Ecophysiology as a tool for evaluating invasive-plant based bioenergies: physiological and ecological case-studies of *Arundo donax* and *Elaeagnus angustifolia*

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Abstract

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In recent years, environmental and social pressures have fostered the development of biomass based energies. Development of 'ideal' biomass feedstocks has led to characterizations of physiological traits that are similar to many of the physiological traits that typify number of invasive grasses. This has led to controversy about the sustainable implementation of invasive-grass based bioenergy feedstocks. While the debate has continued for much of the past decade, little work has been published quantifying the arguments on either side of the controversy. The work presented herein addresses the lack of evidence regarding the potential for increasing plant invasions by utilizing known invasive plants as biomass feedstocks. The first three chapters deal specifically with the invasive grass species *Arundo donax*. These chapters describe experiments in which *A. donax* was grown under a variety of treatments which have been predicted as potential environmental alterations associated with global climate change such as: carbon dioxide enrichment, drought, resource limitations, and soil salinization. The effects of these treatments were quantified at the whole-plant and leaf-level scales. Results show that *A. donax* is able to persist and grow in environmental conditions that typically limit growth of most crops and some native plant species. Furthermore, its growth was found to be improved under elevated carbon dioxide. These findings suggest that it is unlikely *A. donax* will be constrained by the agricultural setting, and therefore cultivating it will disperse it to new environments and will likely lead to future invasions. Alternatively, this dissertation addresses the potential for bioenergy markets to help reduce the spread of invasive plants by partnering with on-going ecological restoration. In the last chapter of the dissertation, titled Bioenergy that Supports Ecological Restoration, a case-study experiment in central Washington State reveals how wood-waste for ecological restoration of Russian olive (*Elaeagnus angustifolia*) can be converted into bioenergy. These findings suggest that a restoration costs can be offset from the sale of Russian olive wood for bioenergy. Yet, the quality of the Russian olive wood is divergent from the typical biomass resources which will limit its incorporation into the bioenergy market. The Russian olive findings present the first supply and demand analysis of invasive plant biomass for bioenergy.

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Dedication

Forgoing sentimentality, I would like to dedicate this work to the unknown ‘ecologists and agronomists’ I regularly refer to throughout my dissertation. To you it is whom I am often speaking. May your decisions regarding the management of *Arundo donax* be better informed by the pages of this text.

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Introduction:

Bioenergy and Invasive Grasses:

Grasses constitute a major group of invasive plants that pose a threat to ecosystem-services that societies depend upon. Grasses may also be well adapted to exploit the theorized conditions associated with global climate change (S. J. E. Wand, Midgley, Jones, & Curtis, 1999), including a wide range of temperatures and light levels, extended droughty periods, and increased carbon dioxide. Anthropogenically accelerated climate change alters the proportions of essential plant resources, water, nitrogen, and carbon dioxide. Changes in the availability of these critical resources, particularly on a global scale, not only affect plant biology but also entire ecosystems (L. H. Ziska, James A. Bunce, 2006). The 'greenhouse effect' and land-use changes associated with fossil-fuel combustion destabilize ecosystems and make native plant communities less resilient to the growth and spread of non-native plants. There have been few studies of invasive plant responses to carbon dioxide (CO_2), the gas most attributed to climate change; yet in general, these reports reveal consistent positive growth responses to CO_2 enrichment (Rogers et al., 2008; Smith et al., 2000; L. H. Ziska, 2003).

Many invasive grasses are 'cool season' grasses, a group of plants that has consistent positive growth responses to increasing CO_2 (Strain & Smith, 1985). This positive response is one of the reasons temperate grasslands are thought to be among the most responsive ecosystems to increased CO_2 concentration (Mooney, Drake, Luxmoore, Oechel, & Pitelka, 1991). Invasive grass species (e.g., *Agropyron desetrorum, Arundo donax, Bromus tectorum, Cortederia jubata, Pennisetum setaceum, Phalaris arundinacea, Spartina* spp., and *Taeniatherum asperum,*) have invaded most North American ecosystems from arctic to arid and aquatic. Invasive grasses are known to alter drastically the ecosystems they colonize and 'engineer' natural cycles (Crooks, 2002) to the detriment of native biological communities. Invasive grasses can dramatically alter native plant-community structure and ecosystem processes, such as fire frequency, nutrient cycling and water circulation (D'Antonio & Vitousek, 1992). These alterations highlight the feedbacks interactions between plants and the abiotic environment.

From a geomorphological perspective, biological ecosystem engineering by invasive grasses represents an active modification of landforms or a passive modulation of morphogenetic factors (gravity, water flow, wind, frost, chemical alteration) that control matter and energy flows (Corenblit, Gurnell, Steiger, & Tabacchi, 2008). The alteration of fire-cycle frequency and intensity are modifications by invasive grasses that re-engineer matter and energy flows. Another way grasses, such as hybrid *Spartina* spp., alter morphogenetic factors is by modifying the natural hydrology. Invasive *Spartina* spp. have been cited to engineer intertidal basin and regional hydrology through changes in substrate cohesion and surface roughness (Collins, 2002). *Spartina* spp., like other invasive grasses, alter sediment deposition and sequestration by influencing channel-bank erosion and channel-network geometry. The modifications of sedimentation and erosion processes shape the intertidal communities' structure and function. Other invasive grasses, such as *Arundo donax, Axonopus furcatus, Panicum repens, Phragmites australis, Phalaris arundinacea,* and *Schedonorus phoenix* (J. M. DiTomaso, E.A. Healy, 2003), are also believed to be modifying riparian and wetland hydrology throughout North America.

The biological invasions by exotic plants have altered the hydrology of vast areas of western North America (Dukes & Mooney, 2004). The naturally integrated and frequently disturbed riparian systems are especially vulnerable to plant invasions compared to other ecosystems (Hood & Naiman, 2000). Invasions can be accelerated in riparian systems as a response to changing climatic conditions as well as anthropogenic transformation of river regimes (e.g., regulation by dams, pumping, main channel diversions). The invasions by grasses and other non-natives affect the hydrologic cycle, because of the control riparian vegetation exerts on surface and subsurface flow at the local scale (Tabacchi *et al*., 2000). Riparian vegetation primarily influences three distinct hydrological processes: flow regulation, water uptake and storage, and water quality. *Arundo donax*, an ecosystem engineer, is a grass species spreading throughout North American waterways and is believed to impact hydrological regimes.

Since its introduction into North America, *Arundo donax* has become a major invasive weed of riparian habitats throughout warm fluvial ecosystems (Gary P. Bell, 1997; J. M. DiTomaso, E.A. Healy, 2003; Dudley, 2000b). The tall perennial grass quickly colonizes scoured floodplains, achieving dominance along riverbanks and even in some estuaries. The presence of these monoculture stands significantly affects the biotic and abiotic riparian community. The physical impact, through hydraulic interactions along river banks with the living and dead stems, and exposed roots of *Arundo donax*, and other invasive grasses, affects substrate roughness and may increase channel turbulence. Furthermore, *Arundo donax*, similar to most grasses, has a dense rhizosphere, which can clog the top layer of the substrate, purportedly trapping and stabilizing more sediment than native vegetation (M.E. Iverson, 1994), increasing sediment retention, and limiting or diverting water flows. The rhizosphere of riparian plants modifies hydraulic conductivity by affecting soil water and ground-water flows, consequently decreasing channel sizes and increasing flow velocity in the narrowed channels. Contrary to the shallow rooted plants, deep-rooted invasive plants may alter the hydrology by improving drainage or infiltration, by increasing substrate porosity and capillarity. These structural modifications by invasive grasses are more evident than the role invasive plants play in the riparian zone water-cycle. The riparian zone water-cycle is poorly researched for most communities; however, considering the intimate plant-water linkages well studied in other ecosystems (Baird & Wilby, 1999), it is logical that the storage and use of water by the riparian plants, native or invasive, strongly affect the riparian hydrological regime.

The water-cycle in riparian zones depends on three linked areas: water uptake; water storage in different parts of plants; and the return of water to the atmosphere by evapotranspiration (ET). Invasive species can strongly alter the water balance in riparian areas by imposing exceptional water consumption and loss rates, consuming the water from the soil and from the saturated zone (Le Maitre et al., 2002). Lowered water tables in many riparian zones of the south-western United States have been attributed to plant invasions (Dukes & Mooney, 2004). The invasive grass *Arundo donax* purportedly modifies natural hydrologic regimes through heightened rates of evapotranspiration (J. M. DiTomaso, 1998; Dudley, 2000a; Hendrickson, 2005; M.E. Iverson, 1994). It has been recorded that *Arundo donax* infestations may transpire 6-110 times more (up to 18, 206 kg m^{-2} $year^{-1}$) than native vegetation (Abichandani, 2007). Although water loss on a per-unit-leaf-area basis has been measured comparable to that of native plants, the dense monospecific stands have the potential to develop a higher leaf area index. These large stands can significantly increase water loss from underground aquifers in semiarid regions (J. M. DiTomaso, E.A. Healy, 2003).

The predictions of excessive water use by non-native invasive plants dates back to the 1970s and was based on research conducted on the effects of afforestation on stream flow and water resources (Gorgens, 2004). Invasive plants, especially trees such as *Tamarisk* spp. and *Eleagnus angustifolia* in North America and *Pinus pinaster* and *Acacia* spp. in South Africa, began to attract more attention to the catchment-scale hydrological ramifications in the 1980s (Everitt, 1980) and 1990s (Le Maitre, Wilgen, Chapman, & McKelly, 1996). Since this time catchment-scale experiments have focused on quantifying changes in stream flow and, to a lesser extent, ground water resources (Gorgens, 2004). Stream flow comparisons ostensibly seem the logical venue for contrasting native and invaded stream reaches. These records, which have been maintained for years for agricultural purposes, can provide a good history of the change in water levels. However, attributing decreases in flows to an invasive plant is challenging because baseline consumption rates by native plants were rarely recorded, and the multiple alterations throughout a developed watershed, for agriculture and energy, further confound the relationship between flora and flow. Comparisons of the hydrology of invaded and pristine plant communities would provide valuable insight into how invasive grasses and other plants are reshaping water resources and could provide links to how the invasion process is restructuring biotic communities.

The rapid perennial growth of *Arundo donax* is believed to impact the riparian community by reducing habitat availability (Herrera & Dudley, 2003) and biological productivity (J. M. DiTomaso, E.A. Healy, 2003), as well as by altering natural nutrient cycling (M.E. Iverson, 1994), related to the dynamics of carbon release from plant debris. This ecosystem engineer produces dense monotypic stands, which impede wildlife migrations through the ecological corridor (Kisner, 2004) and substantially reduce the carrying capacity of small waterways. Unlike biologically diverse native plant communities, invasive grasses such as *Arundo donax* provide limited structure and habitat (Franzreb, 1989) and are poor food sources; the unpalatable leaves contain a wide array of noxious chemicals, including silica (Jackson, 1964), tri-terpines and sterols (Chandhuri, 1970), cardiac glycosides, curare-mimicking indoles (Ghosal, 1972), hydroxamic acid (Zuniga, 1983), and numerous other alkaloids which may protect it from herbivory (Miles, 1993; Zuniga, 1983). Furthermore, riverine areas colonized by invasive grasses receive insignificant shading from the slender blades compared to waterways shaded by the reaching canopies of complex native hardwood galleries (G.P. Bell, 1997). The reduced shading tends to increase water temperatures. Warmer water with more sunlight, promotes algae, which may increase pH (Dubinsky & Rotem, 1974). The higher pH shifts the ammonia equilibrium; from ionized to a more toxic un-ionized form (Thurston, Russo, & Vinogradov, 1981). Furthermore, warmer temperatures and increased algae growth may lower oxygen concentrations and, therefore, aquatic animal populations and diversity (Dunne & Leopold, 1978). The replacement of diverse native communities with monospecific stands of *Arundo donax* or other invasives plants reduces the terrestrial biodiversity as well (Naiman, Decamps, & Pollock, 1993). Biodiversity is expected to control nutrient dynamics at the river-floodplain interface, as well as control the resistance of ecosystems to disturbances induced by human activities, or by physical external agents, such as storms, floods, and fire. Recent studies on the impact of biological diversity on emergent properties of the ecosystem (such as overall plant production) have shown that high diversity promotes resiliency in biological functions (McCann, 2000).

To better address the impacts of invasive grasses on hydrology and how the changes feedback into the ecosystem in ways that reduce the resiliency of the native community to withstand changes, scientific investigations need to move from anecdotal, single-stream reach observations, to mechanistic multi-scale approaches. The general understanding of invasive plant interactions on hydrology are based on research from an exceedingly limited number of taxa in a limited number of ecosystems. For decades, it has been axiomatic that the invasive *Tamarisk* tree uses much more water than native vegetation; yet recent studies have shown its water use may be actually comparable to native phreatophytes (Owens & Moore, 2007). The lack of information regarding system level-fluxes and plant-water relations, or even accurate measurements of native or invasive plant-water use in particular settings, contributes to scaling errors and makes determining the impacts of an invasive plants highly speculative. Therefore, investigations of the processes (such as leaf area, ambient carbon dioxide levels, and ambient temperature) governing water use by invasive plants in a particular setting will complement the flow rate and discharge studies. They will help develop a mechanistic approach, which will become increasingly relevant while considering alterations associated with global climate change.

