. 7a). Plants with added nitrogen grew 3% less root biomass because of plastic allocation toward shoots but grew 382% more root biomass than ambient nitrogen plants that were more limited by nitrogen (Fig. 7a). Because nitrogen additions weakly accelerated ontogeny, plastically changed biomass allocation patterns, and limited plant growth, wild rice's response to nitrogen corresponds with Figure 1h in which all three processes affect allocation (ontogenetic changes in developmental stage, plastic responses, and growth limitations).

Compared to ambient phosphorus, phosphorus additions did not change the developmental rate, root:shoot ratio trajectory, as shown by same intercepts ($F = 0.120$, $p = 0.729$) and slopes ($F = 0.088$, $p = 0.766$) when root:shoot ratio was plotted against total vegetative biomass, or the rate plants traveled along their trajectory (Fig. 7b). Because phosphorus additions did not affect ontogeny or biomass allocation patterns and did not limit plant growth, wild rice's response to phosphorus corresponds with Figure 1a.

Compared to shade, full sunlight significantly accelerated the developmental rate, increased the root:shoot ratio trajectory, as shown by a higher intercept ($F = 32.484$, $p < 0.001$) and shallower slope ($F = 53.538$, $p < 0.001$) when root:shoot ratio was plotted against total vegetative biomass, and accelerated the rate plants travel along their growth trajectory, although only early in the growing season (Fig. 7c). By the fifth sample date during mid flowering, the biomass of shaded plants equaled that of plants in full sunlight, resulting in no sunlight limitation at the final harvest (Fig. 7c). Plants in shade grew 44%

less root biomass because of plastic allocation toward shoots (Fig. 7c). Because light availability affected ontogeny and biomass allocation patterns but was not limiting at later growth stages, wild rice's response to light corresponds with Figure 1e, a combination of ontogenetic and plastic allocation responses without any overall growth limitation.

Graphical Representations of Nutrient Limitations. Timmer-Stone scatterplots of tissue nutrient concentration versus nutrient content (Fig. 8) are graphical representations of toxicity, luxury consumption, limitation or dilution (Timmer and Stone 1978). Adding nitrogen increased shoot and root nitrogen content and concentration and was therefore a limiting resource (Fig. 8a, b). Adding phosphorus alone increased shoot and root phosphorus content only by increasing phosphorus concentration and not by increasing biomass, therefore indicating luxury consumption (Fig. 8c, d). Adding nitrogen, however, led to greater phosphorus uptake, possibly because of more roots, and increased both phosphorus concentration and phosphorus content, therefore indicating a phosphorus limitation after adding nitrogen (Fig. 8c, d). Adding phosphorus and nitrogen together increased shoot and root carbon content and shoot carbon concentration (Fig. 8e, f) and thereby decreased shoot and root nitrogen concentrations relative to tissues from plants with only added nitrogen (Fig. 8a, b). Shade decreased root carbon concentrations resulting in carbon dilution, but shading had little effect on other chemistry parameters (Fig. 8e, f).

Discussion

Response of wild rice to resource availabilities. Nitrogen was the primary limiting resource for wild rice growth, with light and phosphorus as secondary and tertiary limiting resources, respectively and only after sufficient nitrogen had been added (Fig. 4). Nitrogen additions alone increased biomass, but phosphorus additions alone did not. However, phosphorus added with nitrogen increased biomass above nitrogen additions alone indicating a phosphorus limitation after nitrogen limitation is reduced. This interaction between nitrogen and phosphorus availability in wild rice has also been noted by Keenan and Lee (1988), Day and Lee (1989) and Walker et al. (2010).

Ontogenetic and plastic allocation responses to varying resource availabilities as well as allocation responses to growth limiting resource availabilities can occur simultaneously (McConnaughay and Coleman 1999). Determining the presence of a particular allocation response during the course of a plant's life cycle is therefore difficult, but this can be done by plotting a parameter describing allocation patterns, such as root:shoot ratios, against total biomass (Fig. 1). Adding nitrogen to wild rice induced ontogenetic, plastic and growth limiting responses, resulting in plants growing and developing faster and allocating more biomass toward shoots than roots (Figs. 1h, 7a). The plastic response of increased allocation to shoots corresponds to theoretical predictions of increased above ground competition when nutrient availabilities are high (Tilman 1988). Increasing light only induced ontogenetic and plastic allocation responses, resulting in non-shaded plants allocating less biomass toward shoots than roots compared to shaded plants (Figs. 1e, 7c), which corresponds with theoretical predictions of decreased above ground competition when light availability is high (Tilman, 1988).

Adding phosphorus alone did not induce any changes in growth or allocation (Figs. 1a, 7b). Plant responses to nitrogen and light availability in natural stands may be mechanisms for the plastic responses observed by Archibold et al. (1989).

RGR can be dissected into both leaf and root components that represent the physiology, morphology, and tissue mass allocation patterns that together with the acquisition of a limiting resource determine a response in *RGR*. There are three ways for an increase in resource availability to elicit a null response in *RGR* but only one way to elicit an increase in *RGR*. First, a null response can happen when a plant does not take up the available resource and was therefore unable to use it. Second, a null response can happen when a plant takes up the available resource, but no underlying component of *RGR* is affected. Wild rice plants responded to phosphorus in this way. Plants took up the added phosphorus because P concentration increased (Fig. 8c, d), but phosphorus additions minimally affected some of the underlying components of *RGR* (Figs. 5, 6). Third, a null response can happen when a plant takes up the available resource, but the underlying components of *RGR* responded in opposite directions and in magnitudes that compensate for one another. Wild rice plants responded to light in this way. Plants increased carbon concentration and content in full sunlight, especially in roots (Fig. 8f); however, *ULR*, *SLA* and *LWR*, the underlying components of *RGR*, responded strongly to light but in opposite directions (Figs. 5, 6). Finally, an increase in *RGR* results when a plant takes up the available resource and the plant changes either all the underlying components of *RGR* in the same direction or if the magnitude of one or more responses more than compensates for another countering response. Plants responded to nitrogen in

this way. Plants took up added nitrogen because N concentration and content increased (Fig. 8a, b), and nitrogen increased *RGR* because *ULR* and *LWR* strongly increased and more than compensated for the slight decrease in *SLA* (Fig 5).

Null responses in growth and allocation processes are not trivial because a null response can happen by three different mechanisms, whereas a positive response can happen by one mechanism. In this study, changing light or phosphorus both elicited a null response of *RGR* but for two very different reasons. Only after examination of the mechanisms causing these null responses, for example looking at tissue chemistry and growth analysis, can a more complete understanding of growth and allocation processes result.

Implications for Wild Rice Growth and Population Dynamics. According to allocation theory (Bloom et al. 1985, Tilman 1988), a plant will allocate proportionately more of its resources toward tissues used for acquiring the most limiting resource and away from tissues acquiring non-limiting resources. Because roots and shoots have different nutrient concentrations and ratios, changes in allocation patterns between these tissues alters the distribution of nutrients within plants, which will affect nutrient cycling when those plants die and their tissues become litter (Minor et al. 2005).

Shoot and root litter and seeds are a wild rice plant's final products. Delays in the release of nitrogen from wild rice litter because of nitrogen uptake and sequestration in growing microbial biomass during the early stages of decay, known as immobilization, may cause 4-5 year cycles in wild rice production (Pastor and Walker 2006; Walker et al. 2006; Walker et al. 2010). Litter from the previous year's growth contains a considerable

portion of the nitrogen and phosphorus acquired through uptake in the previous year, but the release of nitrogen typically begins after the early summer 10-14 day period during which approximately 50% of total annual nitrogen and phosphorus requirements are taken up by the current generation (Sain 1984; Grava and Raisanen 1978; Walker et al. 2010). The release of nitrogen is especially delayed during the decay of root litter because of the high lignin and cellulose contents of roots (Walker et al. 2010). Since the decomposition of root and shoot litter causes population oscillations of wild rice by delaying the supply of nitrogen to different degrees (Pastor and Walker 2006), allocation responses of individual plants to nutrient and light availability in one year affect the dynamics of entire populations and the cycling of nutrients through the ecosystem in subsequent years. Thus, the short-term responses at the scale of individual plants are ramified through populations and ecosystems at larger scales and over longer time periods.

Wild rice can induce resource limitations not only through the delays in the release of nitrogen from decaying litter but also through differences in light capture down through the canopy through a population cycle of high to low productivities. Plants growing in nutrient rich sediment form dense stands of high above-ground biomass (Keenan and Lee 1988; Wilson and Tilman 1991; Wilson and Tilman 1993; Lee 2002; Walker et al. 2010). Consequently, shading may cause light-limiting environments and influence allocation patterns (Archibold and Weichel 1986). On the other hand, under severe nutrient limitation canopies are less dense because plants may allocate biomass to roots to exploit a larger volume of nutrient poor sediment (Tilman 1988). However,

increasing the proportion of biomass allocated to roots, described by an increase in root:shoot ratio, to obtain more nutrients may exacerbate nitrogen deficiencies in the following year if the absolute amount of root production increases, described by greater root litter biomass, because root litter releases N more slowly than shoot litter (Pastor and Walker 2006; Walker et al. 2010).

Therefore, while wild rice plants adjust their allocation in response to different light and nitrogen availabilities, the availabilities of light and nitrogen also vary during a population cycle in part because of changing allocation patterns. As wild rice populations plastically change the proportion of root biomass as well as the absolute root biomass under varying resource availabilities, the availability of nitrogen may change in the future because of different sizes and composition of litter cohorts. This in turn may cause future generations to plastically adjust their response to changing nitrogen availabilities caused by previous generations' litter cohorts. Thus, the amplitude and period of population oscillations may be exacerbated or suppressed by plastic responses of each generation of plants to changing nitrogen availabilities. Therefore, growth allocations to meet resource capture one year may alter resource availabilities the following year, requiring further allocation responses.

Chapter 3. Effect of Nitrogen, Phosphorus and Light on Reproduction and Fitness of Wild Rice

Overview

Plant fitness is typically correlated with total seed biomass or the number of seeds produced per plant, but the connection between seed production to seedlings the following year is seldom made. Seed production in graminoids, including wild rice (*Zizania palustris* L.) is determined by the number of seed heads produced, the number of seeds per seed head, and the mean weight per seed. To derive seedling number per parent plant, seed filling, predation and germination rates also need to be accounted for because not all seeds are filled, and not all filled seeds are viable. Disregarding these parameters can over estimate plant fitness. It was therefore our objective to evaluate the effects of nitrogen, phosphorus and light on these parameters. Nitrogen addition increased all three components of seed production, resulting in more seedlings produced despite an increased rice worm predation and lower germination rates of seeds compared to seeds from plants grown without nitrogen addition. Phosphorus additions and full sunlight also increased the number of seedlings per plant, but light increased seedling number only after nitrogen was added. In determining plant fitness, the number of seedlings per parent plant derived from the number of germinated seeds was 26%-74% of the total number of seeds produced per plant depending on treatment. Fitness is therefore over estimated when derived from reproductive biomass or seed number. As wild rice populations cycle because of resource availability, seedling cohort densities fluctuate and compete in environments altered by parent generations.

Introduction

A plant's fitness is generally thought to correlate with the number of seeds produced as well as total seed biomass (Harper 1977). The connection to the number of seedlings produced the following year is seldom made, however. The number of seedlings from a parent generation is perhaps a better estimate of fitness because in many species, for example in the graminoid wild rice (*Zizania palustris* L.), not all seeds that are produced are filled (Aiken et al. 1988; Atkin et al. 1992). Furthermore, seeds that are filled may die due to predation during or after development, and not all filled, uneaten seeds germinate. The maternal plant's effects on seed characteristics, such as seed coat chemistry and nutrient stores, can influence the germinate rates and early competitive abilities of seedlings before they respond to their environmental resource availabilities (Simpson 1966, and see review by Wolf and Roach 1987).