Articulating these changes is essential for predicting future invasions that will be affected by changes of the timing of climatic and hydraulic events. An improved understanding of the feedbacks between plants and water resources (e.g., stream flow and discharge, groundwater levels, water temperature, etc.) will also benefit water-resource management.

Multi-scaled, mechanistic research into the hydrological effects of invasive grasses will contribute to water-resource management by identifying regions with lowest resilience to invasions, as well as regions that may have the greatest positive hydrological response to restoration. An improved understanding of the impacts of invasive grasses will also be beneficial when considering the relative importance of invasive plant hydrological alterations, compared with other anthropogenic impacts on catchment and regional scales. The present understanding of invasive plants makes it difficult to discern whether these plants are the drivers or the passengers of hydrological change. In the western United States riparian invasions often follow hydrologic modifications for energy, industry and agriculture (Auble, Friedman, & Scott, 1994; Bunn & Arthington, 2002). Although, invasive plants are a visible symptom of the disturbed ecosystem, it is unclear whether these plants are the greatest detriment to the native community or natural hydrology. In many cases the revitalization of native riparian communities is more dependent on the restoration of natural hydrology than removal of invasive species (Stromberg, 2001). Without a thorough understanding of the hydrological relationship between native plants and the landscape, the impacts by invasive plants compared with anthropogenic diversions remains speculative. The decisions regarding invasive plant management that influence ecosystem services are relevant in light of the future challenges facing water-resource management.

Invasive plants have been well documented as a major component of global environmental change (Vitousek, D'Antonio, Loope, & Westbrooks, 1996) and have the capacity to degrade ecosystem goods and services (Dukes & Mooney, 2004). Degradation of ecosystem goods and services carries great risk for human societies as healthy ecosystems provide countless benefits that directly contribute to human well-being (Millennium Ecosystem, 2005). Social reliance on natural systems for ecosystem goods and services exemplifies the coupling between social and ecological systems. Socio-ecological systems are complex adaptive systems, characterized by nonlinearities, uncertainties, and feedbacks (Bohensky, 2005). Social resilience is an essential element of robust socio-ecological systems because it provides a way of both managing and measuring the adaptive capacity of a community (Folke, 2006). Social resilience is defined by (Adger, 2000) as the ability of groups or communities to cope with external stresses and disturbances as a result of social, political, and environmental change. Unlike current tools, such as economic valuation, which work best in stable environments and have difficulty measuring non-linear natural dynamic systems (Mäler, Xepapadeas, & de Zeeuw, 2003), social resilience incorporates change and considers the capacity for renewal, re-organization and development (Folke, 2006). Possible social resilience metrics (economic, demographic and institutional variables in both temporal and spatial fashions), suggested by Adger in 2000, which could be adopted to improve resource management decisions, are: resource dependency, property rights, food security, and mobility and migration patterns. These metrics can provide great insight into societal relationships with land resources, and consequently how hydrologic modification by invasive plants will alter these relationships. Social and environmental resilience regarding hydrology and water resources is currently being tested by global climate change.

Observed warming over several decades has been linked by numerous workers (*e.g.*, Bates *et al*., 2008) to changes in the large-scale hydrological cycle such as: increasing atmospheric water-vapour content; changing precipitation patterns, intensity and extremes; reduced snow cover; and changes in soil moisture and runoff. The changes are dramatically affecting global vegetation distributions; the area of land classified as very dry have more than doubled since the 1970s. It seems the semi-arid and arid areas (e.g., Mediterranean Basin, western USA, southern Africa and north-eastern Brazil) are particularly exposed to the impacts of climate change and are projected to suffer further decreases of water resources due to climate change (Fischer, 2001). These hydrological attenuations are afflicting intensely farmed areas that are already facing water limitations. Furthermore, it has been projected with high confidence that water supplies stored in glaciers and snow cover will decline, thus reducing water availability during warm and dry periods; another change threatening many farming communities in the arid western United States (Casola, 2005). The aquatic biology as well as fresh water resources may also be affected by global climate change, as the drying and warming in certain regions as well as the higher water temperatures and changes in extremes are projected to affect water quality and exacerbate many forms of water pollution, such as: sediments, nutrients, dissolved organic carbon, pathogens, pesticides and salt (Bates, 2008). The climatic and non-climatic factors, such as population growth, will undoubtedly strain limited water resources. Although it is unclear how great an impact invasive grass has on catchment hydrology, it is clear that riparian plants are intimately linked to catchment hydrology; therefore, alterations of this biotic-abiotic interface precipitate changes throughout the region. In light of the limited understanding of hydrological interactions between invasive grasses, riparian plants in general, and the landscape, current water-management practices may not be robust enough to cope with the impacts of climate change on water-supply reliability, flood risk, health, agriculture, energy and aquatic ecosystems. The ramifications of these impacts will ripple into other policy areas—energy, health, food security and conservation, therefore managing to enhance social and ecological resilience in the context of prudent environment and health policies becoming the most sapient decision.

Chapter I:

Carbon dioxide and nitrogen effects on the growth and biomass allocation for Arundo donax

Abstract

Agriculture and anthropogenic climate change are creating novel ecosystems where exotic species may begin to dominate native plant communities. Atmospheric carbon dioxide enrichment and increased soil nitrogen levels are two key changes altering plant community composition by directly impacting plant growth. *Arundo donax* is a grass species that is being cropped for biomass agriculture and is also known as an invasive species in many riparian ecosystems. An experiment was conducted to examine the interactive effects of carbon dioxide nitrogen enrichment on the growth and allocation of *Arundo donax*. Results show that nitrogen and to a lesser extent carbon dioxide to positively benefit the growth of this plant species. In particular it was shown that nitrogen fertilization in combination with carbon dioxide enrichment leads to an increase in rhizome development. Rhizome development by *Arundo donax* has been linked to stress tolerance and rapid re-growth, which are two functional traits that have been attributed to increased invasion potential. Therefore, these results that show that *Arundo donax* allocated more resources to storage organs under conditions of elevated CO_2 has strong implications suggesting increased growth and resilience in disturbed environments. These implications are highly relevant to ecologists concerned about the growth and spread of this invasive species; and for agronomists interested in modeling growth responses in a changing climate.

Introduction

Novel plant communities that are often populated with exotic species are being created as result of fundamental restructuring of natural systems by agriculture and anthropogenic climate change (Davis, Grime, & Thompson, 2000; Huntley, 1991). An interesting scenario develops when an exotic species, such as *Arundo donax* L., that is known to restructure natural systems (Bossard, Randall, & Hoshovsky, 2000) is selected for use in agriculture. Agriculture is known to foster invasion by increasing propagule pressure and decreasing stochastic events (R. Mack, 2000), how climate change combined with agriculture may additionally foster plant invasions has not been well studied. In general grasses, which predominate grain and bioenergy agriculture, may be well suited to take advantage of the changing global climate (D'Antonio & Vitousek, 1992; S. J. E. Wand, et al., 1999), but the responses are highly species specific. Additionally, climate change involves a complex alteration to a multitude of environmental factors; the alterations of one (*e.g.* temperature or carbon dioxide enrichment) do not necessarily correspond with additive effects on other linked processes (*e.g.* flooding or drought). Thus, studying the interactions of multiple climate change factors is important for understanding the implications of developing invasive plant (*e.g. Arundo donax*) based agriculture.

Anthropogenic climate change is linked to, among other things, an increase in two critical plant resources: carbon dioxide and nitrogen. While both resources originate from the atmosphere, nitrogen (N) uptake is predominately facilitated by the roots whereas carbon dioxide (CO_2) is primarily assimilated through leaf stomata. The atmosphere however contains very little CO_2 approximately 0.039% (or 390 ppm). Therefore the nearly 30% increase in atmospheric CO_2 within the past 150 years, to concentrations not experienced in the preceding half-million years (Petit et al., 1999), represents a dramatic global change phenomenon that has been linked, in many scientific publications, to stimulating photosynthetic capacity in plants (Ainsworth & Long, 2005; Bazzaz, 1996; Wong, 1979). For many plant species, elevated atmospheric CO_2 has increased photosynthesis rates, aboveground biomass accumulation rates, and water use efficiencies (WUE) (A. D. B. Leakey et al., 2009). How these changes alter species distribution has been less studied but is an important area of research.

The range of some plant species is expanding because of intentional (Reichard & White, 2001) or unintentional (Hulme, 2009) transport to new locations were these exotic plants are able to gain competitive advantages (R. N. Mack et al., 2000). Climate change, and the alteration of nutrients and resources, can be considered and ecosystem disturbance which may generate novel ecosystems where non-native species dominate native species. Not only does climate change impact plant distribution and what vegetative communities will dominate in our landscapes—because of changing temperature and precipitation patterns (Walther et al., 2002)—but large scale agriculture also contributes to the development of novel ecosystems. To understand the implication of these changes, my research suggests that it will be important to focus on plant ecophysiological characteristics to be able to detect the potential of plants to take advantage of novel and unique ecosystems. The physiological basis of these changes in plant community composition is supported by decades of research (Huntley, 1991; Parmesan, 2006). Physiologically, elevated CO_2 concentrations enhances carboxylation activity leading to increased sucrose formation (J. F. Farrar & Williams, 1991). This photosynthetic stimulation is attributed to evolutionary optimums in which Rubisco, the primary carboxylase for C_3 species, is thought to have evolved, *i.e*. higher CO_2 concentrations are reflective of pre-historic atmospheric conditions (Bowes, 1996).

While atmospheric CO_2 concentrations have increased by almost one third since the beginning of the industrial revolution, N deposition has increased at least two fold above the presumptive pre-industrial background value (Körner, 2000). Vitousek et al. (1997) documented how higher levels of nitrogen have been fixed anthropogenically than through all natural processes combined. The global nitrogen cycle has been altered through human activities such as industrial fertilizer input into agricultural and urban areas, increased nitrogen fixation from legume-based agriculture, and fossil fuel burning. Consequently, increased nitrogen in ecosystems can disrupt plant community structure and composition, increase soil acidification, and diminish water quality (Bobbink et al., 2010). Nitrogen has a great impact on plant ecophysiological processes (Chapin, Bloom, Field, & Waring, 1987). Therefore, any change in the availability of CO_2 and N, particularly on a global scale, has the potential to not only affect plant biology but also entire ecosystems by altering the structure, function and species composition, leading to the development of novel plant communities.

The impacts of changing N levels on plant physiological processes has received considerable research attention which suggests the importance of including N as part of developing novel plant communities. The ability to use nitrogen efficiently varies widely between plant species and represents an evolutionary strategy that benefits many plants that are adapted to growing in nitrogen poor soils (Berendse & Aerts, 1987; Funk & Vitousek, 2007). Plant are adapted physiologically to changing N levels; plant nitrogen use efficiency (NUE) decreases with increasing nitrogen availability (Vitousek, 1982). Other studies have reported changes in plant community composition—with overall reduced species richness—when nitrogen is a limiting resource for growth (Zavaleta, Shaw, Chiariello, Mooney, & Field, 2003). In addition, species specific responses to CO2 enrichment can also modify competitive interactions, thereby changing plant community composition (Smith, et al., 2000). These plant community changes occur when species adapted to successfully compete for limiting resources under low resource supplies are less able to compete successfully against other species that are not constrained by resource enrichment conditions.

When plant species are highly adapted to grow under low and high resource supply conditions, environmental constraints that normally control species dominance no longer apply and may allow an invasive or novel species to dominate (Blossey & Notzold, 1995). Thus considering that available nitrogen supply is one the greatest limitations on plant growth, and that Rubsico operates more efficiently at higher CO_2 concentration, researchers have speculated that enrichment of either or both of these factors will lead to increased invasion by exotic species (Brooks, 2003; Smith, et al., 2000; Tomassen, Smolders, Limpens, Lamers, & Roelofs, 2004). In addition, it appears that plant invasions are most successful where disturbance coincides with increased fertility (R. J. Hobbs & Atkins, 1988; Richard J. Hobbs & Huenneke, 1992).

Riparian systems are often interlaced in landscapes which also host intensive agriculture or dense urban development. These systems are thusly subject to both frequent disturbance (D. M. Richardson et al., 2007) and high levels nutrient enrichment from surface runoff and subsurface drainage (Paul & Meyer, 2008; Weller, Baker, & Jordan, 2010). This means that these disturbed riparian systems are probably more susceptible to novel species introductions and are particularly vulnerable to colonization by non-native plant species. *Arundo donax* is an ideal species to study because it is an invasive grass that thrives in urban and agricultural riparian areas throughout the Sunbelt region of the United States. Moreover, there is interest to expand its use as a biomass crop (Angelini, Ceccarini, & Bonari, 2005; Pilu, Bucci, Badone, & Landoni, 2012) (De Bari, Liuzzi, Villone, & Braccio).