Seed production in graminoids is determined by three main components: the number of seed heads produced, the number of seeds per seed head and the mean weight per seed (Weir and Dale 1960; Harper 1977; Oelke et al. 1982). But the number of seedlings produced is reduced from the number of seeds by predation and other factors that reduce seed filling and the germination rate, and is given by the following;

Seed head production in wild rice increases with tillering. Tillers are multiple stems from the same plant, and tillering is perhaps the most complex component of new seedling production (Ao et al. 2010). In the same family as wild rice (*Poaceae*), white

rice (*Oryza sativa*) increases tillering with increasing shoot nitrogen concentration and decreases tillering with increasing leaf area index, a measure of total leaf area per unit ground area. This indicates that both below and above ground resource availability can modulate the degree of tillering in graminoids (Zhong et al. 2003). Wild rice increases tillering with fertilizer applications and shallower water depths and decreases tillering at greater densities, hence lower light availabilities (Archibald and Weichel 1986; Atkin et al. 1992; Lee 2002). In addition to resource availability, wild rice tillering can also be affected by climate, with the greatest tillering in intermediate climates (Counts and Lee 1990).

While tillering is controlled by both below and above ground resource availabilities, not every tiller produces a seed head. A ^{14}C experiment with white rice found assimilates translocate from vegetative to reproductive tillers during reproduction (Wang & Hanada 1982), although there was no difference in seed production found when vegetative tillers were either physically removed or suppressed and thus preventing them from contributing to reproductive efforts (Ao et al. 2010). Therefore, the trade-off between vegetative and reproductive growth and its effect on wild rice reproduction remain poorly understood.

The second component of seed production in wild rice, number of seeds per seed head, increases with increasing fertilizer rates (Lee 1983; Atkin et al. 1992; Lee 2002), decreasing density (Lee 2002), and with seed number on the previously formed seed head if a plant produces multiple seed heads (Goldman 1991). Overall, the number of seeds per plant increases with decreasing density, which increases light availability (Lee 2002).

The third component of seed production, individual seed weight, is thought to remain relatively constant within a species (Harper 1977). Evidence of variable individual wild rice seed weight, however, is contradictory in that seed weight is found to increase in high fertilizer treatments (Lee 1983; Atkin et al. 1992) but does not change when different amounts of litter were produced the previous year, changing nutrient availability for the current year's cohort (Walker et al. 2010). In general, larger seeds within a species are presumed to have higher germination rates than smaller seeds because they have larger nutrient stores (Stanton 1984, Harper 1977). Large nutrient stores could increase seedling growth rates (Stanton 1984) which could eventually lead to increased tillering after the plant no longer depends on the seed for nutrition (Edwards & Emara 1970).

Therefore, many of the components of Eq. 3.1 are at least partly determined by nutrient availability, especially the availability of nitrogen. Nitrogen availability in wild rice beds is determined by the balance of nitrogen uptake (immobilization) and release (mineralization) by microbes decomposing previous cohorts of wild rice stem and root litter (Pastor and Walker 2006, Walker et al. 2010). Early in decomposition as the microbial population grows, the balance is towards net immobilization of nitrogen, but at later stages in the decay process, the microbial population declines and nitrogen is released, or mineralized, whereupon it becomes available for plant uptake. The end of the immobilization period and the release of nitrogen from the litter of the previous year's population occurs in mid to late summer (Walker et al. 2010), after the 10-14 day period in late spring or early summer when the current year's plants take up approximately 50%

of their total annual nitrogen requirements (Grava and Raisanen 1978). Thus, immobilization delays the release of nitrogen relative to the period of most rapid uptake. Consequently, the decomposition of previous years' shoots and roots influences the amount of shoots and roots produced in the next year (Pastor and Walker 2006, Walker et al. 2010). Seed production in wild rice is influenced by the proportions of immobilizing or mineralizing litter, as shown by the experiments of Walker et al. (2006), where plants grown with mineralizing litter (therefore increased N availability) had greater seed biomass than plants grown with immobilizing litter (therefore decreased N availability). Therefore, seed production and hence fitness of wild rice could oscillate during biomass oscillations because of self-induced oscillations in nitrogen availability.

By experimentally manipulating availabilities of nitrogen, phosphorus, and light to parent plants, our objective was to determine wild rice's reproduction and fitness responses to the availabilities of nitrogen, phosphorus, and light which simulate those experienced by plants during natural population oscillations. Also examined was the production of new seedlings for the next generation to evaluate how well it estimates plant fitness compared to seed number or total seed weight.

Materials and Methods

Light, nitrogen and phosphorus availabilities were altered to determine their affect on reproductive biomass and fitness of wild rice (*Zizania palustris* L.). In order to obtain seeds for measurements, a growth experiment was conducted at the University of Minnesota Duluth (UMD) Research and Field Studies Station in Duluth, MN. Wild rice was grown in 25 x 20 x 61cm gusseted 3ml plastic bags from U.S. Plastics Corp (Lima,

OH). Each bag was clipped into a 20 x 15 cm rectangle as part of a 12 x 5 rectangle cattle fence grid. Each grid was suspended in a galvanized steel stock tank of dimensions 3.05 x 0.91 x 0.61 m. Each bag contained two liters of washed sand below three liters of sediment, and then watered to 28 cm above the sediment. The sediments were collected from Rice Portage Lake, a natural wild rice lake on the Fond du Lac Chippewa Reservation in Carlton County, Minnesota. Before distributing to plastic bags, the sediment was mixed in large stock tanks. Three sediment grab samples were taken after mixing; the sediments contain total C% = 10.4 ± 0.2 SD, total N% = 1.0 ± 0.02 SD, and organic P% = 0.06 ± 0.003 SD (Harmon and Lajtha 1999). Seeds for sowing were gathered from a wild rice pond on the UMD campus in fall of 2008 and were cold stratified until spring 2009 at 4°C to fulfill dormancy requirements before sowing on 5 June 2009. Two seeds were planted in each bag to ensure that at least one seed germinated to serve as the parent plant.

Nutrient availabilities and light levels were manipulated using fertilizer additions and shade cloths, respectively. Eight treatments of two light, two nitrogen and two phosphorus levels were arranged in a split-plot design. Each of two light levels was applied to three tanks as the whole plots, and each of four nutrient treatments was randomly assigned to twelve bags within each tank as subplots. Each treatment's sample size was twelve plants, four from each tank within a light treatment for each fertilizer treatment.

Cumulative nitrogen and phosphorus additions of 300mg and 110mg respectively per plant were added to fertilized bags over the growing season, based on quantities

contained in a mature, 30g plant (Grava and Raisanen 1978). Also, 50mg of potassium was added to every bag twice during the growing season to minimize possible potassium limitation effects. Nutrients were added weekly over a sixteen week fertilizer schedule, and the elemental weights of nutrients given to fertilized treatments changed each week to follow the phenology of wild rice nutrient uptake (Grava and Raisanen 1978) (Table 4). Fertilizer solutions were prepared using NH_4Cl , $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and KCl . Using a syringe and for all fertilizer solutions and concentrations, which changed weekly, 10ml of solution were injected into designated bags at the sediment/ sand interface.

Full sunlight and 25% of full sunlight represent light saturated net photosynthesis (A_{max}) and the half saturation point of wild rice A_{max} respectively (Zhoa et al. 2004; Sims and Hildebrandt unpubl.). Wild rice's A_{max} , between 15 to 35°C, occurs under photosynthetically active radiation (PAR) levels ranging from 1000 to 1040 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Zhoa et al. 2004), and half saturation of net photosynthesis is around 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Sims and Hildebrandt unpubl.). The shaded environment was created by one wooden/rope frame over three adjacent stock tanks, and shade cloth from American Nettings and Fabric Inc. (Ferndale, WA) was draped over this frame. When averaged over different weather conditions, the shade structure let through $25.3\% \pm 1.6$ s.e. of full sunlight, and the air temperature was $0.34^\circ\text{C} \pm 0.03$ s.e. lower than outside the shade structure. At natural wild rice stands in Grand Rapids, MN and Superior, WI, 13.3% and $19.5\% \pm 0.7$ s.e. of full sunlight reached the water surface, respectively, and 63.3% and $37.9\% \pm 0.9$ s.e. of full sunlight penetrated halfway through the canopy, respectively (Clay and Oelke 1987; Sims observation). Therefore, the shade cloth transmitted similar

amounts of light as do natural wild rice canopies, and plants grown under the shade cloth faced similar light regimes as small rice plants growing under a canopy of larger plants in natural stands.

Seeds were collected from mid-September to mid-October when they were ready to shatter, or fall off the seed head. Seeds collected from six of the twelve plants per treatment were immediately dried after collecting. Dried seeds were separated into filled, unfilled and rice worm infested categories. Because not every seed produced per plant was collected because of natural shattering, seed production was determined by comparing the number of seeds collected to the number of pedicels on a seed head, and to account for the mass of seeds lost due to rice worm infestation, total seed biomass was estimated by the following equation:

Seeds from the remaining six plants per treatment were immediately placed in 100mL plastic bottles, filled with well water after collection, and stored in darkness in a cold room (4°C) for seven months until the germination study (Oelke et al. 1982). To begin the germination study, wet seeds were separated into filled, unfilled and rice worm infested categories, and all filled seeds from one plant were placed in one pint size Mason jar with deionized water (for a total of 6 jars/treatment) on 7th June, 2010. Jars were placed randomly on an East facing window sill of the Swenson Science Building at UMD and were rotated every day. Seeds were considered germinated and were removed from

the jar when their radicle broke through their seed coat (Harper 1972). Jars were filled with deionized water when needed, and the germination study was ended on 6th July, 2010. Germination rate was calculated per plant as the number of full seeds that germinated by the number of full seeds collected. The number of seedlings per plant was calculated as the product of the average germination rate per treatment and the estimated number of full seeds not eaten by rice worm per plant for the same treatment.

Tiller number per parent plant was documented twice each week beginning on Julian day 194 when aerial tillers were present and ending when all remaining plants were harvested between Julian days 173-187 (Table 4). The number of plants measured for tiller number decreased throughout the growing season as plants were sequentially harvested for a different study (Table 4).

The field experiment was a completely random split-plot design as described above. All treatments were considered fixed. In the ANOVA, tanks were random effects nested within light, and there were no significant tank effects. When necessary, data was log transformed to better fit normality and homoscedasticity assumptions. Tukey's HSD was used to determine differences among individual treatments. Results from different methods of deriving seedling number per plant were compared using *t*-tests at $\alpha = 0.05$.

Results

Total Seed Biomass

Wild rice seed biomass production, calculated from Eq. 3.2, responded differently to nitrogen, phosphorus and light levels (Fig. 9). Nitrogen addition strongly increased total seed biomass ($F_N = 231.261$, $p < 0.001$; Fig. 9). Phosphorus addition increased total

seed biomass, but only after nitrogen was added to the same plants ($F_{N * P} = 11.350$, $p = 0.002$; Fig. 9). Full sunlight increased seed biomass ($F_L = 7.105$, $p = 0.011$) by 40% over shade, but this increase was greater with added nitrogen (46%) than without (13%).

Tillering and Seed Head Production

Compared to ambient nitrogen, nitrogen addition strongly increased the mean number of tillers per plant ($F_{N(\text{tillering})} = 197.8$, $p < 0.001$; Fig. 2) and the number of seed heads produced per plant ($F_{N(\# \text{ of seed heads})} = 470.3$, $p < 0.001$, data not shown). With added nitrogen, the number of tillers per plant peaked around Julian day 238 then generally declined to the final harvest (Fig. 2). Nitrogen addition also increased the proportion of tillers produced that were reproductive compared to plants that did not receive extra nitrogen ($F_{N(\text{proportion})} = 10.505$, $p < 0.002$; Fig. 3).

Compared to ambient phosphorus, phosphorus addition increased the mean number of tillers per plant and the number of seed heads produced per plant but only when nitrogen was also added ($F_{N * P(\text{tillering})} = 18.68$, $p < 0.001$; Fig. 2; $F_{N * P(\# \text{ of seed heads})} = 9.74$, $p = 0.002$, data not shown). When only phosphorus was added, the number of tillers for plants peaked earlier than with added nitrogen, at Julian day 219 as compared with Julian day 238 (Fig. 2). Phosphorus had no effect on the proportion tillers produced that were reproductive.

Compared to shade, full sunlight increased the mean number of tillers per plant and the number of seed heads produced per plant but only when nitrogen was also added ($F_{N * L(\text{tillering})} = 11.94$, $p < 0.001$; Fig. 2; $F_{N * L(\# \text{ of seed heads})} = 9.74$, $p = 0.002$, data not shown). Full sunlight also decreased the proportion of tillers produced that were

reproductive compared to plants grown in shade, but full sunlight only decreased this proportion with added nitrogen ($F_{N * L(\text{proportion})} = 4.74, p = 0.032$; Fig 3).

Seeds per Seed Head

Nitrogen additions strongly increased the mean number of seeds per seed head ($F_N = 20.58, p < 0.001$; Fig. 12), but phosphorus additions and full sunlight had no effect. Seed heads of plants with added nitrogen produced on average sixteen more seeds than seed heads of plants with ambient nitrogen.

Individual Weight of Full Seeds

Assuming seeds eaten by rice worm were filled, plants from different treatments did not differ in their proportions of filled or unfilled seeds; however, because rice worm selected seeds from plants grown with added nitrogen and/or in shade ($F_{N(\text{rice worm})} = 8.28, p = 0.006$; $F_{L(\text{rice worm})} = 10.72, p = 0.002$, respectively; Fig. 13), plants grown with ambient nitrogen and/or in full sunlight ($F_{N(\text{uneaten filled})} = 11.30, p = 0.002$; $F_{L(\text{uneaten filled})} = 15.25, p < 0.001$, respectively; Fig. 13) had a greater proportion of uneaten filled seeds. Phosphorus additions had no effect on rice worm infestation or seed filling rates.

Nitrogen additions strongly increased the mean dry weight of individual full seeds ($F_N = 7.79, p = 0.008$; Fig. 14) by 20%, but phosphorus additions and light levels had no effect.

Germination Rates and Seedlings

Nitrogen additions decreased the germination rate of full seeds ($F_{N(\text{germ. rate})} = 5.74, p = 0.022$; Fig. 15), but phosphorus additions and light levels had no effect. Full seeds

from plants grown with added nitrogen had a 10% lower average germination rate than full seeds from plants grown with ambient nitrogen.

The predicted number of seedlings produced per plant, calculated from Eq. 3.1 as the number of seeds that germinated, was between 26% - 74% of the total number of seeds produced per plant. Nitrogen additions strongly increased the predicted number of seedlings produced per parent plant ($F_{N(\text{seedlings})} = 61.276, p < 0.001$; Fig. 16). Plants grown in full sunlight produce more seedlings the following year ($F_{L(\text{seedlings})} = 32.889, p < 0.001$; Fig. 16) but only with added nitrogen ($F_{N*L(\text{seedlings})} = 6.618, p = 0.014$; Fig. 16). Phosphorus additions slightly increased the predicted number of seedling compared to plants grown at ambient P ($F_{P(\text{seedlings})} = 5.438, p = 0.025$; Fig. 16).

Discussion

Total seed biomass has generally been used to measure a plant's fitness (Harper 1977). Seedling number the following year, however, is a better estimate of plant fitness: not all seeds produced are viable because of predation and seed filling rates, and those that are viable do not always germinate (Simpson 1966; Aiken et al. 1988; Atkin et al. 1992). The predicted number of seedlings per plant derived by accounting for seed filling rates, predation and germination rates depended on treatment and was 26% -74% of the total number of seeds produced per plant which includes seeds that are not filled, are eaten, or do not germinate and is therefore nonviable biomass.

Compared to ambient nitrogen, nitrogen additions strongly increased total seed biomass by increasing the number of seed heads produced, the number of seeds per seed head and the mean weight per seed. In spite of decreased germination rates and selection

by rice worms for plants grown with nitrogen additions, nitrogen additions increased the number of seedlings produced per plant the following year because so many seeds per plant were produced. Phosphorus additions increased total seed biomass only after nitrogen was added, indicating phosphorus became limiting to seed production only after nitrogen limitations were reduced. Phosphorus additions also slightly increased the number of seedlings produced per plant the following year. Full sunlight increased the number of seedlings produced per plant the following year in part because of increased total seed biomass (primarily because of more seed heads per plant) but also because rice worms selected seeds from shaded plants. Because greater nitrogen, phosphorus and light availability increased the number of seedlings produced the following year, they increased plant fitness compared to plants grown under ambient P and N and reduced light.

Nitrogen availability to the parent generation influences the number of seedlings produced the following year through its effects on seed production, rice worm predation, and germination success. However, nitrogen availability changes from year to year because of immobilization of nitrogen in first-year cohorts of litter also produced by the parent generation (Pastor and Walker 2006; Walker et al. 2006; Walker et al. 2010). Nitrogen immobilization in decaying first-year litter cohorts delays nitrogen release from the decomposition of litter relative to the 10-14 day period in late spring-early summer when wild rice plants take up 50% of their total annual nitrogen; this delay in nitrogen availability may cause 4-5 year biomass oscillations (Grava and Raisanen 1978; Sain 1984; Keenan and Lee 1988; Archibold et al. 1989; Pastor and Walker 2006, Walker et al

2010). For example, during a year of high nitrogen availability large amounts of biomass are produced, which becomes a large cohort of nitrogen immobilizing litter the following year, as well as large amounts of seeds. The large cohort of litter results in low nitrogen availability the following year, and the increased nitrogen limitations consequently yield small amounts of biomass. Seedlings growing the year after a high biomass year may experience great competition for above and below ground resources because of large seedling densities and the concurrently low nitrogen availabilities (Walker et al. 2010). Environmental maternal effects therefore extend to the next generation directly through seed production and germination and indirectly through changes in nitrogen availability caused by maternal litter.

Even though the number of seedlings produced is a better measure of fitness than seed number or total seed biomass, an even better measure would be the number of offspring reaching reproductive maturity that result from that seedling population. Seedlings are subject to a different nitrogen environment than their parents because of the changes in nitrogen availability caused by immobilization into the litter of the parent generation (Walker et al. 2010). As fluctuations in nutrient availability drive fluctuations in biomass and canopy density, they may also induce fluctuations in light availability to smaller plants and seedlings (Keenan and Lee 1988; Wilson and Tilman 1991; Wilson and Tilman 1993). Therefore, the new seedling population will undergo self-thinning, where plants failing to meet their needs for limited resources die and remaining plants have greater access to those resources (Yoda et al. 1963; Westoby 1984).

The degree of self-thinning may, however, depend on changes in nitrogen and light availability during a population cycle (Lee 2002). Wild rice stands meet several self-thinning criteria (Yoda et al. 1963) including: monotypic stands (Weir and Dale 1960; Oelke et al. 1988) similar age composition, sufficiently high initial plant density (Weiner and Whigham 1988; Lee 2002), similar plant shape (Weiner and Whigham 1988; Lee 2002), and high leaf coverage (Clay and Oelke 1987; Sims observation). Low shoot nitrogen concentration, resulting from low sediment nitrogen availability, and low light availability, resulting from high leaf area indices, cause tiller death (Zhong et al. 2003), and competition for below and above ground resources may, therefore, cause the death of whole wild rice plants. Although, conclusive evidence of self-thinning in wild rice remains wanting, some seedlings are assumed to die because of competition for above and below ground resources. Seedling decomposition may, therefore, play a role in the immobilization/ mineralization processes of litter decomposition, perhaps in the timing of nitrogen release.

Because some seeds are eaten, not all seeds are viable, and each seedling is not guaranteed survival to reproductive maturity, a plant's fitness is therefore determined by the number of its offspring that reach reproductive maturity. In the case of wild rice, the parent plant's influence on its offspring extends beyond the time when seed nutrition is exhausted by virtue of the problems of nutrient immobilization into the parent plant's litter. Therefore, not only is the reproductive output of the parent generation influenced by their nutrient and light environment, the parent generation also influences their own

fitness by altering the nutrient and light environment of their offspring through the decomposition of their litter.

Table 1. Wild rice fertilizer and harvest schedules during summer 2009. The fertilizer schedule provides the elemental weights (mg) of nitrogen (N) and phosphorus (P) applied per plant in treatments that received N, P or both per application, and the harvest schedule lists corresponding average developmental stages for the entire experiment and sample sizes. Nitrogen and phosphorus fertilizer solutions were prepared using NH_4Cl and $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, respectively.

Week	<i>Fertilizer Schedule</i>				<i>Harvest Schedule</i>			
	Date	mg N plant ⁻¹	mg P plant ⁻¹	% of total	Date (Julian Day)	Harvest	Mean Life Stage	<i>n</i> per treatment
1	5-Jun	0.0	0.0	0.0	5-Jun (156)		Planting	
2	15-Jun	0.0	0.0	0.0				
3	24-Jun	7.5	2.75	2.5				
4	1-Jul	15.0	5.5	5.0	30-Jun (181)	1	Floating Leaf	35-36
5	8-Jul	15.0	5.5	5.0				
6	15-Jul	30.0	11.0	10.0	13-Jul (194)	2	2nd Aerial Leaf	6
7	23-Jul	45.0	16.5	15.0	24-Jul (205)	3	Jointing	6
8	29-Jul	45.0	16.5	15.0				
9	6-Aug	45.0	16.5	15.0	7-Aug (219)	4	Early Flowering	6
10	14-Aug	30.0	11.0	10.0				
11	19-Aug	15.0	5.5	5.0				
12	27-Aug	15.0	5.5	5.0	26-Aug (238)	5	Mid Flowering	6
13	2-Sep	15.0	5.5	5.0				
14	9-Sep	15.0	5.5	5.0				
15	16-Sep	7.5	2.75	2.5				
16	23-Sep	0.0	0.0	0.0	30-Sep - 14- Oct	6	Maturity	12
	Total	300.0	110.0	100.0	(273-287)			

Table 2. Wild rice (*Zizania palustris* L.) developmental stages from germination through seed shattering and plant death including descriptions.

		Developmental Stage	Stage Description
Growth Stages	Vegetative	0 Emergence	germination and ribbon-like leaf erect in water column
		1 Floating Leaf	ribbon-like leaves floating on water's surface
		2 2nd Aerial Leaf	2nd leaf erected in air off water's surface
		3 Early Tillering	tiller grows one aerial leaf
		4 Mid Tillering	tiller(s) grow more than one aerial leaf
Growth Stages	Reproductive	5 Jointing	internodes of stem begin to elongate
		6 Early Boot	panicle and female ovaries begin to emerge from stem
		7 Early Flowering	few male flowers are mature, other still in stem
		8 Mid Flowering	most male flowers are mature, none are in stem
		9 Late Flowering	few male flowers remain on panicle
		10 Maturity	no male flowers present and seed filling occurs
		11 Shattering	seeds shatter off panicle and plant tissue dies

Table 3. Effect of nitrogen, phosphorus and light on relative growth rate (*RGR*) and its leaf and root components. Unit leaf rate (*ULR*), specific leaf area (*SLA*) and leaf weight ratio (*LWR*) are leaf components and unit root rate (*URR*), specific root area (*SRA*) and root weight ratio (*RWR*) are root components. Repeated measures main effect *F*-values between becoming significant to 25, from 25 to 75 and >75 are represented by one, two and three arrows respectively. Six of twenty-eight interactions for all parameters were significant, but are not shown because none were significant for *RGR*. An arrow's direction is towards the response of added N, added P and full sunlight. An equal sign indicates no significant difference.