A. donax is a rhizomatous, C_3, grass that was introduced to the United States in the 1800's and has expanded into large, self-sustaining populations. It has been classified as an ecological and economical pest (Dudley, 2000b). *A. donax* populations expand through the distribution of vegetative propagules typically in the form of stem and rhizome fragments (Gary P. Bell, 1997). *A. donax* forms monospecific stands that can inhibit the establishment or growth rate of other species; and as *A. donax* increases dominance within a watershed regional species diversity and richness decreases (Reiger, 1989). Improving control strategies for *A. donax* requires greater understanding of its mechanisms for colonization and dominance over native species, including both innate biological characteristics and environmental factors, including N and CO2.

Predicating the effects of global change on plant species, or even plant communities, is inherently complex. While some work has been done to characterize *A. donax*'s growth responses in relation to N (Quinn, Rauterkus, & Holt, 2007), no studies have been published relating the growth of this invasive species in relationship to a combination of resources. It is important to study the simultaneous effects of N and CO_2 because it has been shown that plant responses to each aspect of climate change are often species dependent and cannot necessarily be extrapolated from the plant responses to each climate change factor applied individually (Craine et al., 2003; Mittler, 2006). The impact and/or benefits of *A. donax* are still being debated. This experiment will contribute to these debates by showing the (1) links between CO_2 and N at the physiological level and (2) discussion how alterations in resource availability will impact certain plant plants, e.g., *A. donax*, in novel ecosystems. Specific aims of this study were to determine if the interaction between CO_2 enrichment and nitrogen limitations on the growth and allocation of A. donax. It was hypothesized that the fertilization effect of CO_2 would partly offset the growth reductions when nitrogen was limiting, and that the greatest differences would be detected in belowground structures (*i.e.* roots and rhizomes).

Materials and Methods

Experimental location

To test the interaction of CO_2 and N on the growth of and allocation of *A. donax* a two-way factorial experiment was conducted at the CO_2 growth chambers at the Douglas Research Conservatory, at the University of Washington Botanic Gardens, Seattle, USA.

Plant material

On June 22th, 2010, 48 rooted stem cuttings from a wild type *Arundo donax* were propagated for this study from an *A. donax* stand growing, unmanaged, along the bank of San Anselmo Creek, CA, USA. Of the 48 rooted stem cuttings that were propagated, twelve propagules we set aside for destructive harvesting to create a baseline description of the plant biomass allocation patterns. The remaining 36 rooted cuttings were individually potted (one plantlet per pot) in 10.9 liter '*300 Series'* black plastic nursery containers (Haviland Plastics Products, Co., Haviland, OH, USA) filled with washed building sand (Salmon Bay Sand & Gravel, Seattle, WA, USA). The sand-filled nursery containers were placed in 11.36 L buckets (the buckets acted as large drip pans). The nursery containers/buckets – here after referred to as pots — were placed into one of the glasshouses at the Douglas Research Conservatory (DRC), Seattle, WA, USA. Daylight was supplemented in the glasshouse by high pressure sodium 400 watt single phase bulbs (Phillips Electronics North America Corp., Andover, MA, USA) during 0800 to 2200 hrs. Upon transfer to the pots the rooted cuttings were watered once with half-strength Hoagland's fertilizer solution. After eight days, when all the plants passed visual inspection of successful transplanting, the pots were placed into the experimental treatment groups.

Treatments

The 48 pots were randomly split into three groups of 12 pots. Each group was assigned a nitrogen treatment: full–N, ½–N, or $^1/_8$th –N. The nitrogen was delivered as ammonium nitrate at concentrations 0.643 g/L, 0.321 g/L and 0.080 g/L, corresponding to the respective nitrogen treatment group: full–N, ½–N, and $^1/_8$th –N. The ammonium nitrate was applied in a modified Hoagland's fertilizer solution. The fertilizer treatment was applied once a week for the duration of the experiment: 24 weeks. Three pots from each nitrogen group were randomly placed in one of four CO_2 chambers located inside the glasshouse.

The CO_2 chambers (100cm *x* 100cm *x* 200cm) are closed-topped, PVC-framed units, surrounded with Mylar polyester sheeting. Fans forced air from outside the greenhouse into the chambers through a system of flexible aluminum ducting and PVC piping. Two chambers were randomly assigned ambient air, while the remaining two were fumigated with additional CO_2 delivered from a 22.70 kg tank (Praxair, Seattle, WA, USA). The elevated CO_2 concentrations were maintained by using bubble flow meters (FL-2000, Omega, Stanford, CT, USA), at approximately 370 μmol mol^{-1} of CO_2 above ambient levels. The concentration for the elevated CO_2 reflects a near doubling of the ambient levels and represent levels projected to be the atmospheric concentration at the end of this century (Bernstein et al., 2007). For additional details of the chamber construction please refer to Kinmonth-Schultz and Kim (2011).

The CO_2 concentrations in the elevated chambers were monitored and automatically collected every 15 minutes by a CIRAS-1 infrared gas analyzer (PP Systems International, Inc., Amesbury, MA, USA). Ambient CO_2 from the air pumped into the chambers was measured by a CARBOCAP® carbon dioxide probe (Vaisala Inc., Boston, MA, USA) located outside of the glasshouse. Temperature in each chamber was monitored every 15 minutes using a pair of thermocouples placed, 20 and 100 cm from the tops of the chambers. The CO_2 and temperature data were stored automatically in a CR1000 data-logger (Campbell Scientific, Logan, UT, USA).

The *A. donax* plants were grown under these conditions for 88 days. On September 17th, 2010, all of the pots from the ½–N treatment group were destructively harvested, and removed for separate analysis. At this time the aboveground biomass was also clipped and removed from the full–N (HN) and $^1/_8{}^{th}$ –N (LN) nitrogen groups. The pots containing the roots and rhizomes of the HN and LN groups remained in their assigned CO_2 chambers and were allowed to re-grow for an additional 90 days, when on December 16th, 2010, the HN and LN groups were removed from the CO_2 chambers and destructively harvested.

At each harvest, plant parts were separated and sorted into leaf, stem, root, and rhizome tissues. For leaf parts, a SPAD leaf chlorophyll meter (SPAD-502, Konica Minolta, Ramsey, NJ, USA) was used as a proxy measurement for nitrogen content. An average of five SPAD readings were taken from the uppermost fully expanded leaf of every plant harvested. Following this, leaf surface areas were measured by scanning the leaves with a leaf-area meter (LI-3000; LI-COR, Inc., Lincoln, NE, USA) and then placed in paper bags. The stems, roots and rhizomes were also bagged and all the plant parts were dried for 48 hours in a forced air oven heated to 80^{o} C. The oven-dry biomass was weighed and analyzed to compare the effects of CO_2 and N on biomass accumulation and allocation.

Experimental design and statistical analysis

The nitrogen pot treatments within the CO_2 chambers constituted a split-plot factorial, with CO2 as the main plot treatment and nitrogen as the subplot treatment. Accordingly, CO2 and N were considered as fixed effects and the paired chamber blocks were considered as random effects. All data analyses were calculated using R 2.12 statistical software (Team, 2011). The split plot treatemt was balanced across the whole plots in the replications, yet unlike a randomized complete block design the treatment is not fully crossed. The model used was an analysis of variance (ANOVA) for split-plot designs (Casella, 2008), in which theF-tests for the treatment effects on the biomass allocation were first tested at the main plot level; and then the variance was partitioned for the subplot treatment effects and the interaction between Nitrogen and CO_2. Orthogonal pairwise contrasts were used to further differentiate results within and between particular treatment groups. All biomass growth responses were plotted using the bar-chart function with standard error of the means in Sigma Plot 12.0 (Systat software, INC., San Jose, CA, USA).

Results

Overall, analysis of the biomass revealed very strong positive growth effects from the nitrogen treatments and some detectable growth enhancement from the CO_2 enrichment as well. The high nitrogen treatment had significantly greater biomass than the low nitrogen treatments ($P<0.001$) for all response variables including: leaf nitrogen content (SPAD), aboveground harvests, total biomass, and allocation ratios (above- to below-ground; rhizome to root) (Table 1-1.). While the effects of CO_2 were not as uniform as the N-treatments, main effects were attributable to the CO_2 enrichment in all of the measured biomass response variables except for the allocation ratios.

Growth enhancement resulting from CO_2 enrichment was evidenced in whole-plant biomass (Fig. 1-1) with plants grown in elevated CO_2 having 112% of the biomass compared to the biomass of plants grown in ambient CO_2 ($P<0.01$) (Table 1-1). Likewise a nitrogen effect was evidenced by whole plant biomass. Plants grown with full-N (HN) fertilizer group had a mean mass 159% of that if the mean biomass of the plants grown in 1/8th-N (LN) fertilizer group ($P< 0.001$) (Table 1). In response to the experimental manipulations the allocation of carbohydrates for biomass production was predominately into aboveground plant parts (stems and leaves) compared to belowground plant parts (rhizomes and roots) (Fig. 1-1). This was evidenced in the belowground to aboveground allocation ratio, where belowground biomass represents approximately 1/3rd and 1/5th of the aboveground biomass for LN and HN Nitrogen groups, respectively (Fig. 1-3). The belowground to aboveground ratio was primarily related to the N-treatments ($P <0.001$), and there were no biomass allocation changes in response to the CO_2 treatment. When the treatment effects on total biomass accumulation were analyzed by plant component part (leaf, stem, rhizome, root), CO_2 enrichment could be attributed to increases in biomass in the leaf ($P <0.05$), stem ($P <0.01$), and rhizome growth ($P <0.1$). While increased N levels especially improved aboveground biomass accumulation, N effects were also strongly evident in the changes in leaf ($P < 0.001$), stem ($P < 0.001$), rhizome ($P < 0.05$) and root growth ($P < 0.1$) (Fig. 1-2).

When considering only the belowground components, the rhizome: root ratio (Fig. 1-4) differences could largely be attributed to nitrogen treatment ($P <0.001$) and the interaction between CO_2 and N ($P<0.05$), yet there was not an attributable influence from the main effects of CO_2. Pairwise contrasts of the groups revealed that the interaction term was significant within the elevated CO_2 treatment group ($P <0.05$) because there were positive growth increases in the rhizome compared to roots within the HN, whereas this positive enrichment to rhizomes was not evident within the LN treatments within the enriched CO_2 group. Unlike the contrasting allocation responses between enriched and ambient CO_2 groups within HN, there were not attributable differences between CO2 treatments within the LN group (Table 1-1). Contrasts between nitrogen treatments were showed increased biomass ($P <0.05$) in the high nitrogen group compared with the low nitrogen group for all response variables. However, results between CO_2 treatments groups were not as clear. Between CO_2 within LN group no clear relationships were detected for any of the response variables. Conversely, between the CO_2 treatments within the HN group differences ($P <0.05$) for all but the 1st harvest above-ground and belowground to aboveground ratios.

Discussion

Anthropogenic changes including CO_2 and N enrichment of ecosystems are altering the natural resource availability and thus fostering the creation of novel ecosystems that may be dominated by plant communities with compositions distinctly different than those that are present today. Plants that are more competitive to the altered resource conditions may gain competitive advantages. This release from nutrient and resource limitations may be evidenced by what appears to be invasions of plants previously undocumented or uncommon in nutrient limited ecosystems. Some studies have shown that other C_3 invasive grasses, like *A. donax,* have also increased plant biomass as a response to CO_2 enrichment (Nagel, Huxman, Griffin, & Smith, 2004; L. H. Ziska, 2003). Likewise reports of the beneficial effects of nitrogen fertilization on plant growth are ubiquitous and are even documented specifically for *A. donax* (Quinn, et al., 2007). However, because of the potentially non-additive effects of global change factors it was important to look at the interplay of these two essential plant resources. For instance, some studies have shown that CO_2 enrichment can alter internal plant nitrogen balance, decreasing leaf nitrogen concentrations at both sufficient and deficient nitrogen levels (Larigauderie, Hilbert, & Oechel, 1988). Contrarily, SPAD analysis—a proxy measurement for nitrogen content, which has been shown to have strong correlation with *A. donax* leaf chlorophyll content (Spencer et al., 2008)— indicated no change within the HN group and actually slightly increased ($P < 0.1$) values in for the CO2 enriched within the LN group. Although leaf nitrogen concentration did not seem to be affected by CO_2 the growth response results indicated that aboveground biomass stimulation was only responsive when nitrogen is abundant. When N was limiting there were no significant differences between elevated or ambient CO_2 treatment groups (Table 1-1). This finding is not all that unusual, considering a lack of CO_2 response when N is deficient has been reported for other C_3 grasses (Kinmonth-Schultz & Kim, 2011; Zanetti et al., 1997), and illustrates the importance of nutrient supply for carbon fixation, primary production, and plant biomass accumulation. Nutrient supply also plays an important role in determining carbon allocation.

Studies have shown that when plants grow in nitrogen deficient conditions carbohydrates produced in the leaves during photosynthesis do not appear to be stored in the leaf tissue and are instead allocated to carbon sinks (*e.g.* rhizomes, roots) (Hermans, Hammond, White, &

Verbruggen, 2006). Such a response to low N conditions stimulates an increased root to shoot or belowground to aboveground ratio. This research showed that a significant increase in belowground to aboveground ratio would occur under low N conditions. It also resulted in highly significant increases in the belowground to aboveground ratio within each CO_2 treatment (Fig. 4). Interestingly, some researchers have also attributed CO_2 enrichment as a cause of increased allocation to roots in C_3 grasses (P. S. Curtis, Balduman, Drake, & Whigham, 1990; Drake, Muehe, Peresta, GonzalezMeler, & Matamala, 1996; Larigauderie, et al., 1988). However, this research suggested an increased allocation to belowground sinks was not attributable to CO_2 within either nitrogen group treatment for *A. donax*. Although this finding contradicts some earlier studies, it did provide supportive evidence that similar with other findings that the CO_2 effects on the belowground to aboveground ratio are at most small (John F. Farrar & Gunn, 1996). Additionally, conflicting results published regarding root growth response disparities have been attributed to pot size variation (Poorter, Bühler, van Dusschoten, Climent, & Postma, 2012) as well as the experimental treatments.

While *A. donax* did not significantly exhibit a CO_2 meditated belowground to aboveground biomass allocation change, the allocation of carbohydrates to rhizomes compared to roots was correlated ($P < 0.05$) to CO_2 enrichment within the HN group (Fig. 1-5). Interestingly the CO_2 enriched HN treatment group (H+) had a nearly one-to-one rhizome to root biomass relationship. Surprisingly, *A. donax*'s increased allocation to rhizomes when nutrients are abundant is contrary to the results for *Phalaris arundinacea* (another invasive rhizomatous grass) that exhibited a decrease in the proportion of carbohydrates allocated to rhizome compared with roots when grown under similar conditions (Kinmonth-Schultz & Kim, 2011). Ecologically, the regrowth ability for *A. donax* has been directly associated with its rhizome size (Boose & Holt, 1999; Quinn & Holt, 2008). Therefore, *A. donax* will likely be able regrow more quickly and vigorously, if rhizome size increases with CO_2 enrichment in nutrient-rich ecosystems. Moreover, fracturing and re-sprouting of rhizomes and stems is fundamental to the colonization and dominance of this asexually propagated invasive plant. Therefore, increased aboveground biomass and increased rhizome mass will likely lead to an increased propagules, especially in nutrient rich systems like the agricultural and urban drainages (Paul & Meyer, 2008; Weller, et al., 2010). Whether or not increases in propagules and regrowth ability will necessarily result in increased invasions is as of yet unproven, albeit a likely consequence. Furthermore it is also plausible that the biomass stimulation to aboveground plant parts and rhizomes will also interact with flood and fire disturbance regimes.

A. donax is an invasive grass that has been implicated with increased fire regimes (Gary P. Bell, 1997; Scott, 1994). *A. donax*, like other grasses, perpetuates the fire cycle by accumulating abundant dry-standing biomass (Coffman, Ambrose, & Rundel, 2010). Because *A. donax* is rhizomatous it is able quickly re-sprout, often before native hardwoods, it is likely to be well adapted to respond to conditions of increased CO_2 enrichment. In relation to flood disturbance, the increased rhizomes and aboveground biomass create more propagules to be dispersed by scouring floodwaters. Additionally, rhizomes are used to tolerate persistent or periodic drought stress. Thus larger rhizomes resulting from anthropogenic CO_2 enrichment could create potentially more stress tolerant *A. donax*. While the competitive success of *Arundo donax* over co-occurring native plant species has been observed in a variety of riparian systems, the physiological factors that influence this success are often are not well understood. The results of this experiment indicate nitrogen and to some extent CO2, and an interaction between both factors, may stimulate the growth of *A. donax* because it is so physiologically adapted to the new environments developed with climate change and N saturation.

The findings of the research presented herein are relevant to at least two groups. The first group is agriculturalists who are using *A. donax* for bioremediation (Idris, Jones, Salzman, & Allinson, 2012; Kausar et al., 2012) and industrial purposes (Basso, Cerrella, Buonomo, Bonelli, & Cukierman, 2005). These scientists and engineers will be interested in the findings for crop scheduling and modeling purposes. Agriculturalists will also be interested in these results because they directly impact planting and production of *A. donax* as a biomass. First, it is commonly thought that *A. donax* is a model crop because like other perennial grasses it can be grown in marginal conditions with little nutrient input (Dohleman, Heaton, & Long, 2010). However, the results herein show that nitrogen fertilizer significantly increases biomass levels and thus it is unlikely, given the profit driven nature of agriculture, that growers would not fertilize and forgo increased yields. Secondly, agricultural production of *A. donax* will be benefited by increased rhizome size, because rhizome propagation is the primary method for *A. donax* cropping (Angelini, et al., 2005).

The second group of people who may be interested in these findings are invasion-biologists who monitor the spread of *A. donax.* Invasion-biologists are interested about the competitive advantages *A. donax* may gain in regards to CO_2 and N enrichment. *A. donax* is already known as a fierce competitor because of its high growth rates (Reiger, 1989; Rossa, Tuffers, Naidoo, & von Willert, 1998). The results suggest that this competitive advantage may be stimulated by N pollutions and CO_2 enrichment. The combination of these two resource enrichments is a highly plausible scenario for the canals, and drainages water-ways surrounding fertilized *A. donax* biomass plantations; for urban streams that are typified by nitrate runoff; and in riparian systems where seasonal flood pulses are often laden with N rich material

Lastly, opportunities exist to study *A. donax* invasion feedbacks with flood and fire disturbance regimes. These could also be investigated given the interplay of N and CO_2 enrichment. Additionally, evaluating the effects of N and CO_2 on both nonindigenous and co-occurring native plant species in intact ecosystems could provide insight useful in developing reliable prediction of how these plant communities could change in the future.

Figures:

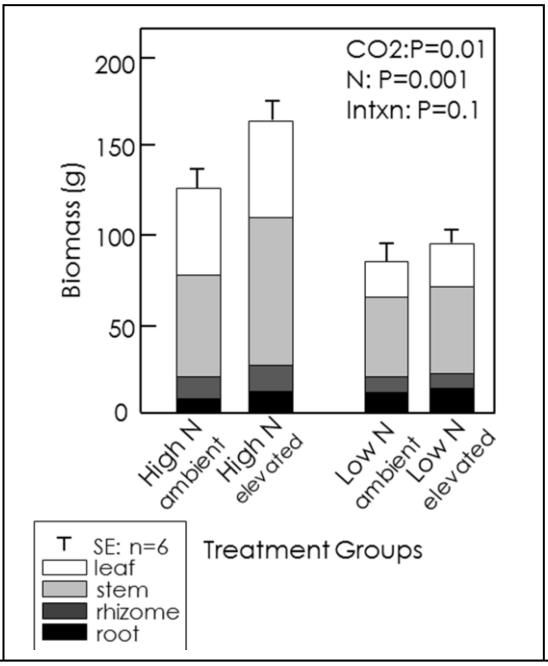


Figure 1-1: Stacked bar chart plot showing the proportion of plant organ (root, rhizome, stem, leaf) as part of the total biomass harvested from the plants. Statistical analysis revealed that CO_2 and nitrogen enrichment improved (P=0.01, and P=0.001, respectively) the biomass differences. The interaction between the two treatments was found to only have a mild stimulation for total biomass.

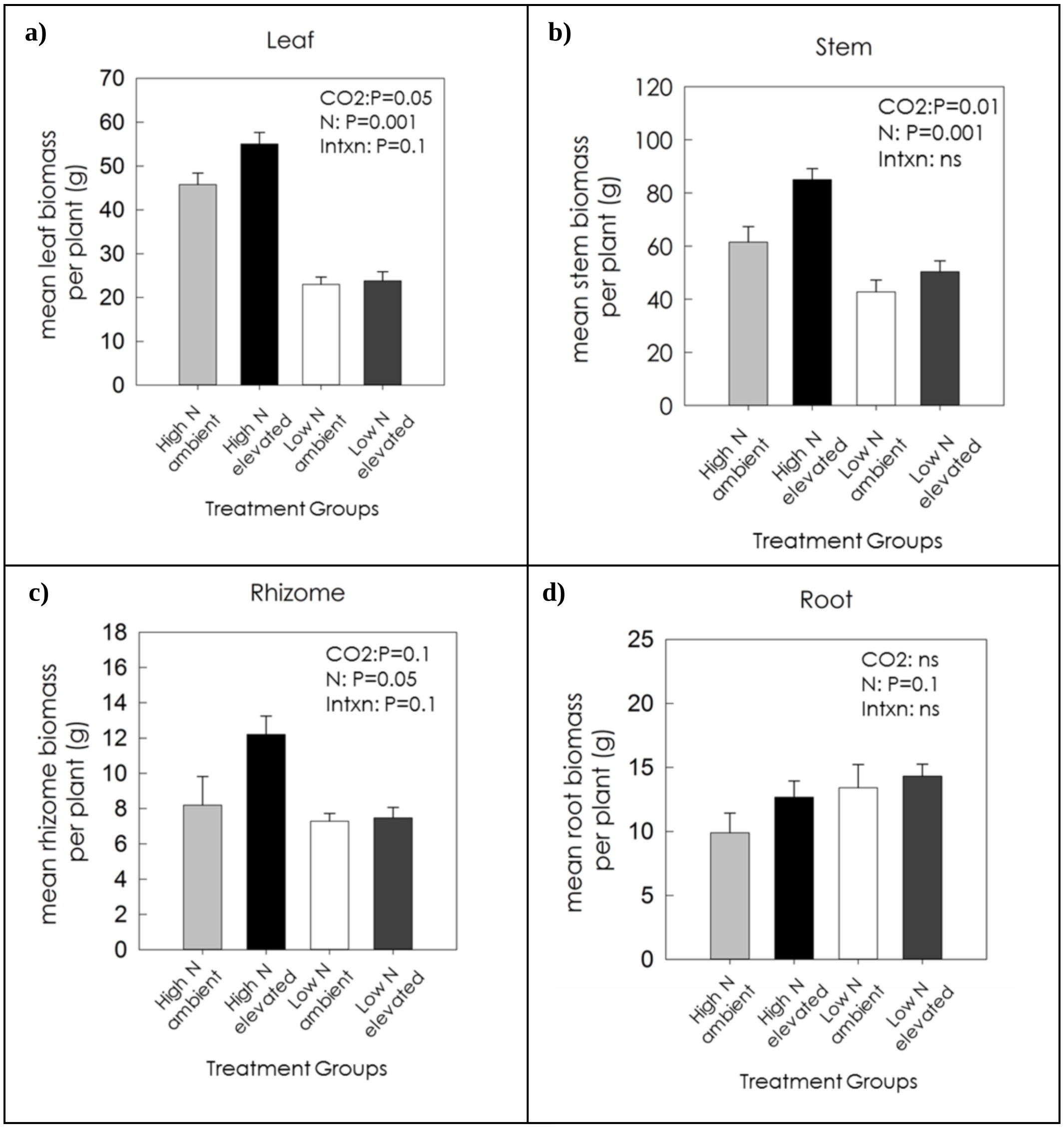


Figure 1-2: Bar charts showing mean biomass (n=6, SE error bars) of individual plant parts. CO_2 and Nitrogen effects were attributable to increases in growth in elevated chambers and high nitrogen treatments in the aboveground portions (leaf and stem, **1-2a** and **1-2b** respectively). Belowground (rhizome and root, **1-2c**, and **1-2d** respectively) nitrogen had a greater influence in the variability between growths. Also when CO_2 was enriched rhizome growth was greatly increased in the high nitrogen group, while CO_2 enrichment did not significantly increase growth of rhizome in low nitrogen group.

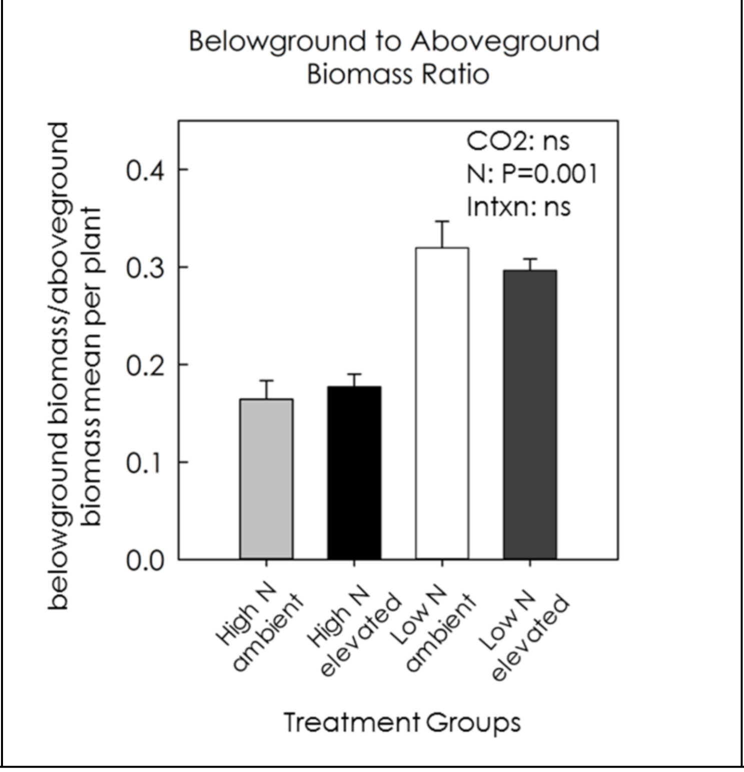


Figure 1-3: plot of the mean (n=6) belowground to aboveground biomass ratio with SE error bars illustrates that for all treatments biomass was primarily allocated to aboveground parts (i.e. stems and leaves) compared to roots and rhizomes. This was especially evident when nitrogen was plentiful, shown by the < 20% values within the high nitrogen (HN) treatment groups. When nitrogen was limiting there was an increase (P=0.001) in belowground biomass compared with that allocated belowground in the HN treatment groups.