Main Effect	<i>RGR</i>	<i>ULR</i>	<i>SLA</i>	<i>LWR</i>	<i>URR</i>	<i>SRA</i>	<i>RWR</i>
Nitrogen	↑↑	↑↑	↓	↑↑	↑↑↑	↓↓↓	↓↓↓
Phosphorus	=	↑	=	=	↑	↓	=
Light	=	↑↑↑	↓↓↓↓	↓↓↓↓	↓	=	↑↑

Table 4. Wild rice fertilizer schedule with elemental weights (mg) of nitrogen (N) and phosphorus (P) applied per plant and sample sizes for tiller counts during summer 2009. Nitrogen and phosphorus fertilizer solutions were prepared using NH₄Cl and NaH₂PO₄·H₂O, respectively. Tiller number per plant was documented twice each week beginning on Julian day 194 when aerial tillers were present.

Week	<i>Fertilizer Schedule</i>					<i>Sample Size for Tiller Counts</i>
	Date	Julian Day	mg N/plant	mg P/plant	% of total	# of plants
1	5-Jun	156	0.0	0.0	0.0	0
2	15-Jun	166	0.0	0.0	0.0	0
3	24-Jun	175	7.5	2.75	2.5	0
4	1-Jul	182	15.0	5.5	5.0	0
5	8-Jul	189	15.0	5.5	5.0	0
6	15-Jul	196	30.0	11.0	10.0	36
7	23-Jul	204	45.0	16.5	15.0	30
8	29-Jul	210	45.0	16.5	15.0	24
9	6-Aug	220	45.0	16.5	15.0	24
10	14-Aug	226	30.0	11.0	10.0	18
11	19-Aug	231	15.0	5.5	5.0	18
12	27-Aug	239	15.0	5.5	5.0	18
13	2-Sep	245	15.0	5.5	5.0	12
14	9-Sep	252	15.0	5.5	5.0	12
15	16-Sep	259	7.5	2.75	2.5	12
16	23-Sep	266	0.0	0.0	0.0	12
	Total		300.0	110.0	100.0	

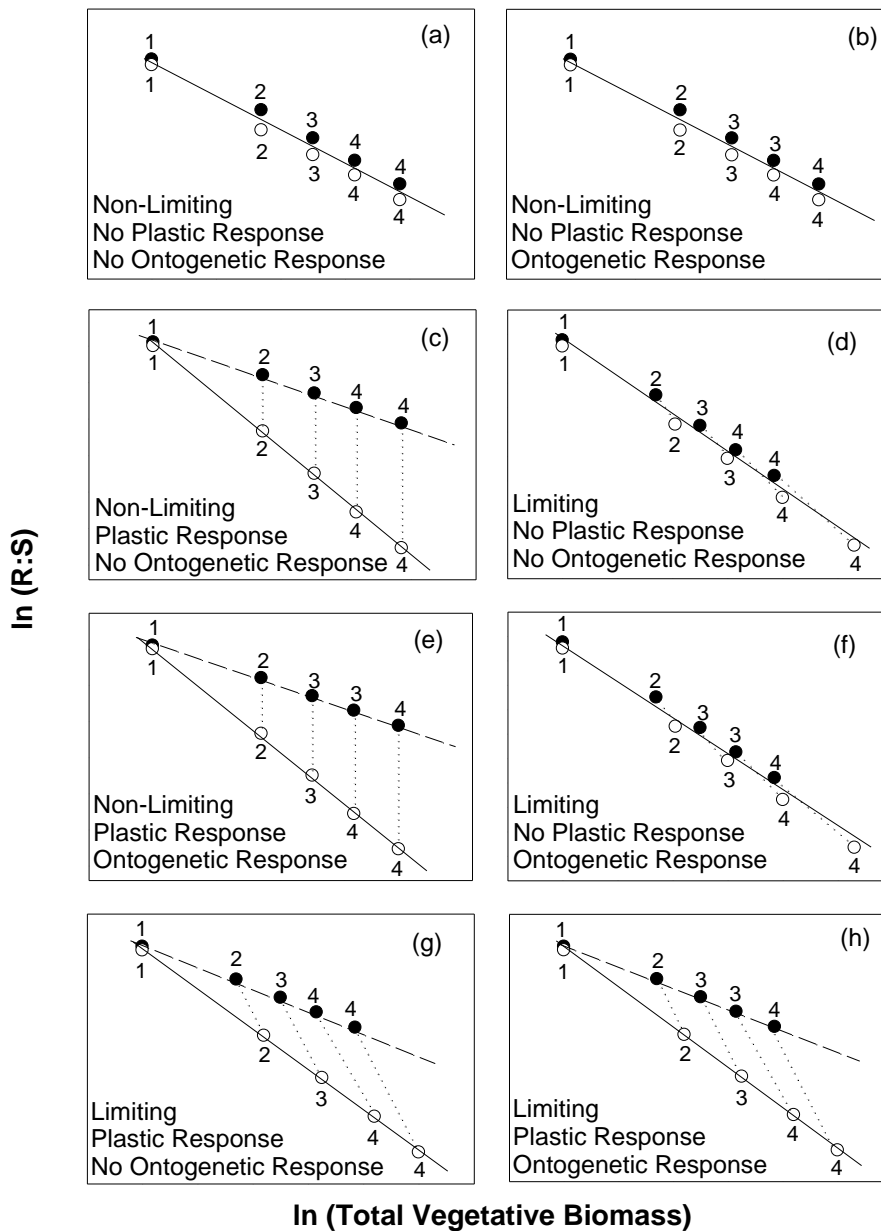


Figure 1. Eight possible scenarios relating changes in plant biomass and allocation to ontogenetic, plastic, and growth limitation responses. Each scenario compares resource limitations to biomass production, plastic allocation, and ontogeny (numbered developmental stages) for two genetically similar groups of plants grown under high (open circles) or low (closed circles) resource availability. Dotted lines connect plants sampled at the same time but grown under different resource availabilities. A limitation response is shown by a difference in biomass on the same sample date (d, f, g and h). A plastic response is shown by plants following different trajectories over multiple sample dates (c, e, g and h). An ontogenetic response in developmental stage is shown by plants being at different developmental stages on the same sample date (b, e, f and h).

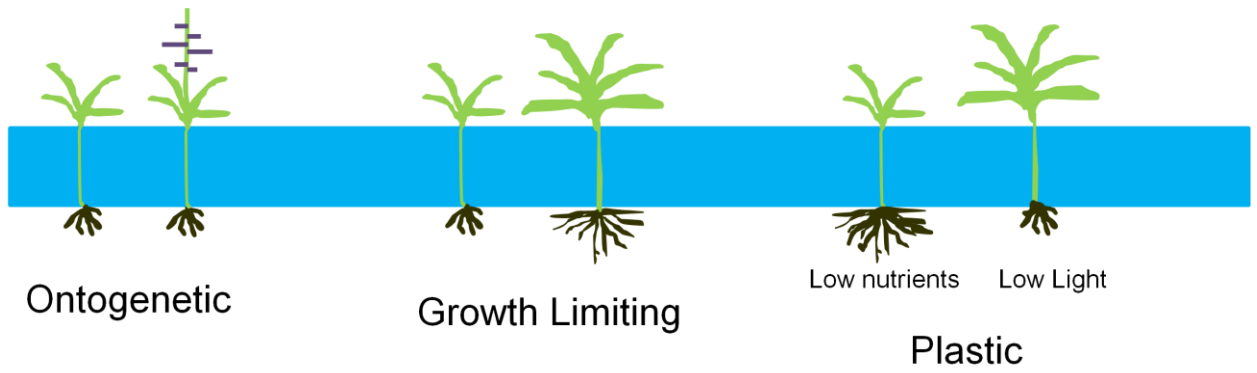


Figure 2. Allocation pattern response mechanisms for wild rice plants under different resource availabilities. An ontogenetic response occurs when plant developmental rates change. A growth limiting response occurs when plant growth rates change. A plastic response occurs when plants allocation biomass toward tissues responsible for acquiring limiting resources.

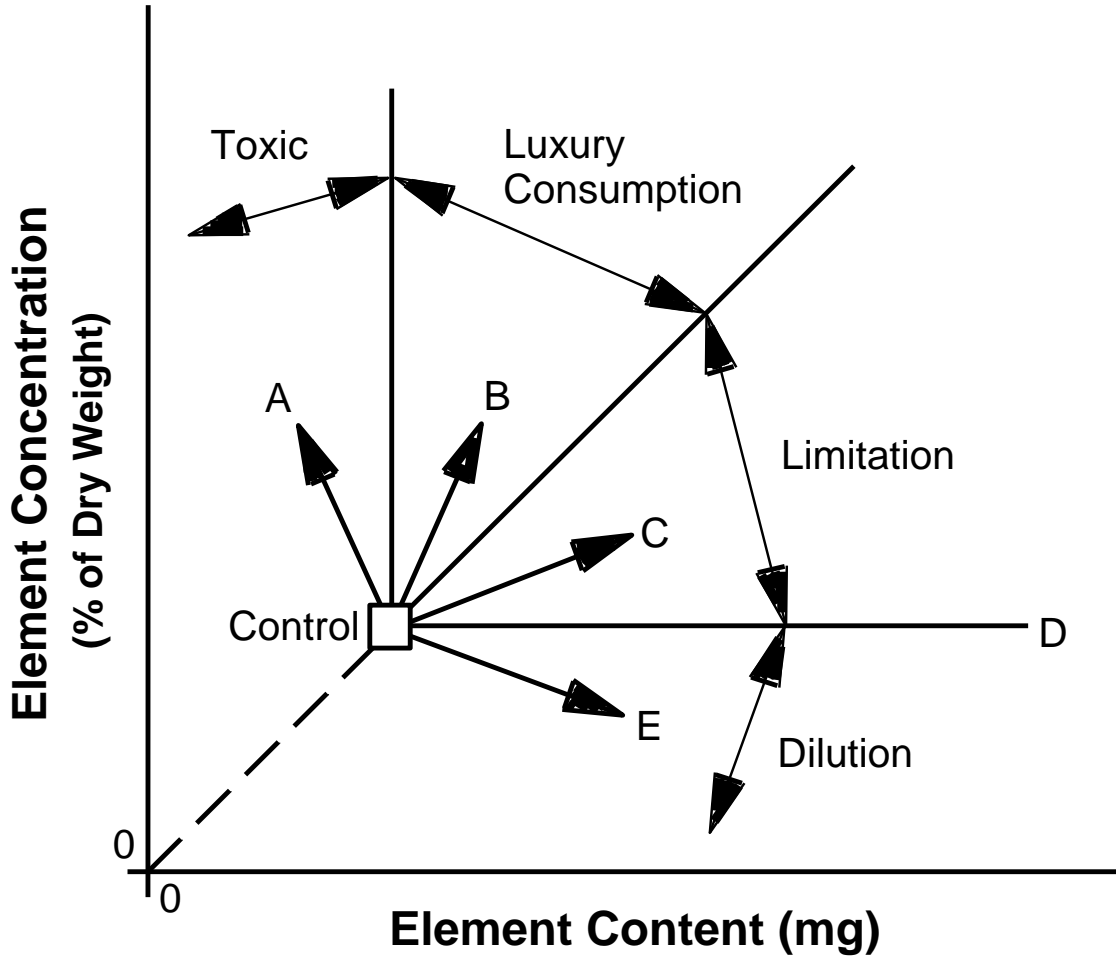


Figure 3. Changes in element concentration (%) and content (mg/plant) compared to the trajectory from the origin through a control (Timmer and Stone 1978). Tissues from plants grown in different resource availabilities may vary in both element concentrations and content. Changes may indicate toxicity (A), luxury consumption (B), limitation (C), minimum percent (D), or dilution meaning not limiting (E).