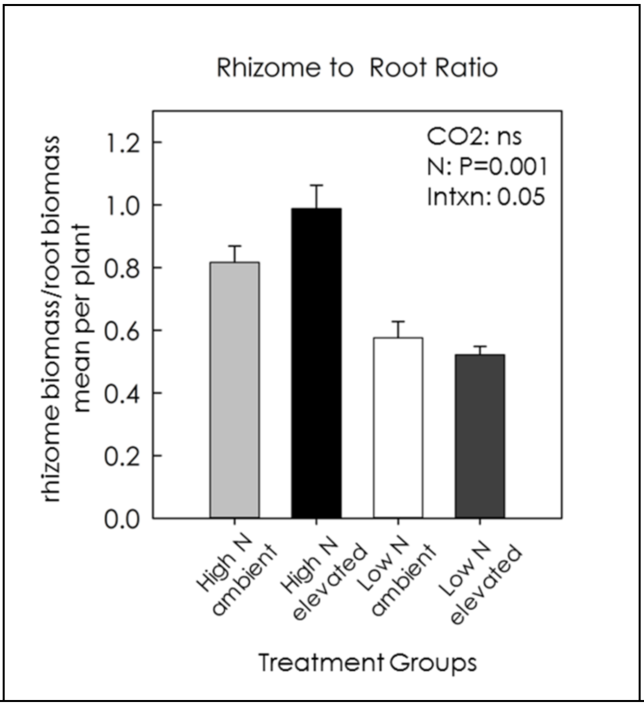
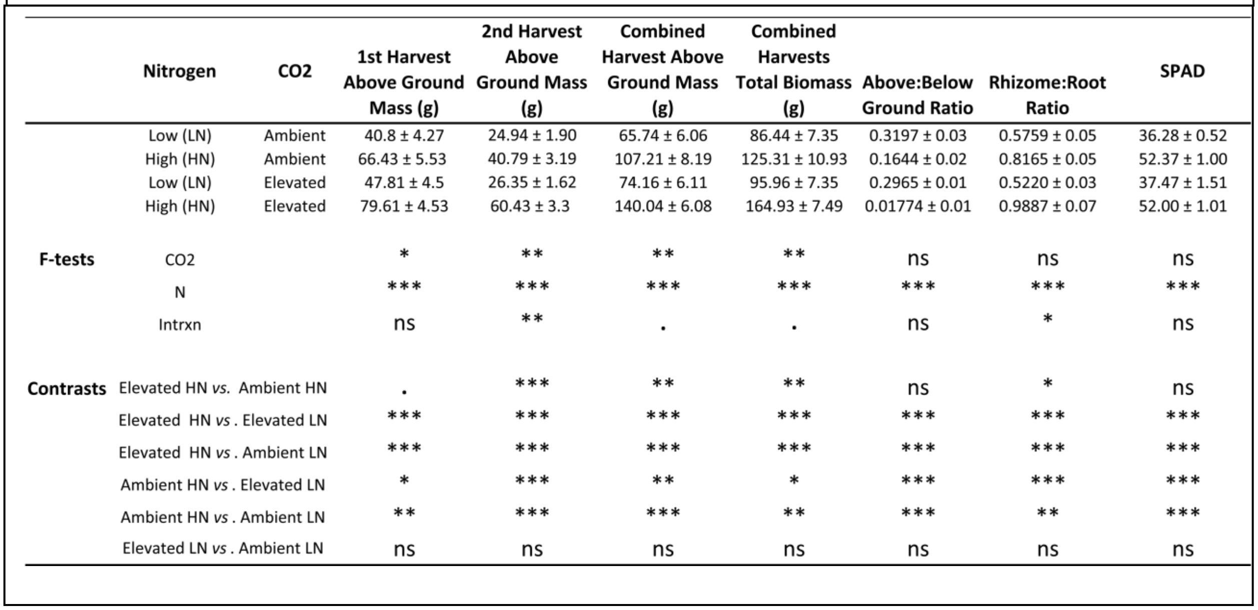


Figure 1-4: Plot of belowground biomass ratios. For the high nitrogen (HN) group the allocation of biomass between rhizome and roots are nearly equal (i.e. >80%) whereas when nitrogen is limiting (LN) biomass allocation to rhizomes is close to 50% that of the roots. The interaction term is indicative of the different relationship within nitrogen treatments. When nitrogen is abundant CO_2 enrichment increases rhizome size. When nitrogen is limiting CO_2 increases root size.

Tables:

Table 1-1: Statistical analyses of biomass harvests suggest main effects of CO2 ($p<0.05$) and subplot effects from nitrogen treatment ($P=0.001$) improved growth of above-ground biomass at each harvest and also total biomass accumulation. An interaction between the two treatments was only shown to have an influence on 2nd harvest above-ground biomass ($P=0.01$). The interaction was evidence of the much greater gains by the elevated CO$_2$ groups between high nitrogen (HN) treatments compared with the growth gains by the ambient CO$_2$ groups at with low nitrogen (LN). The other interaction term that showed greater than random chance ($P=0.05$) was evidence in the rhizome to root ratio. In this case, the interaction was significant because within the HN treatment, elevated CO2 increased rhizome development, whereas within the LN groups CO2 did not change the allocation relationship



	Nitrogen	CO2	1st Harvest Above Ground Mass (g)	2nd Harvest Above Ground Mass (g)	Combined Harvest Above Ground Mass (g)	Combined Harvests Total Biomass (g)	Above:Below Ground Ratio	Rhizome:Root Ratio	SPAD
	Low (LN)	Ambient	40.8 ± 4.27	24.94 ± 1.90	65.74 ± 6.06	86.44 ± 7.35	0.3197 ± 0.03	0.5759 ± 0.05	36.28 ± 0.52
	High (HN)	Ambient	66.43 ± 5.53	40.79 ± 3.19	107.21 ± 8.19	125.31 ± 10.93	0.1644 ± 0.02	0.8165 ± 0.05	52.37 ± 1.00
	Low (LN)	Elevated	47.81 ± 4.5	26.35 ± 1.62	74.16 ± 6.11	95.96 ± 7.35	0.2965 ± 0.01	0.5220 ± 0.03	37.47 ± 1.51
	High (HN)	Elevated	79.61 ± 4.53	60.43 ± 3.3	140.04 ± 6.08	164.93 ± 7.49	0.01774 ± 0.01	0.9887 ± 0.07	52.00 ± 1.01
F-tests	CO2		*	**	**	**	ns	ns	ns
	N		***	***	***	***	***	***	***
	Intrxn		ns	**	.	.	ns	*	ns
Contrasts	Elevated HN *vs.* Ambient HN		.	***	**	**	ns	*	ns
	Elevated HN *vs* . Elevated LN		***	***	***	***	***	***	***
	Elevated HN *vs* . Ambient LN		***	***	***	***	***	***	***
	Ambient HN *vs* . Elevated LN		*	***	**	*	***	***	***
	Ambient HN *vs* . Ambient LN		**	***	***	**	***	**	***
	Elevated LN *vs* . Ambient LN		ns	ns	ns	ns	ns	ns	ns

Chapter II:
Carbon dioxide effects on the water-use efficiency and drought tolerance for Arundo donax

Abstract

Freshwater is an essential resource—integral to all ecological and societal activities— a resource that the availability, timing, and quality have become vulnerable to climate change. Contention for limited freshwater resources is inevitable as increasing the land area is used by agriculture to supply biomass for bioenergy. Directing water toward crop production is necessary whether the biomass crops are rain-fed or irrigated, because all plants require water to survive and grow. Such a decision becomes even more challenging for a crop like *Arundo donax* (*A. donax*) that is considered a 'water-user' because of reported high transpiration rates. An experiment was initiated at the University of Washington Botanic Gardens CO_2 growth chambers, in which *A. donax* plants were supplied with adequate nutrients and water at ambient and elevated CO_2 levels and measured for intrinsic, instantaneous, and whole-plant water-use efficiency (WUE). In a parallel experiment conducted at the same time in the same chambers *A. donax* plants were grown under similar conditions and then restricted from water to determine how drought influenced WUE under ambient and elevated CO_2 conditions. The results of this study confirm that *A. donax*, like most C_3 grasses, will increase its biomass growth while using less water when atmospheric carbon dioxide is elevated. Additionally, the results show that WUE improved associated with elevated CO_2 which delayed the impacts of drought-stress in effect making *A. donax* more stress tolerant. The findings presented herein represent a critical missing piece in the understanding of *Arundo* invasions.

Introduction

To mitigate the impacts associated with climate change governments are putting into place policies that reduce fossil fuel dependence by increasing the use of sustainable bioenergy crops (Perlack et al., 2005; Powlson, Riche, & Shield, 2005). ‘First-generation’ bioenergies that depended primarily on food crops provided negligible carbon gains and created social conflicts (Naylor et al., 2007; Pimentel & Pimentel, 2008). Societal pressure, and poor energy economics by food crops forced researchers to seek alternative biomass sources; grasses became some of the most desirable non-food ‘second generation’ bioenergy feedstocks (Dohleman, et al., 2010). However, grasses also constitute a major group of invasive plants and thus there have been alarming reports that bioenergy developments will lead to the cultivation of plants which will subsequently invade surrounding ecosystems (Barney & Ditomaso, 2008; Raghu et al., 2006). *Arundo donax* L. is a grass species at the nexus of the bioenergy and invasive plant debate. Agronomists and industrial engineers promote *A. donax* as one of the most desirable crops because of reported greater yields and energy gains than available from maize (*Zea maize*) or other grasses (Angelini, et al., 2005). Conversely, ecologists are concerned that cultivating *A. donax* will foster its naturalization. Surprisingly, some basic questions about this species physiology remain unclear; in particular what is this species water-use efficiency, *i.e.* how much water must to be used per unit of biomass gain? Such a question is fundamental to both agronomists who claim of minimal inputs for great gains, and for ecologists who predict reductions in water resources should *A. donax* escape cultivation. Understanding this plant’s water-use efficiency (WUE) is ever more relevant in a changing climate, where acquisition of freshwater resources may become less predictable.

Freshwater is an essential resource—integral to all ecological and societal activities (Gleick, Pacific Institute for Studies in Development, & Security, 2012) — a resource that the availability, timing, and quality have become vulnerable to climate change. Global climate change has been linked by numerous reports to changes in the global hydrological cycle such as: increased atmospheric water-vapor content; changed precipitation patterns, intensity and extremes; reduced snow cover; and changed soil moisture and runoff (Kundzewicz et al., 2007; Vorosmarty, Green, Salisbury, & Lammers, 2000). Contention for limited freshwater resources is inevitable as increasing the land area is used by agriculture to supply biomass for bioenergy.

Directing water toward crop production is necessary whether the biomass crops are rain-fed or irrigated, because all plants require water to survive and grow. Thus diverting limited water resources away from food production or other ecosystem services is a highly contentious decision in arid lands and in regions where water may be less available in the future. Such a decision becomes even more challenging for a crop like *Arundo donax (A. donax)* that is considered a 'water-user' because of reported high transpiration rates (M.E. Iverson, 1994; Watts & Moore, 2011).

Predictions of excessive water use by non-native invasive plants dates back to the 1970s and was based on research conducted on the effects of afforestation on stream flow and water resources (Gorgens, 2004). Invading non-native plants, especially trees such as *Tamarisk* spp. and *Eleagnus angustifolia* in North America, and *Pinus pinaster* and *Acacia* spp. in South Africa, began to attract more attention as catchment-scale hydrological studies were conducted in the 1980s (Everitt, 1980) and 1990s (Le Maitre, et al., 1996). In more recent years catchment-scale experiments have focused on quantifying changes in stream flow and, to a lesser extent, ground water resources (Gorgens, 2004). While most of the research relating invasive plant transpiration and water-cycle disruption has focused on invasive trees, there is concern by researchers and land managers that invasive grasses may also be negatively impact the riparian water-cycle through their high transpiration rates. In the south-western United States invasive plants have been associated with lowered water tables in many riparian zones (Dukes & Mooney, 2004). Additionally, invasive grasses have been cited to re-engineer intertidal basin and regional hydrology by changing substrate cohesion and surface roughness (Collins, 2002). When invasive grasses alter sediment deposition and sequestration they also influence channel-bank erosion and channel-network geometry. Altered sedimentation and erosions illustrate structural modifications. While structural modifications by invasive grasses are evident, an equally important, yet less obvious modification is the role invasive plants play in altering the riparian zone water-cycle.

The water-cycle in riparian zones depends on three linked areas: water uptake; water storage in different parts of plants; and the return of water to the atmosphere by evapotranspiration (ET). Although the riparian zone water-cycle is poorly researched considering the intimate plant-water linkages well studied in other ecosystems (Baird & Wilby, 1999) it is logical that storage and use of water by riparian plants also impacts the water-cycle. Thus, the theory holds that the riparian water-cycle will be altered if invasive plants impose exceptional water consumption and loss rates, consuming greater rates of water from the soil and from the saturated zone (Le Maitre, et al., 2002). This theory may be confounded in a changing climate as transpiration rates decrease in response to atmospheric enrichment of carbon dioxide.

Atmospheric enrichment of carbon dioxide (CO_2) directly affects plants by inducing partial stomata closure (Morison, 1985). The closure effects plant water use and carbon gain because nearly all of the water transpired as well as CO_2 absorbed pass through stomatal pores. Stomata attempt to balance plant water and carbon status by permitting sufficient CO_2 to enter the leaf for photosynthesis while minimizing transpirational dehydration. The amount of carbon gained per unit water lost is also known as plant ‘water use efficiency’. While the rate of diffusion of gases into or out of the leaf from/to the surrounding environment depends upon many factors including the concentration gradient and the resistance of diffusion along the pathway, the typical response to CO_2 enrichment is increased WUE (Lawson, Caemmerer, & Baroli, 2011). Additionally, atmospheric enrichment of CO_2 typically increases carboxylation efficiencies, especially in C_3 plants. While there have been a few studies showing the positive interaction between invasive plants and carbon dioxide enrichment (Rogers, et al., 2008; Smith, et al., 2000; L. H. Ziska, 2003), grasses in general have shown consistently positive growth responses to increasing CO_2 (S. Wand, J. E., Gu, Michael, & Peter, 1999). Therefore, to understand the ecological impacts, or agricultural yields, associated with *A. donax* WUE it is important to study this species responses at current and elevated CO_2 conditions. Towards this goal of quantifying *A. donax* WUE an experiment was initiated at the University of Washington Botanic Gardens CO_2 growth chambers, in which *A. donax* plants were supplied with adequate nutrients and water at ambient and elevated CO_2 levels and measured for intrinsic, instantaneous, and whole-plant WUE. In a parallel experiment conducted at the same time in the same chambers *A. donax* plants were grown under similar conditions and then restricted from water to determine how drought influenced WUE under ambient and elevated CO_2 conditions. I hypothesized that (1) CO_2 enrichment would decrease stomatal conductance and increase carboxylation efficiencies resulting in increased WUE. Further, I hypothesized that (2) increased WUE would mitigate the effects of drought.

Materials and Methods

Study Location

The experiment was conducted at the University of Washington Botanic Gardens CO_2 growth chambers located in the Douglas Research Conservatory (DRC), Seattle, Washington, USA. The CO_2 growth chambers (100cm *x* 100cm *x* 200cm) are closed-topped, PVC-framed units surrounded with Mylar polyester sheeting. Fans forced air from outside the greenhouse into the chambers through a system of flexible aluminum ducting and PVC piping. For additional details of the chamber construction please refer to (Kinmonth-Schultz & Kim, 2011). Two chambers were randomly assigned ambient air, while the remaining two were fumigated with additional CO_2 delivered from a 22.70 kg tank (Praxair, Seattle, Washington, USA). The elevated CO_2 concentrations were maintained by using bubble flow meters (FL-2000, Omega, Stanford, Connecticut, USA), at approximately 800 μmol mol^{-1} CO_2 above ambient levels. The concentration for the elevated CO_2 reflects a near doubling of the ambient levels and represent levels projected to be the atmospheric concentration at the end of this century (Bernstein, et al., 2007). The CO_2 concentrations in the elevated chambers were monitored and automatically collected every 15 minutes by a CIRAS-1 infrared gas analyser (PP Systems International, Inc., Amesbury, Massachusetts, USA). Ambient CO_2 from the air pumped into the chambers was measured by a LI-6200 infrared gas analyser (LI-COR, Inc., Lincoln, Nebraska, USA). Temperature in each chamber was monitored every 15 minutes using a pair of thermocouples placed, 20 and 100 cm from the tops of the chambers. CO_2 and temperature data were stored automatically in a CR1000 data-logger (Campbell Scientific, Logan, Utah, USA). Daylight was supplemented in the DRC by high pressure sodium 400 watt single phase bulbs (Phillips Electronics North America Corp., Andover, Massachusetts, USA) during 0800 to 2200 hrs.

Plant Material

On June 1th, 2009, rhizomes—collected from population of *Arundo donax* growing wild along the San Anselmo Creek (San Anselmo, California, USA)—were divided into 5-10 cm lengths, containing at least three internodes. The rhizome divisions were set in perlite on propagation trays and then placed in a mist bench in the DRC. Water was applied from overhead micro-spray sprinklers for five seconds every thirty minutes. After two weeks most of the rhizome pieces had sprouted and developed 2-3 leaves. Eight of these rooted and leafed divisions were set aside for destructive harvest to create a baseline description of the plant biomass allocation patterns. Thirty-two of the remaining rooted and leafed divisions were individually potted (1 propagule per pot) in 10.9 liter ‘*300 Series’* black plastic nursery containers (Haviland Plastics Products, Co., Haviland, Ohio, USA) filled with washed building sand (Salmon Bay Sand & Gravel, Seattle, WA, USA). The sand-filled nursery containers were placed in 11.36 L buckets (the buckets acted as large drip pans). The nursery containers/buckets—hereafter referred to as pots — were placed into one of the glasshouses at the DRC.

After being moved to the pot, the rooted rhizome divisions were watered with half-strength Hoagland’s fertilizer solution (Epstein & Bloom, 2005). The macronutrients were provided as follows: KNO_3, $Ca(NO_3)_2*4H_2O$, $NH_4H_2PO_4$,and $MgSO_4*7H_2O$ (concentrations for K^+, NO_3^-, Ca^{2+}, NH^{4+},PO_4^{3-}, Mg^{2+} and SO_4^{2-} were 3.0, 7.0, 2.0, 1.0, 1.0, 0.5 and0.5 µM . The micronutrients were in the forms of: KCl, H3BO3, $MnSO_4*H_2O$, $ZnSO_4*7H_2O$, $CuSO_4*5H_2O$, H_2MoO_4, and NaFeDTPA (concentrations of Cl^-, B^{3+}, Mn2+, Zn^{2+}, Cu^{2+}, Mo^{6+} and Fe chelate were 25, 12.5, 1.0, 1.0, 0.25, 0.25 and 9 µM respectively). On June 24th, 2009, ten days after transplanting, the propagules all passed visual inspection of successful transplanting, and were placed into the experimental treatment groups.

Experimental design

For the greenhouse experiment, a total of thirty-two pots planted with *A. donax* were randomly split in into two groups of sixteen pots. Each group was assigned a watering treatment, either watered or drought. Watered pots were constantly provided adequate water by maintain between 1-5 cm of water or fertilizer solution in the bucket at all time. *A. donax* is a riparian and estuarine plant and thus able tolerate saturated soil conditions. The drought pots were watered in the same fashion up to a certain point (described later) when the drought stress was applied. Four pots from each watering treatment group—watered and drought—were randomly placed in one of four CO_2 chambers located inside the glasshouse. An additional pot was placed in the center of each CO_2 chamber to act as an evaporation pan. This evaporation pan was identical to all of the other pots except it did not include an *Arundo* propagule. Thus each CO_2 chamber contained four pots from each watering treatment and one evaporation pan. When the water level dropped to near the 1cm mark on the pot they were refreshed with half-strength Hoagland's solution. All pots were watered, periodically, in this manner for fifty days.

On August 12th, 2009, the 50th day after the pots were placed in the CO2 chambers, drought stress was initiated by withholding the water supply to the drought treatment group. Drought was imposed for 28 days of the experiment (the experiment was conducted for 78 days in total). During the drought treatment the non-drought plants continued to be watered with the Hoagland solution. The quantity of water added to pots within the watered treatment group, and the evaporation pans, were recorded for the entirety of the experiment.

The effects of drought stress were monitored by a variety of metrics described below including whole plant pot lysimetry, leaf relative water content, leaf gas-exchange, and leaf chlorophyll fluorescence. Twenty-eight days after the imposition of the drought treatment, all the plants were harvested. Each plant harvested was separated and sorted into the following categories: leaf, stem, root, and rhizome tissues, for determining their dry weights. Before drying the leaves, leaf surface areas were measured for each plant using a leaf-area meter (LI-3000; LI-COR, Inc., Lincoln, Nebraska, USA). After leaf areas were recorded, all of the plant parts were dried for 48 hours in a forced air oven heated to 80° C.

Ecophysiological measurements

Non-drought measurements of water-use efficiency

The water-use efficiency of the non-drought experimental group measurements include whole plant water-use efficiency (WUE), leaf level WUE and stable carbon isotope analysis.

Whole plant WUE

For 12 randomly selected non-drought plants, the whole plant WUE was calculated as the ratio of oven dry biomass gained against the amount of water used as shown below.

$$\text{WUE} = \text{grams mass} / \text{ml water used}$$

Biomass values were collected from the harvest data and consisted of the total oven dry biomass per plant. Water-use was calculated by summing the water applied to each pot throughout the 78 day experiment; and subtracting from this summed value the amount of water remaining plus the pot and soil and the mean value of water evaporated from the evaporation pans.

Water use per plant = Water applied –

(water remaining + mean value of water evaporated from evaporation pans)

Leaf level gas exchange

Leaf level gas exchange was determined with a portable photosynthesis meter (LI-6400; LI-COR, Inc., Lincoln, Nebraska, USA). The ‘*A/C$_i$*’ auto-program’ was used to record gas-exchange measurement across changing carbon dioxide concentrations. Stable parameters were set at the following: temperature at 25^{o} C, light was set at 1500 photon flux density, and flow rate at 300 µmol m^{-2} sec^{-1}. During the measurement period, CO_2 was automatically changed at nine levels from 50 to 1500 $\mu molmol^{-1}$ CO_2. Leaves were allowed to acclimate to the change in the CO_2 levels for a minimum of five minutes and a maximum of ten minutes. Measurements were recorded from 0700 hrs to 1300 hrs. A/C_i curves were created for three plants from each CO_2 treatment group, i.e., growing condition CO_2 concentrations were 400 and 800 $\mu molmol^{-1}$ CO_2. The A/C_i curves produced the following information: photosynthetic assimilation (*A*), transpiration rates (*E*), stomatal conductance (g_S) and the differences between atmospheric (C_a) and internal CO_2 concentrations (C_i). These measurements were used to evaluate instantaneous WUE (*A*/*E*) which is a leaf level version of carbon gained compared with water loss. Additionally the gas-exchange measurements were used to evaluate the intrinsic WUE (A/g_S) which compares the photosynthetic properties independent of evaporative demand. Lastly, gas-exchange measurements were used to evaluate the C_i/C_a ratio which reflects the balance between net assimilation and stomatal conductance according to Fick’s law: $A = g_S (C_a - C_i)$.

Drought metrics

Pot lysimetry

Lysimetry is a method for measuring transpiration that accounts for the weight of water lost and attributes that loss to evaporation and plant transpiration. For this experiment, pots were removed from the CO2 chambers and weighed on a 50 kg x 0.02g electric balance (Acculab S-50; Acculab, Bradford, MA). Mass was recorded for each of the drought treatment pots on the last day of watering and on subsequent days during the drought period. The evaporation pans were also weighed throughout the duration of the drought period. The difference in grams of pot mass between the beginning of the drought and the conclusion of the experiment is equivalent to amount of millilitres water transpired and evaporated, and was used to estimate whole plant water use.

Leaf relative water content

Relative water content (RWC) is the ratio of the leaf water content compared to the fully hydrated conditions, and is shown as an equation below.

$$RWC = (\text{Fresh weight} - \text{Dry weight}) / (\text{Fully hydrated weight} - \text{Dry Weight})$$

Obtaining these values required clipping a fully developed young leaf from each drought treatment plant at the beginning of the drought stress treatment and at seven different days as drought progressed. Clipped leaves were bagged in pre-moistened, re-sealable, plastic bags and brought to the lab in less than 30 minutes after harvesting to prevent desiccation. The leaves were then weighed to obtain a per leaf fresh weight and scanned to determine the leaf-area of each leaf using the LI-3100. The fresh leaves were then placed back in the plastic bags which were filled with di-ionized water then re-sealed. The leaves were allowed to soak for 24 hours at which point they were removed from the plastic bags, surface dried using a paper towel and then re-weighed. After the fully hydrated leaf weights were recorded, leaves were dried at 80° C for 48 hours in a forced air drying oven. The oven-dry leaf mass was then weighed and recorded. The oven-dry mass and leaf-area measurements of the all of the clipped leaves were added to the final harvest biomass totals.