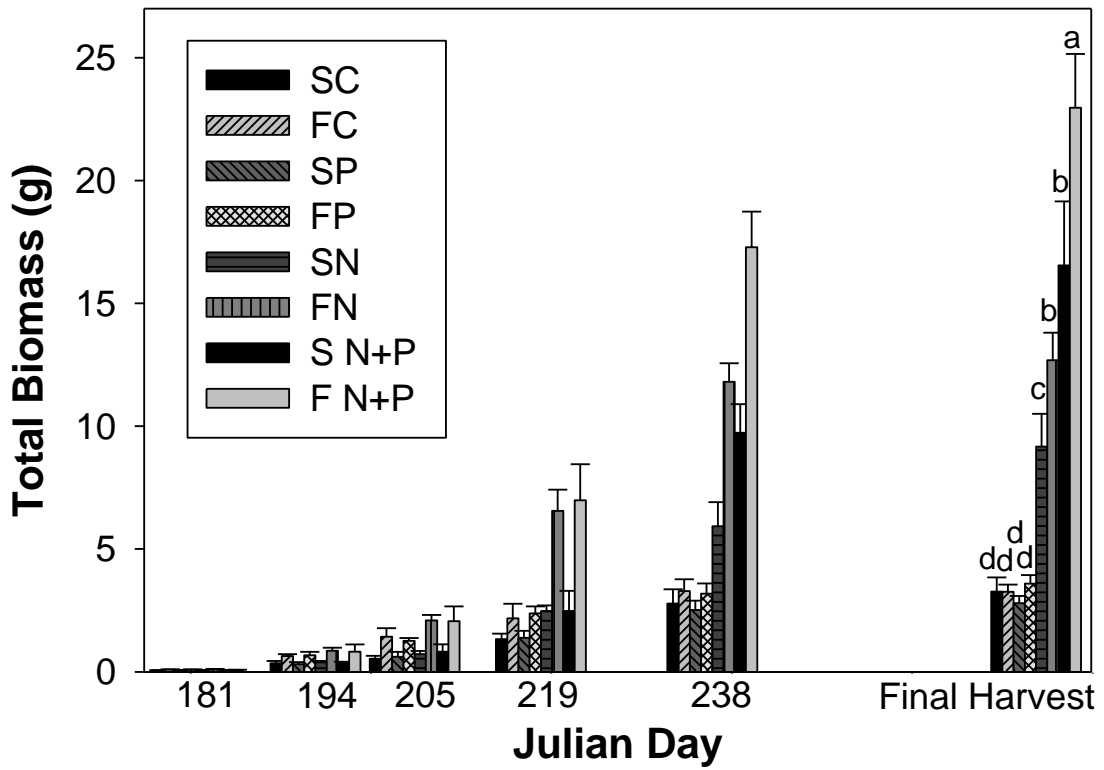


Figure 4. Mean total biomass of wild rice for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Error bars are 95% C.I. Darker bars are shade treatments, and lighter bars are full sunlight treatments. Sample sizes for each harvest date follow Table 1 except for the final harvest with a sample size of six plants. Mean, final harvest dates for individual treatments at the shattering stage varied between Julian days 273-287. For the final harvest, treatments with the same letter are not significantly different according to Tukey HSD.

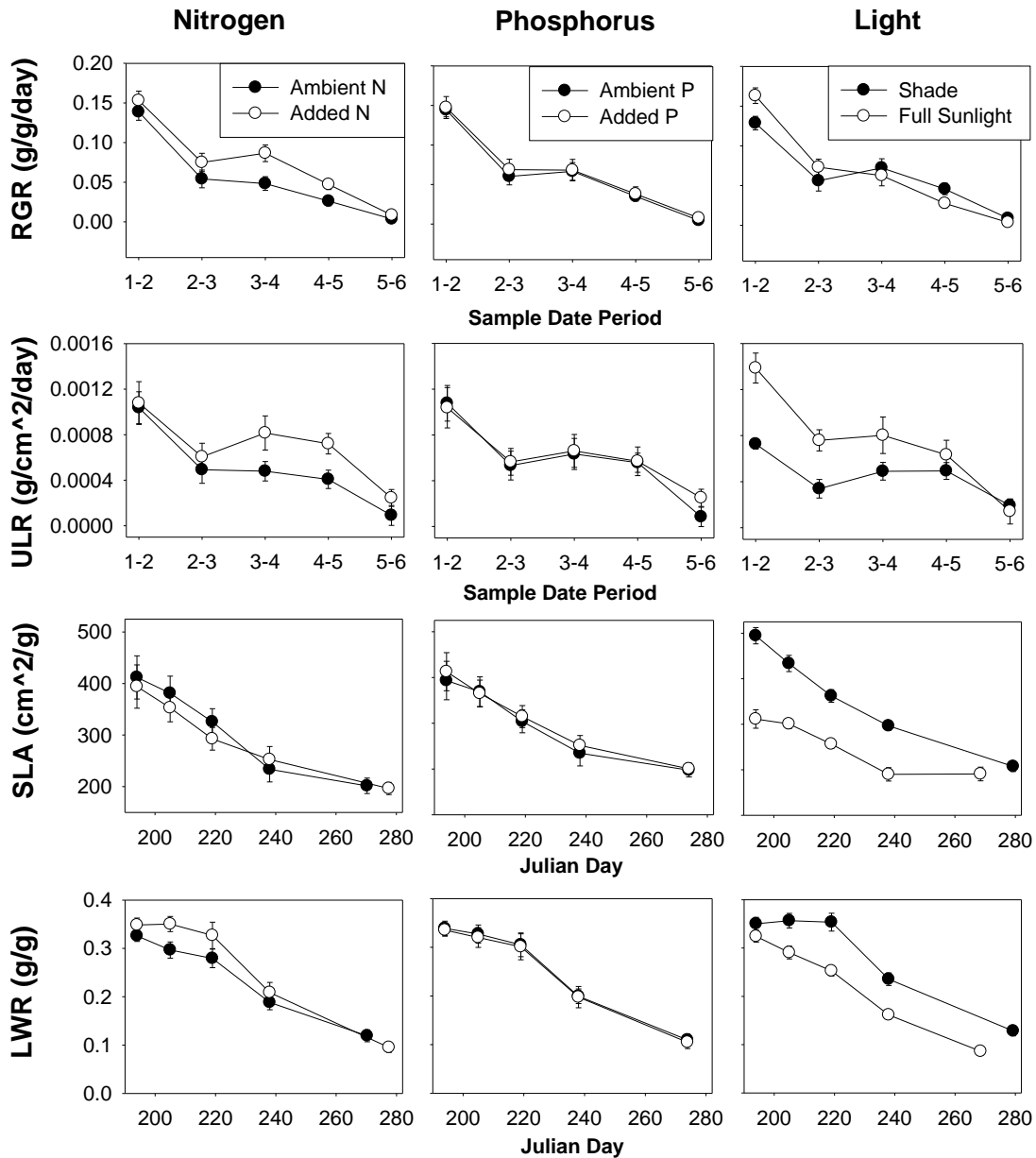


Figure 5. Mean relative growth rate (*RGR*) and leaf component, unit leaf rate (*ULR*), specific leaf area (*SLA*) and leaf weight ratio (*LWR*), responses of wild rice to nitrogen, phosphorus and light levels. Points are means of six plants for each harvest date, except for the sixth harvest for *SLA* which is a mean of twelve plants. Leaf measurements were not collected on plants from harvest 1 because only the floating leaf was present. Filled circles are lower resource availability whereas open circles are higher resource availability. Error bars are 95% C.I. for each harvest date.

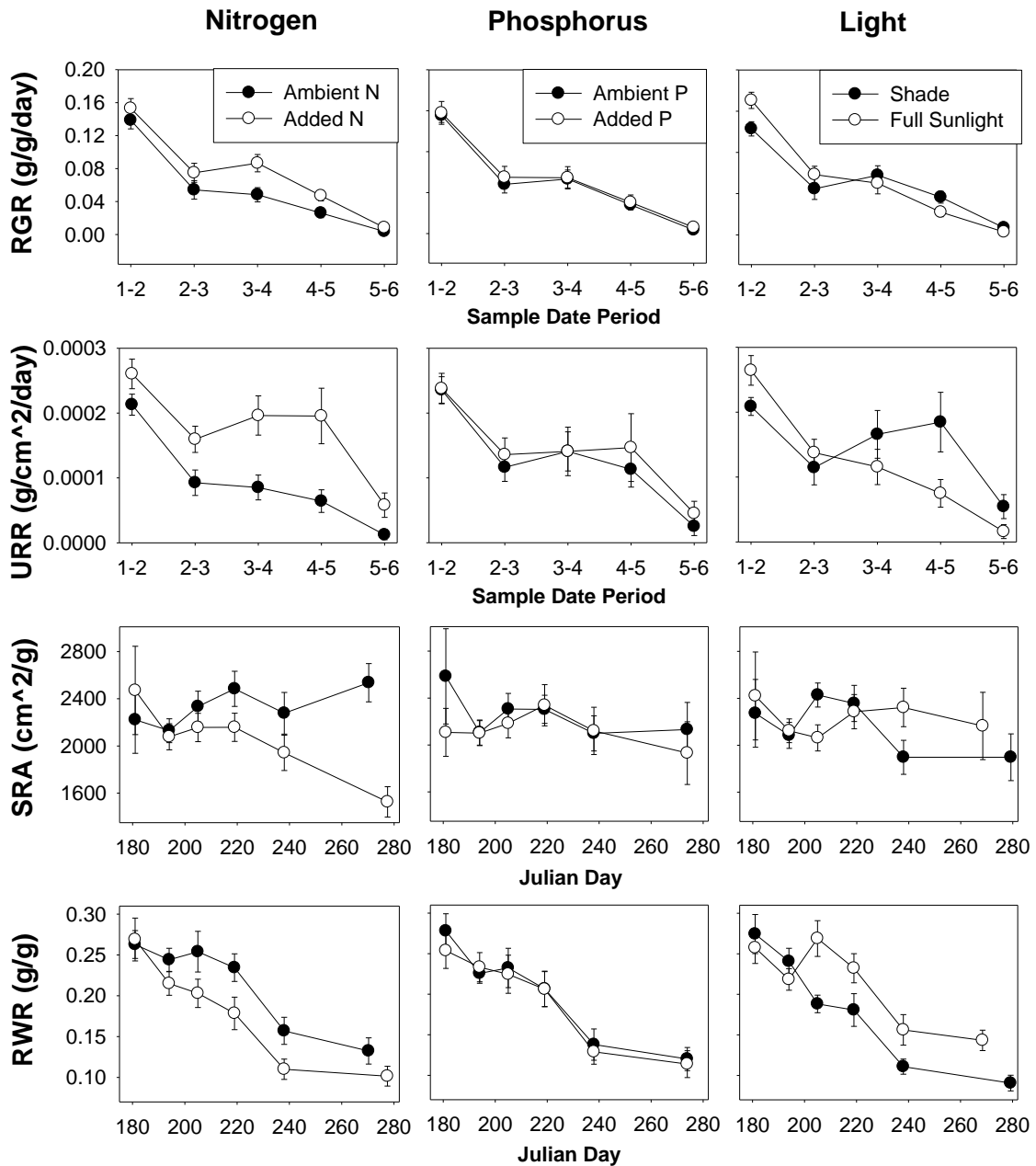


Figure 6. Mean relative growth rate (*RGR*; same as in Figure 5) and root component, unit root rate (*URR*), specific root area (*SRA*) and root weight ratio (*RWR*), responses of wild rice to nitrogen, phosphorus and light levels. Points are means of six plants for each harvest date, except for the sixth harvest for *SRA* which is a mean of twelve plants. Filled circles are lower resource availability whereas open circles are higher resource availability. Error bars are 95% C.I. for each harvest date.

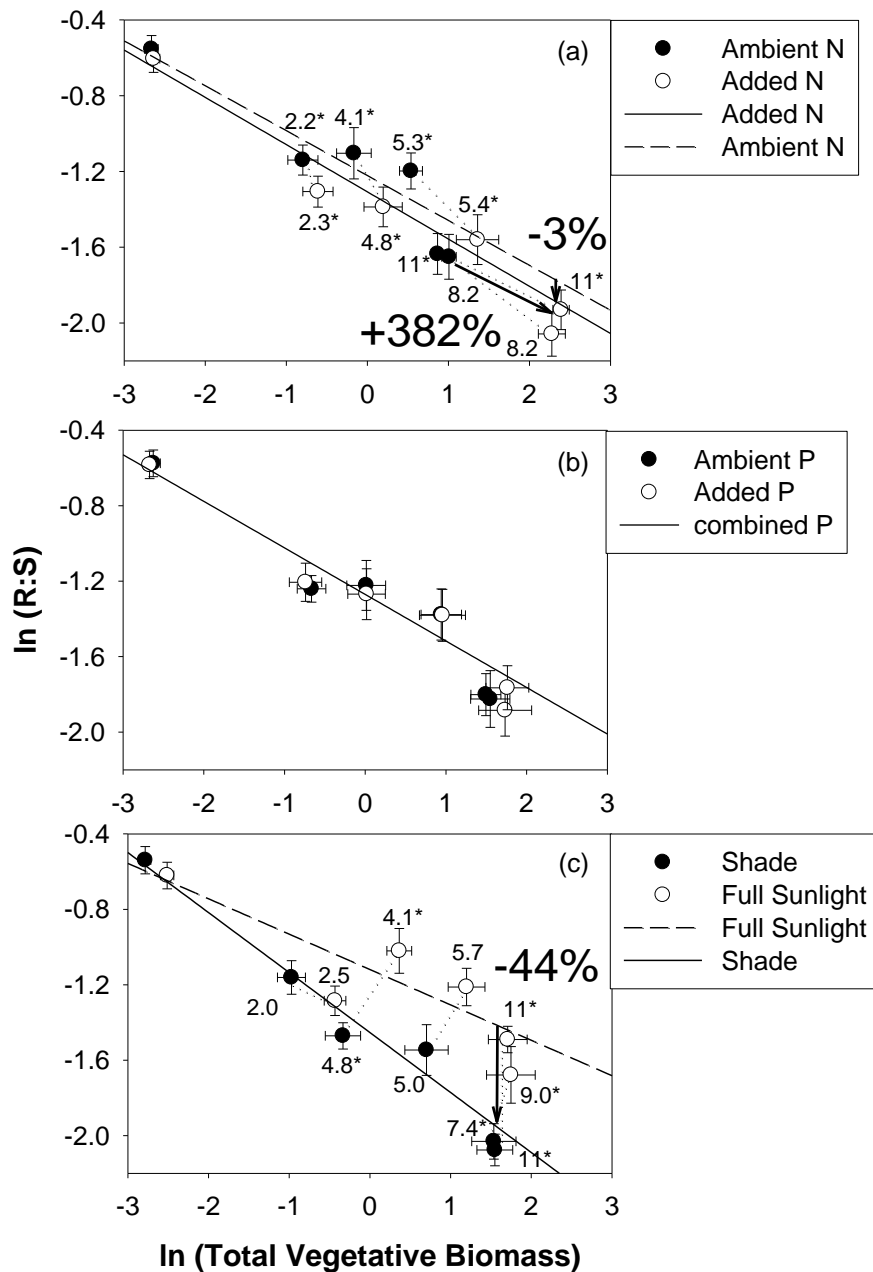


Figure 7. Relationship of $\ln(\text{root:shoot } (R:S))$ to $\ln(\text{total vegetative biomass})$ in wild rice for nitrogen (a), phosphorus (b), and light (c) main effects. Vegetative biomass includes roots and shoots but not seeds. Filled circles are lower resource availability and open circles are higher resource availability. Regression equations are derived from data for all harvest dates, whereas individual circles represent means for each harvest date. Error bars are 95% C.I. Dotted lines connect means from the same harvest. Nitrogen and light (but not P) affected plant developmental rates, which are denoted by developmental stage numbers and followed by an asterisk when significant as determined by ANOVAs for each harvest (Table 2). Bolded percent values and arrows indicate changes in root biomass because of plasticity or growth limitation.

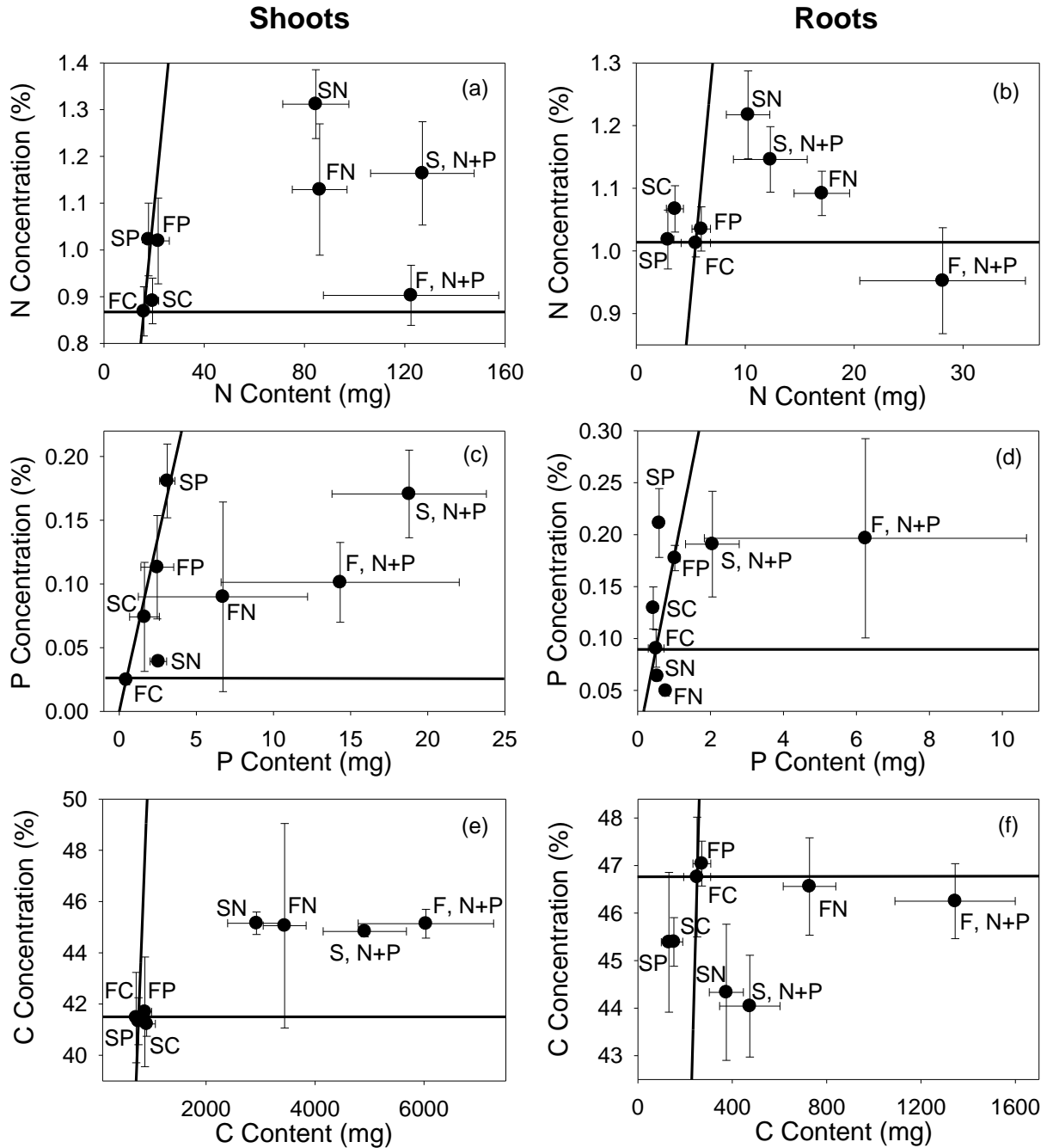


Figure 8. Relationship between tissue element concentration (%) and element content per plant for the shoots (mg/shoot) and roots (mg/root) of wild rice plants grown under different nitrogen (C for ambient control, N for added N), phosphorus (C for ambient control, P for added P) levels, and light (S for shade and F for full sunlight). Elements shown are nitrogen (N) (a, b), phosphorus (P) (c, d) and carbon (C) (e, f). Each point represents mean values for 6 randomly chosen plants of the 12 from the final harvest. A linear line is drawn from the origin through the full sunlight ambient N and P treatment (FC) mean. Other treatment means along this line show a change in concentration but not

in biomass indicating luxury consumption response for those treatments. Treatment means along a line parallel with the x -axis and through FC indicate a minimum concentration requirement for those treatments. Treatment means that lie between these two lines indicate a limitation response for those treatments. Treatment means that lie below the minimum concentration indicate a dilution response for those treatments.

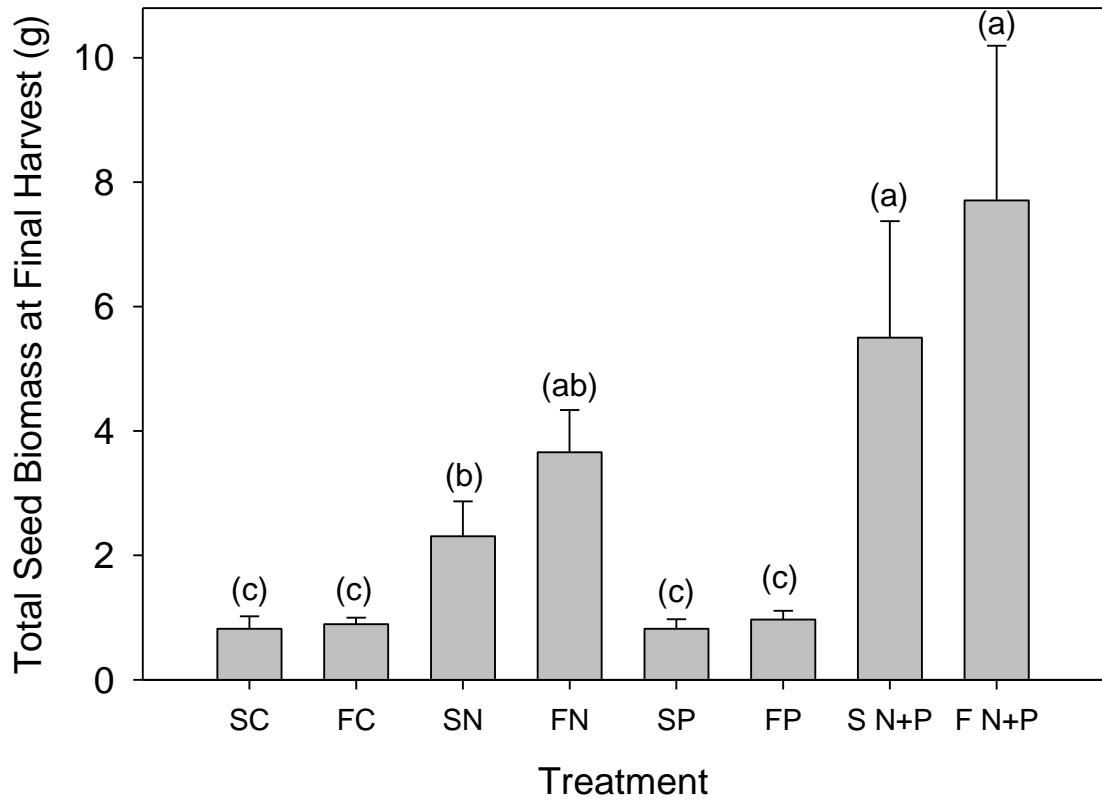


Figure 9. Estimate of total seed biomass per plant for wild rice at final harvest using Eq. 3.2 for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Sample size was six plants for each treatment, and y-error bars are 95% C.I. Treatments with the same letter are not significantly different according to Tukey HSD at $p < 0.05$.