Leaf gas-exchange and chlorophyll fluorescence

Leaf carbon assimilation and water vapor loss through transpiration was measured using the LI-6400. The '*A/Ci* auto-program' was used again to record gas-exchange measurement across changing carbon dioxide concentrations. The same stable parameters that were used for the non-drought plants: temperature at 25^{o} C, light was set at 1500 photon flux density, and flow rate at 300 μmol m^{-2} sec^{-1}. Like before, CO_2 was automatically changed at nine levels from 50 to 1500 μmolmol^{-1} CO_2. Leaves were allowed to acclimate to the change in the CO_2 levels for a minimum of five minutes and a maximum of ten minutes. Measurements were recorded from 0700 hrs to 1300 hrs. Each *A/Ci* auto-program required approximately one hour to record and thus only one drought plant could be recorded from each chamber each day. The *A/Ci* curve was used to decompose photosynthetic carbon assimilation by ETR (maximum rate of electron transport), carboxylation efficiency (RuBP-saturated rate of Rubisco) and Amax (maximum rate of assimilation).

The LI-6400 was also used to record chlorophyll fluorescence since chlorophyll fluorescence is as an inverse relationship to photosynthetic performance. Fluorescence data were collected at pre-dawn from 0400 hrs to 0600 hrs. Dark-adapted leaves can be used to evaluate potential photochemical efficiency (the maximum quantum yield of PSII). The potential quantum yield as measured in dark-adapted leaves (F_v/F_m) is a ratio of the maximal fluorescence (F_m) minus the resting or null fluorescence (F_o) divided by the maximal fluorescence. The F_v/F_m ratio is calculated automatically by the LI-6400 from F_o and F_m values. The procedure required using the 'Do FoFm' option on the LI-6400 which exposed the dark adapted leaf surface to a saturating pulse of light and measuring the energy fluoresced response using the LI-6400. Measurements from three leaves from all of the thirty-two plants were recorded on five different days throughout the drought treatment.

$$F_v/F_m = (F_m - F_o)/F_m$$

Data analysis

The experimental design which includes a watering treatment within the CO_2 chambers represents completely randomized block split-plot design, where the CO_2 is the main (or whole-plot) effect and the water is the sub-plot effect. CO_2 and water (H_2O) were considered fixed effects and the chambers were considered as random effects. Initially SigmaPlot 12.2 (Systat software, INC., San Jose, California, USA) was used to create charts illustrating whole-plant WUE, leaf WUE, pot lysimetry, leaf RWC, photosynthetic characteristics (Amax, ETR, carboxylation efficiency), and F_v/F_m ratio. A linear regression of the relationship between the independent variable, water-use, and the dependant variable, biomass gained, was fitted, by Sigmaplot's curve fitter tool, to the non-drought whole plant WUE. Additionally, R 2.12 statistical software (Team, 2011) was used to conduct an analysis of covariance (ANCOVA) where again biomass represented the dependant variable and water-use and this time the covariate CO_2 treatment represented the independent variables.

Results

Non-drought

The results of the experiments conducted suggest that CO_2 enrichment increases water-use efficiency (WUE) of *A. donax* at the whole plant level (Figure 2-1). The linear regressions between the dependence of biomass and water use showed a very strong correlation for the elevated (R^2=0.94) and ambient (R^2=0.99) chambers. Furthermore, the interaction term ($P < 0.05$) between H_2O and CO_2 in the ANCOVA comparing the influence of CO_2 on the WUE suggests that WUE is being positively affected by the CO_2 treatments. However, the *A/Ci* curves for the non-drought from either chamber did not show evidence of CO_2 enrichment increases net assimilation (Figure 2-2). The lack of photosynthetic enhancement was shown by the similar carboxylation efficiency (initial slope) as well as Amax (asymptote) (Figure 2-2). Regardless, the WUE improvement evidenced at the whole plant was also evidenced in the leaf gas exchange measurements (Figure 2-3). Each of the three sub-charts (Figures 2-3a-c) present the mean values for intrinsic WUE (A/g_S), instantaneous WUE (A/E) and the C_i/C_a ratios for both CO_2 treatments at CO2 levels 400 μmol mol^{-1} CO_2 and 800 μmol mol^{-1} CO_2, representing the growing conditions for both treatments. The increases in A/g_S and A/E and the decrease in C_i/C_a ratios suggest higher water-use efficiencies for plants grown in elevated CO_2. The effects of elevated CO_2 on water-use efficiency were most pronounced at the 800 μmol mol^{-1} CO_2 treatment. Considering that A was not shown to be directly affected by the CO_2 (Figure 2-2) these leaf level improvements of WUE can be attributed to decreases in stomatal conductance and transpiration.

Post-drought

The measurements taken suggest that the responses to drought were delayed for plants grown under elevated CO_2 conditions compared with plants growing in ambient CO_2 conditions. First, pot lysimetry (Figure 2-4) showed how the plants grown in ambient CO_2 conditions used a greater percentage of the available water more quickly than the plants grown under elevated CO_2 conditions. Half-way through the experimentally induced drought (*i.e.* tenth day of the drought), plants growing in the elevated CO_2 and ambient conditions had no significant differences in their water-use. After this point in the drought, the plants grown under ambient CO_2 conditions no longer absorbed water whereas the plants grown under elevated CO_2 continued to take up a small fraction of the available water. The delayed effects of drought were also evident in leaf relative water content results (Figure 2-5).

Leaf RWC results show that both treatment groups had a pre-drought leaf RWC of approximately 80%. Unlike the pot lysimetry, which indicated an immediate difference between the treatment groups, RWC did not show signs of the drought until sometime after day seven. During the second week of drought, the plants grown under ambient CO_2 conditions had a lower RWC compared to *A. donax* grown in the elevated CO_2 group. At the end of the drought treatment, desiccated leaves from both treatment groups had a similar RWC (~15%). Additional divergent responses to the drought treatment as a result of CO_2 growing conditions were evidenced in leaf level gas exchange.

The A/C_i measurements recorded by the LI-6400 after the drought was imposed were decomposed to display key values representing stages of photosynthetic supply/demand limitations. The slope of the *A/Ci* curve is representative of the carboxylation efficiency (RuBP-saturation rate of Rubisco) when CO_2 is limiting the photosynthesis. The maximum assimilation rate (A_{max}: net CO_2 assimilation rate when PPFD and CO_2 are saturating) was taken from the greatest values for *A* from the A/Ci curve. Lastly, ambient assimilation rates A_{amb} were taken from values representing the assimilation rates occurring under growing condition carbon dioxide level, 400 μmol mol^{-1} CO_2 for the ambient plants and 800 μmol mol^{-1} CO_2 for the elevated plants. Although there was individual variation within treatment groups, A/C_i measurements suggest that the between treatment drought response is conserved, in which plants grown at ambient CO_2 concentrations are more quickly affected (in this case by decreased photosynthesis) than plants grown at elevated CO_2 conditions. This is illustrated by the earlier responses and more rapid rate of change within the ambient group and the delayed responses within the elevated CO_2 group. Chlorophyll fluorescence is the final metric that indicated the delayed response to drought by the elevated CO_2 group.

The F_v/F_m ratio has been shown to provide a reliable estimate the yield of PS II photochemistry and frequently shows a strong, quantitative relationship with the quantum yield of CO_2-assimilation as determined through infrared gas analysis (Oxborough & Baker, 1997). The F_v/F_m

results illustrate the divergent stress responses between CO_2 treatments with the characteristic sigmoidal decay that was evident in the RWC and photosynthetic assimilation values (Figure 2-8). Similar to the results obtained for RWC within both treatment groups, there is a delayed response to the drought, e.g., fluorescence values do not detect the experimental drought until ten days into the drought. After the fluorescence values detect the effects of the drought, the F_v/F_m ratio decreases which indicates a decrease in photosynthetic efficiency. F_v/F_m decay occurs rapidly for A. donax growing in the ambient CO2 group but occurs later for plants growing in the elevated CO2 group.

Discussion

Understanding the WUE of *A. donax* is increasingly important for ecologists and agronomists in a changing climate where freshwater resources may become more limited. The results of this study show that atmospheric carbon dioxide enrichment improves *A. donax* WUE. This was evident at the whole-plant and leaf-level. The gas-exchange results, the similar *A/Ci* curves (Figure 2-2) and the leaf-WUE (Figure 2-3) indicate that the increases in WUE were a consequence of decreased transpiration by the plants grown in elevated CO_2 rather than an increases in net-assimilation by plants in this group. One of the primary responses by plants to CO_2 is to reduce stomatal conductance (Lawson, et al., 2011; Morison, 1985). As stomatal conductance decreases with CO_2 enrichment there are subsequent diffusion limitations which alter the ratio of intercellular to ambient CO_2 concentrations (C_i/C_a), concomitantly reducing transpiration rates which then alters instantaneous WUE (*A/E*). Alternatively, while increased WUE could have been a result of greater carbon assimilation, acclimatization, or down-regulation, is a common phenomenon of C_3 plants with CO_2 enrichment (Cure & Acock, 1986), whereas CO_2 does not cause acclimation of stomatal conductance (Andrew D. B. Leakey, Bernacchi, Ort, & Long, 2006). Other than a recent paper which used stable carbon isotopes to represent WUE (Watts & Moore, 2011) these findings represent the only work published regarding *A. donax* whole plant or leaf level WUE at different levels of CO_2. WUE can be measured in a variety of ways (*i.e.* stable-isotope, transpiration, sap-flux etc.) which presents challenges for creating inter-species comparisons between studies, such work can be accomplished. Although this type of meta-analysis is outside of the scope of this study it would be a worthwhile step for scientists interested in plant community or watershed level interactions of *A. donax*, or to compare its WUE against other biomass feedstocks. The latter investigation is highly relevant considering that agronomists determine biomass harvests based on rain or irrigation inputs. Additionally, *A. donax*'s response to drought also presents new information regarding how this species tolerates stress. Chlorophyll fluorescence is an excellent indicator or stress.

From chlorophyll fluorescence the F_v/F_m ratio has been shown to provide a reliable estimate the yield of PS II photochemistry and frequently shows a strong, quantitative relationship with the quantum yield of CO_2-assimilation as determined through infrared gas analysis (Oxborough & Baker, 1997). The F_v/F_m results illustrate improved stress tolerance by plants grown in enriched CO_2 compared with plants grown in ambient chambers this characteristic sigmoidal decay that was evident in the RWC and photosynthetic assimilation values (Figure 2-8). The results suggest that the photosynthetic function is maintained for a greater period in the enriched chambers. Low water availability, like CO_2 enrichment, is another environmental cue that triggers stomatal closure. The enriched supplies of CO_2 compensate for the increasingly decreased stomatal conductance a response of the increasing drought. Stomatal closure—mediated by the aforementioned osmotic stress signals—affects photosynthesis and plant growth by restricting CO_2 diffusion into the chloroplasts, or altering leaf photochemistry and carbon metabolism. Enhancements to water-use efficiencies associated with CO_2 enrichment provide limited drought avoidance evidenced in the by the results (Figure 2-4) in which enhanced WUE by plants grown in the elevated CO_2 chambers used less quickly preserving the diminishing resource. Interestingly the elevated CO_2 plants were able to use a greater percentage of the available water as shown by the lower values after day ten (Figure 2-4). This result is likely due to the fact that the improved cost of water loss to carbon gain from CO_2 enrichment allowed this group of plants to assimilate for a longer period of time. Similar extended assimilation periods because of CO_2 enrichment during periods of drought has been confirmed in other studies of C_3 plants (Galmés, Medrano, & Flexas, 2007).

As photosynthetic function is protected the F_v/F_m ratios are unaffected. The effects of drought in the leaves are evidenced at an earlier date by the RWC and then by the F_v/F_m. This would suggest that as the stomatal limitations are overcome and the leaf begins to desiccate then the biochemistry begins to decline as more energy is directed toward chlorophyll fluorescence as photoprotection and away from photosynthesis. Alternatively, earlier cessation of transpiration by the ambient CO_2 group could be a result of damaged photosynthetic apparatus. The damage is typically caused as oxidative stress, which can seriously affect leaf photosynthetic machinery especially during warm and bright conditions. Considering that drought was applied during August, when light and temperature are greatest, the potential for oxidative stress to damage the photosynthetic apparatus of *A. donax* is a distinct possibility. Damage to PSII is indicated by the dark fluorescence values (Figure 2-7) in which, the photosynthetic quantum conversion declines as evidenced in the strong decline in F_v/F_m ratio.