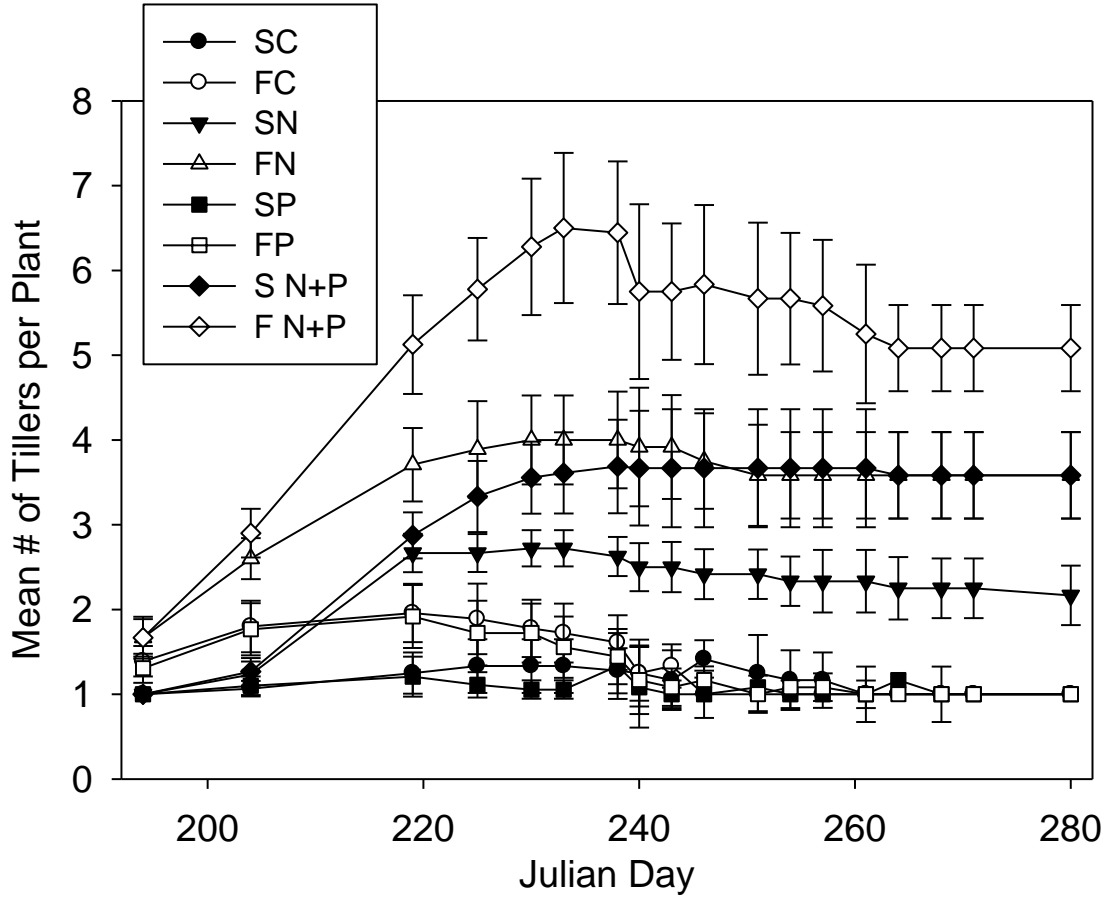


Figure 10. Mean number of tillers per plant over the growing season for wild rice for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Sample sizes for each mean varied between 12 – 36 (Table 4). Tiller number per plant was documented twice each week beginning on Julian day 194. Planting was on Julian day 156 and plants were harvested between Julian days 273-287. Y-error bars are 95% C.I.

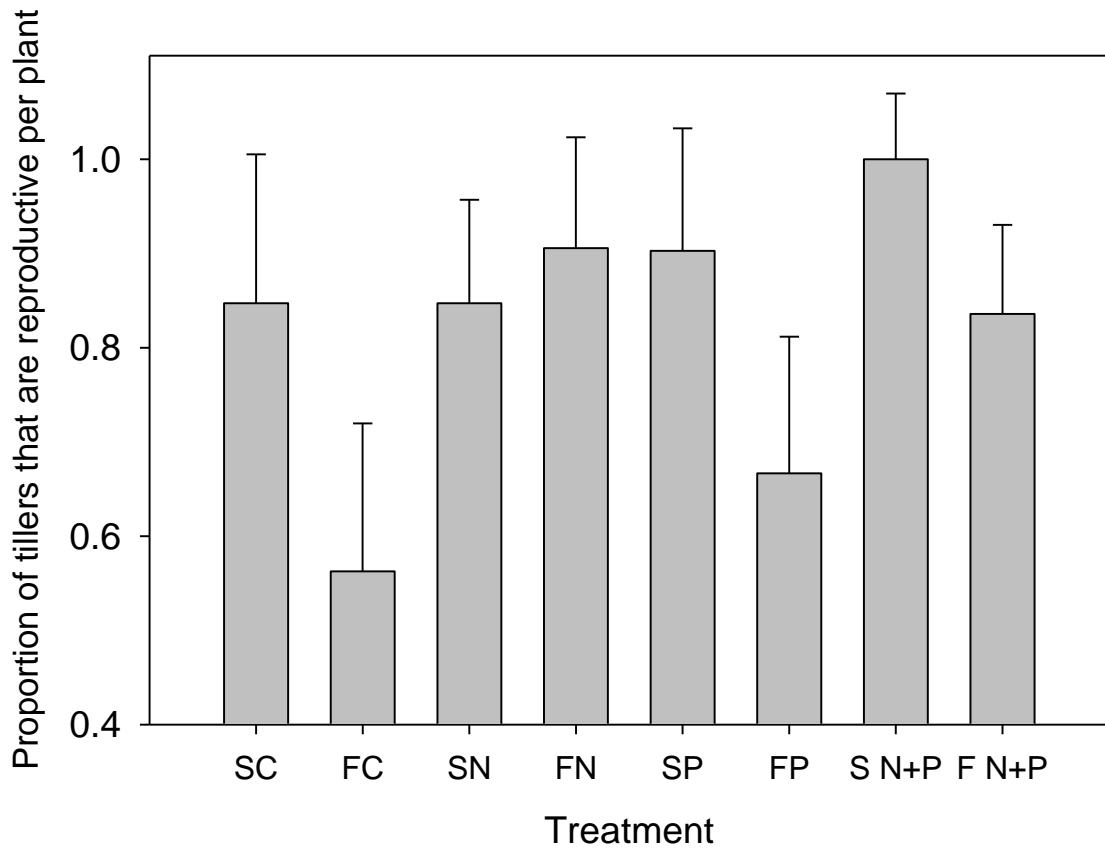


Figure 11. Mean proportion of the maximum number of tillers that were reproductive for wild rice for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. The remaining proportion of tillers did not produce seed heads. Bars are means of 12 plants and y-error bars are 95% C.I. ANOVA was significant for nitrogen ($F_N = 10.505, p < 0.002$) and nitrogen*light interaction ($F_{N*L} = 4.74, p = 0.032$).

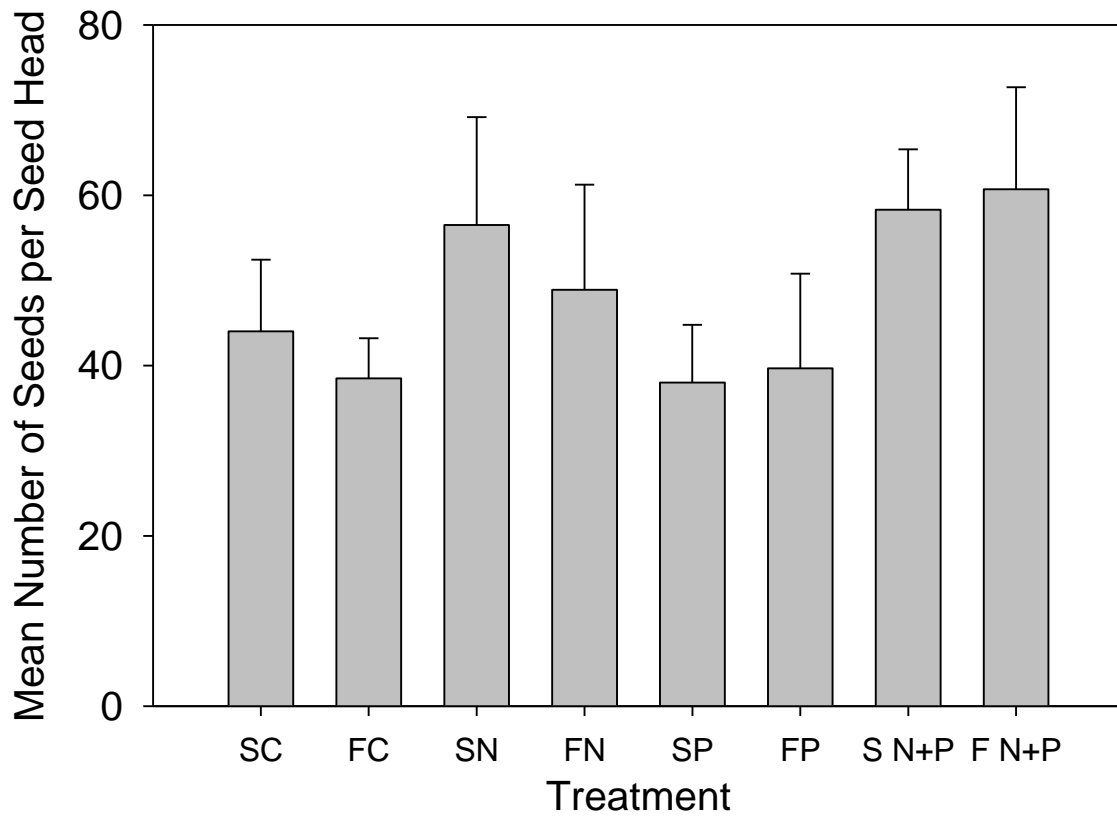


Figure 12. Mean number of seeds per seed head for wild rice determined by counting each pedicel on each seed head for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Sample size is six plants for each treatment, and y-error bars are 95% C.I. ANOVA was significant for nitrogen ($F_N = 20.58, p < 0.001$).

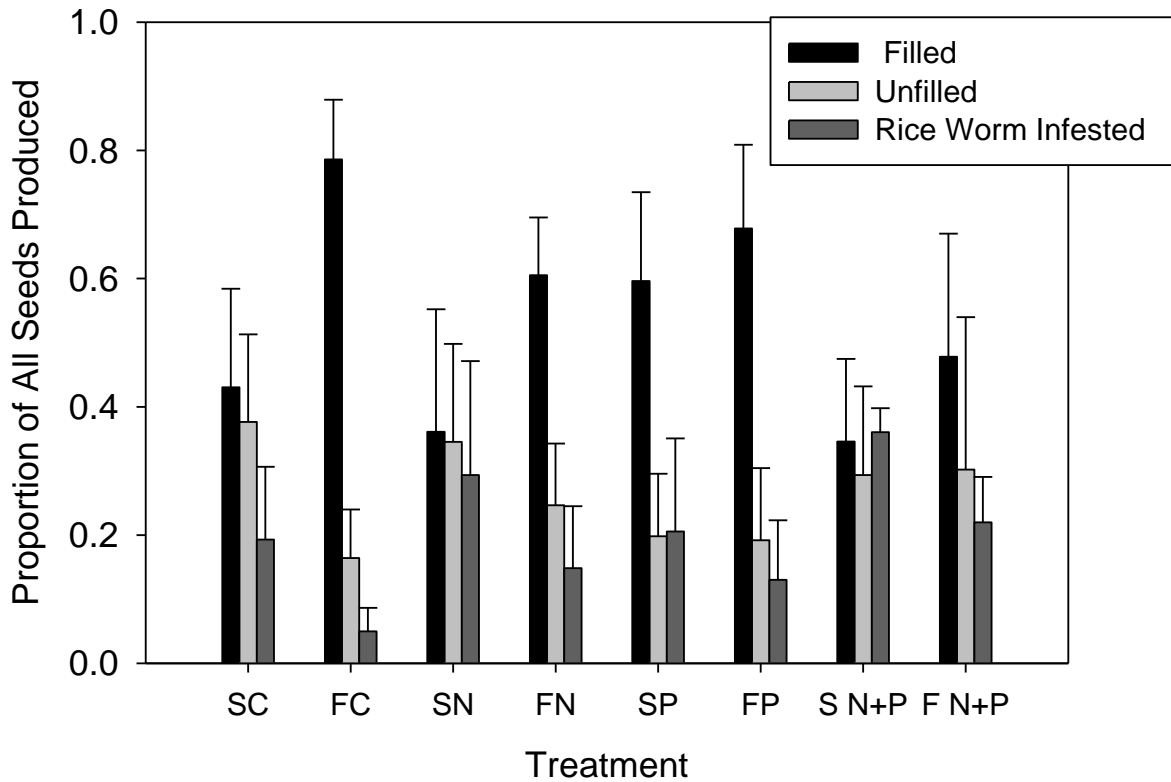


Figure 13. Comparison of the proportion of all wild rice seeds produced that were filled, unfilled, or assumed filled but then eaten by rice worm (“rice worm infested for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Sample size is six plants for each treatment, and y-error bars are 95% C.I.

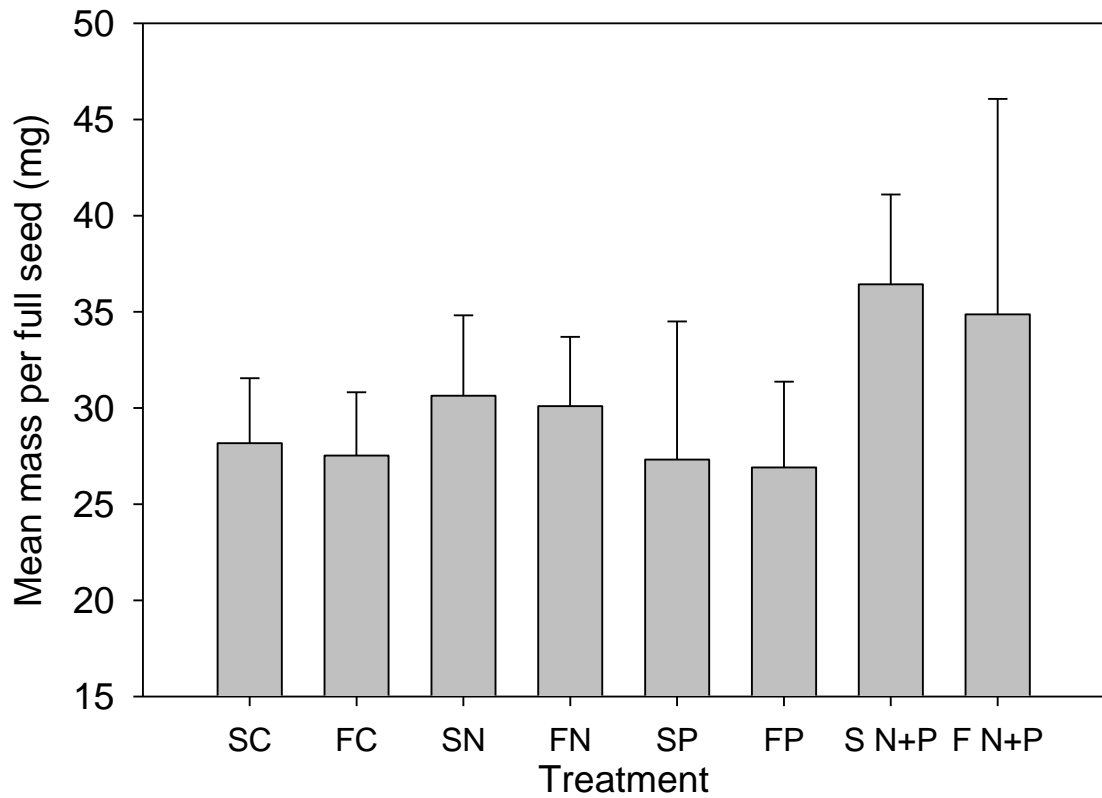


Figure 14. Mean dry mass of individual, full, wild rice seeds for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Sample size is six plants for each treatment, and y-error bars are 95% C.I. ANOVA was significant for nitrogen ($F_N = 7.79$, $p = 0.008$).

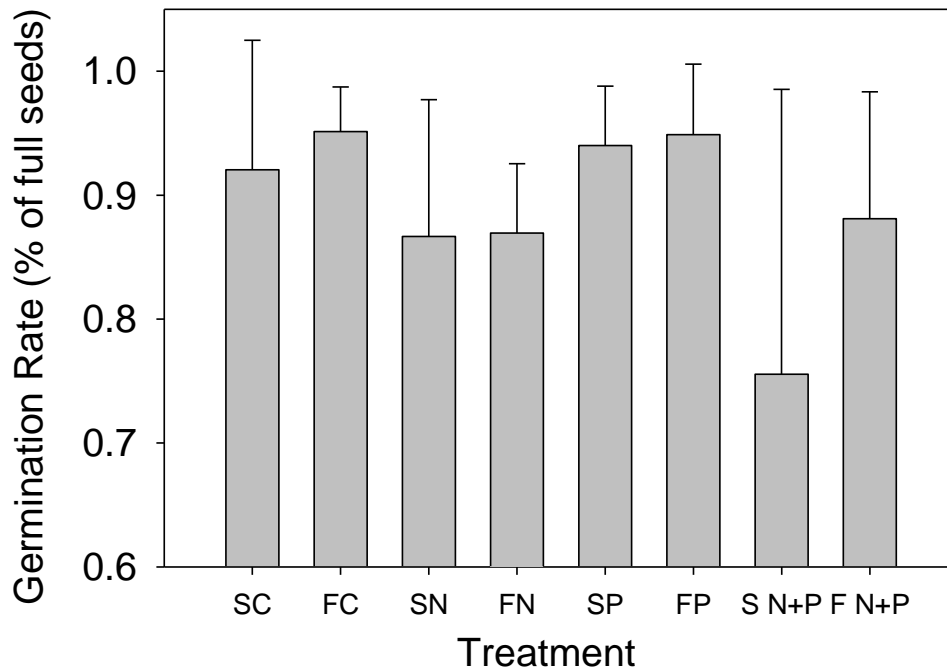


Figure 15. Germination rates of full wild rice seeds for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Unfilled or rice worm infested seeds were assumed nonviable and therefore not included in the germination study. Sample size is six plants for each treatment, and y-error bars are 95% C.I. ANOVA was significant for nitrogen ($F_N = 5.74$, $p = 0.022$).

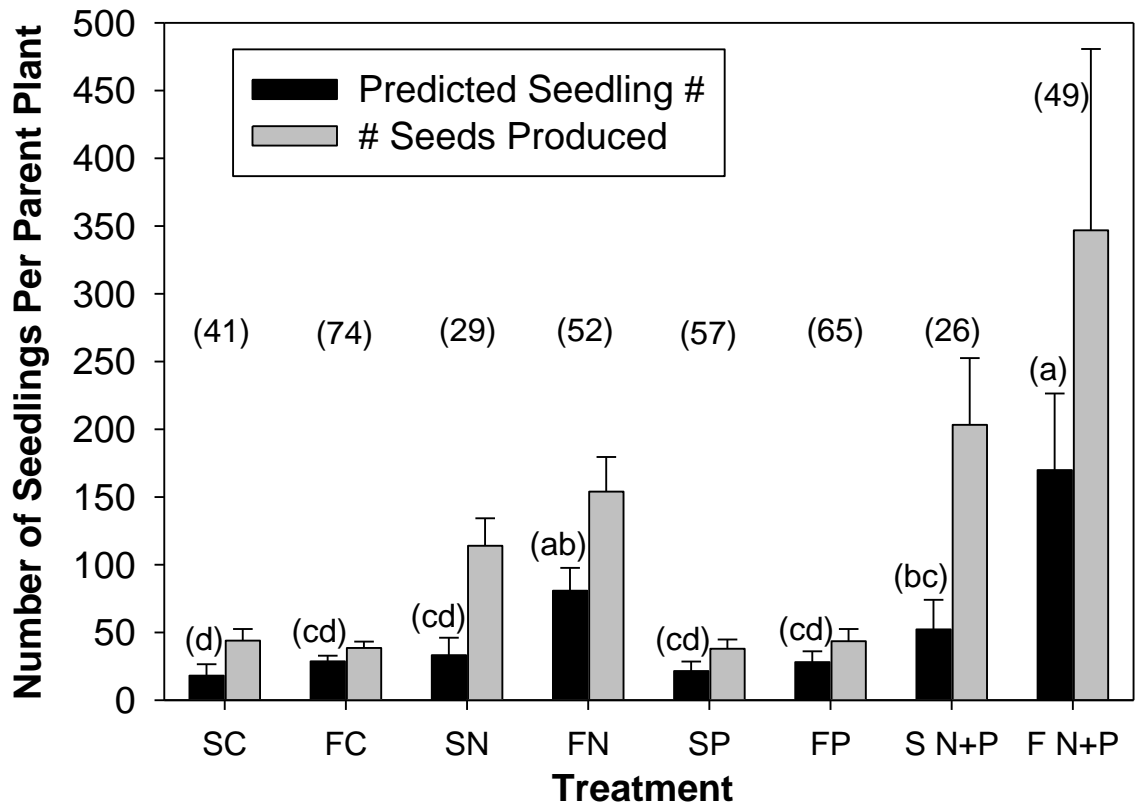


Figure 16. Comparison of the number of seedlings per parent plant calculated using Eq. (3.1) for eight treatments of light (S for shade and F for full sunlight), nitrogen (C for ambient control, N for added N) and phosphorus (C for ambient control, P for added P) level combinations. Predicted seedling number was derived by multiplying the average germination rate per treatment by the estimated number of full seeds not eaten by rice worm per plant for the same treatment, and therefore accounts for filling (filled and unfilled) and rice worm infestation rates. Number of seeds produced is the total number of seeds produced per plant. Sample size is six plants for each treatment, and y-error bars are 95% C.I. For predicted seedling number only, treatments with the same letter are not significantly different according to Tukey HSD at $p < 0.05$. All pairs of derivations for each treatment are significantly different according to t-tests at $\alpha = 0.05$. Numbers in parentheses are the percentage of seeds that germinated compared to the number of seeds produced.

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