The results of this study confirm that *A. donax*, like most C_3 grasses, will increase its biomass growth while using less water when atmospheric carbon dioxide is elevated. While there will likely be general stimulation in WUE across C_3 species within the invaded plant communities it has been shown that species specific responses to CO_2 enrichment are highly variable (Poorter, 1993). Thus considering that little is known about the effects of CO_2 enrichment on the native species in communities *A. donax* spp. invades it remains unclear whether it will have a distinct advantage over its competitors. This realization presents an opportunity to determine how this response will play out in the ecosystem by establishing competition studies between *A. donax* and native species grown in elevated CO_2 conditions. Understanding if *Arundo* WUE will be enhanced disproportionately to the native plants WUE is important because it has been projected with high confidence that water that many of the areas that *Arundo* currently invades in the United States will become more arid (Seager et al., 2007) (Casola, 2005). Other semi-arid and arid areas invaded by *Arundo* (*e.g.*, Mediterranean Basin, southern Africa and north-eastern Brazil) are particularly exposed to the impacts of climate change and are projected to suffer further decreases of water resources due to climate change (Fischer, 2001). Considering that low water availability is signature abiotic limitation to plant growth and yield in semi-arid areas the interaction of drought and CO_2 are very relevant.

Quantifying the impacts of invasive plants on water resources is paramount in arid and semi-arid environments, where *A. donax* commonly invades (*i.e.* Southwestern U.S., Australia, and South Africa). The association between invasive plants and limited water resources has spurred national level programs to eradicate invasive ‘water-users’ (Van Wilgen, Le Maitre, & Cowling, 1998). While *A. Donax is* hypothesized excessive transpiration rates have resulted in 'water-user' labels as of yet very little has been published to validate these claims. Without the scientific justification to dedicate resources towards widespread *Arundo* eradication the invasion goes unchecked. Therefore quantifying *A. donax* WUE is important step in understanding the hydrological impacts of this invasive species. The results from this study show how improved WUE, associated with elevated CO_2, delays the impacts of drought-stress in effect making this invasive grass more stress tolerant. The findings presented herein represent a critical missing piece in the understanding of *Arundo* invasions.

Figures

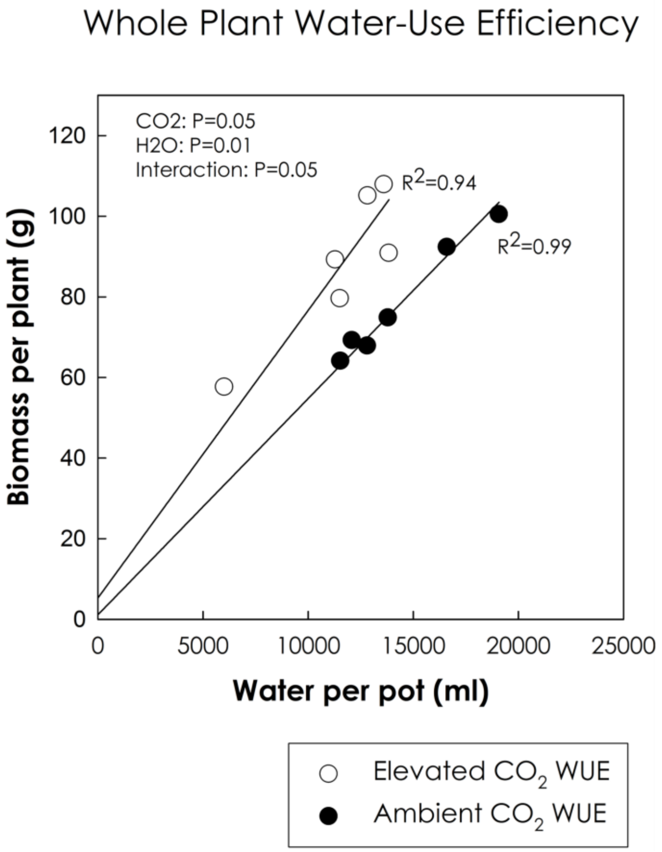


Figure 2-1: Non-drought whole plant water-use efficiency (WUE). Linear regression of relationship between water used and biomass grown per plant per pot for plants grown elevated (R^2=0.94) and ambient (R^2=0.99) chamber shows that water use is strong determinant of biomass gained. Analysis of covariance revealed an interaction between water-used and CO_2 (P=0.05) indicating that the slopes of the lines are different, which suggests that there is a significant improvement in WUE in the plants grown in enriched CO_2.

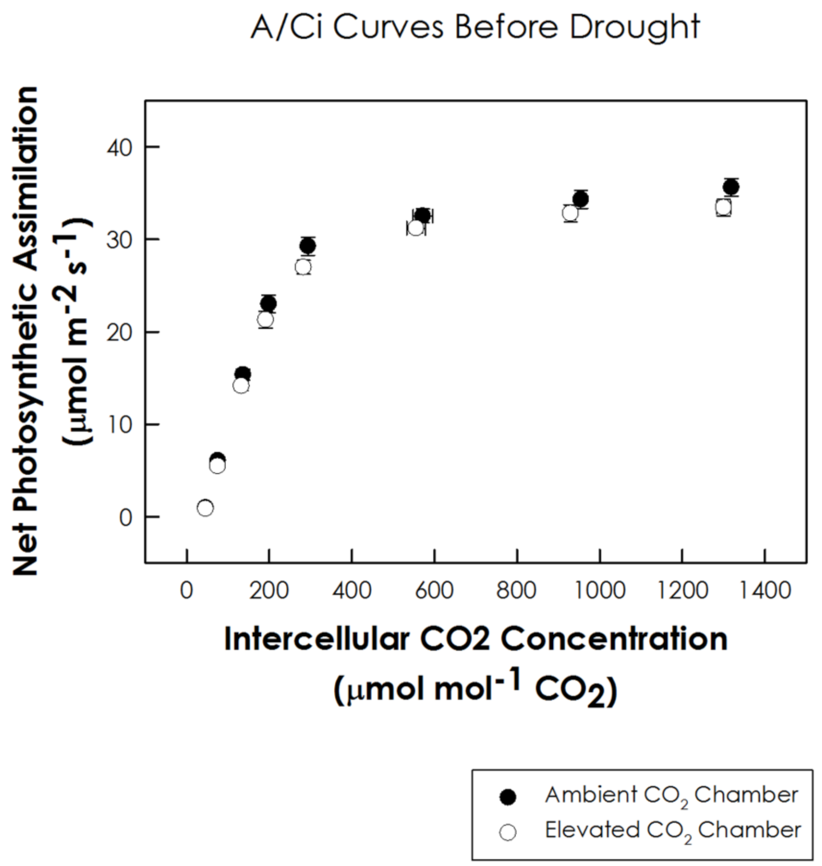


Figure 2-2: *A/Ci* curves recorded with the Li-6400 auto-program illustrates that pre-drought plants grown in ambient CO_2 chambers (n=9) had similar assimilation rates as plants grown in elevated CO_2 chambers (n=9). The lack of photosynthetic stimulation by plants in elevated CO_2 chambers suggests acclimatization responses to CO_2 environment.

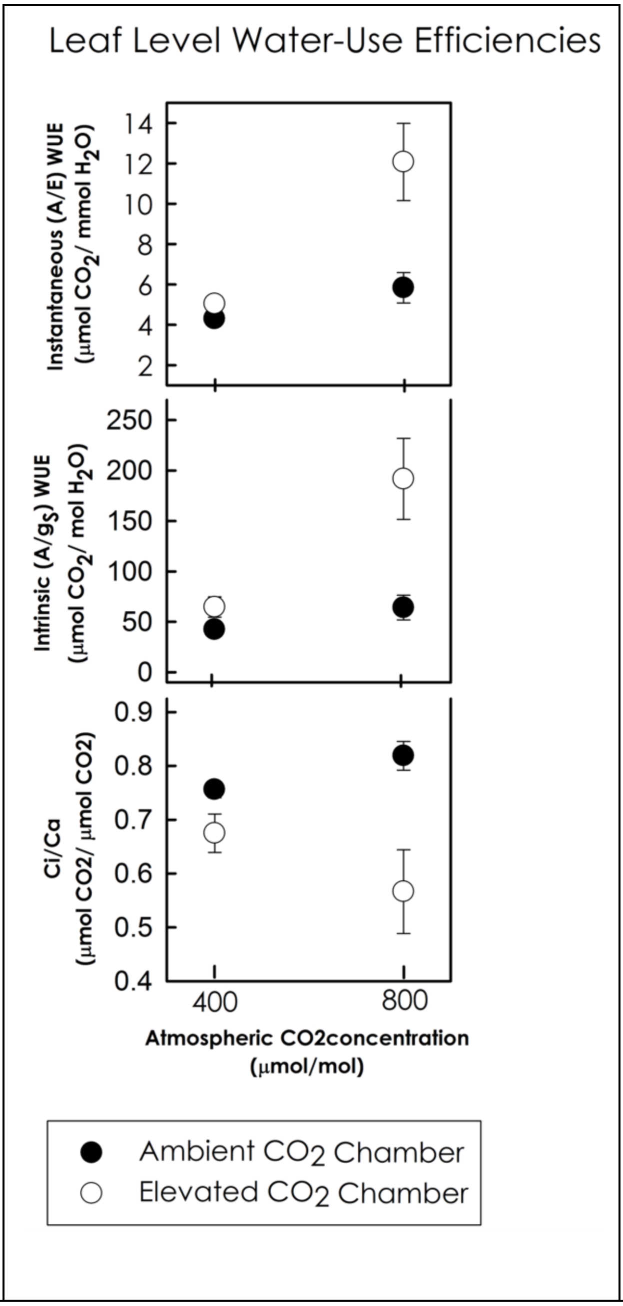


Figure 2-3: Each of the three sub-charts present data of the mean value (n=3) with SE error bars for both CO_2 treatments at CO_2 levels 400 mmol mol^{-1} and 800 mmol mol^{-1}, representing the growing conditions for both treatments. The leaf gas-exchange shows elevated intrinsic (*A/g_S*) and instantaneous (*A/E*) WUE for the plants grown in chambers enriched with CO_2; *Ci/Ca* ratios were lower for plants growing under elevated CO_2 conditions suggesting higher water-use efficiency. The effects of elevated CO_2 on water-use efficiency were most pronounced at the 800 mmol mol^{-1} CO2 treatment

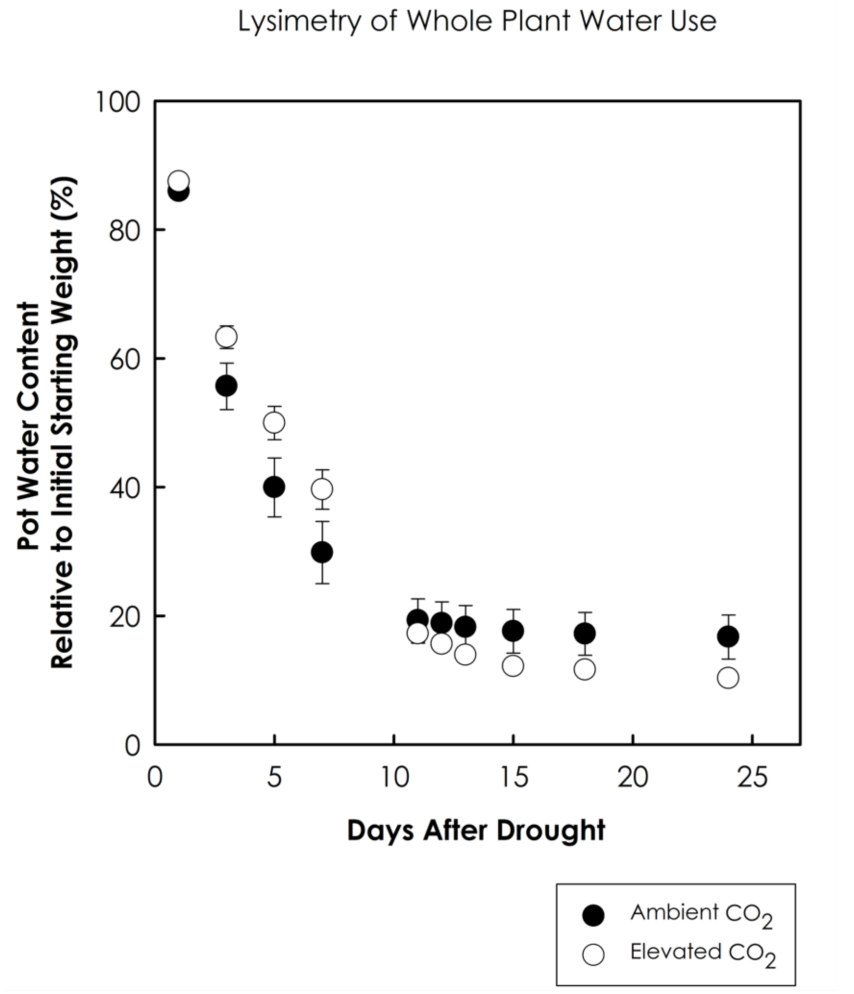


Figure 2-4: Pot weights represented as a proportion of the weight on the day of last watering. Values represent the mean relative weight for plants (n=8, SE error bars) grown in ambient and elevated CO_2 chambers, 400 mmol mol^{-1} and 800 mmol mol^{-1} respectively. Plants grown in elevated chambers use less water at first, as result of decreased stomatal conductance. Increased WUE by plants in elevated CO_2 allows these plants also to use greater total amount of water available, represented by the lower mean values after day ten.

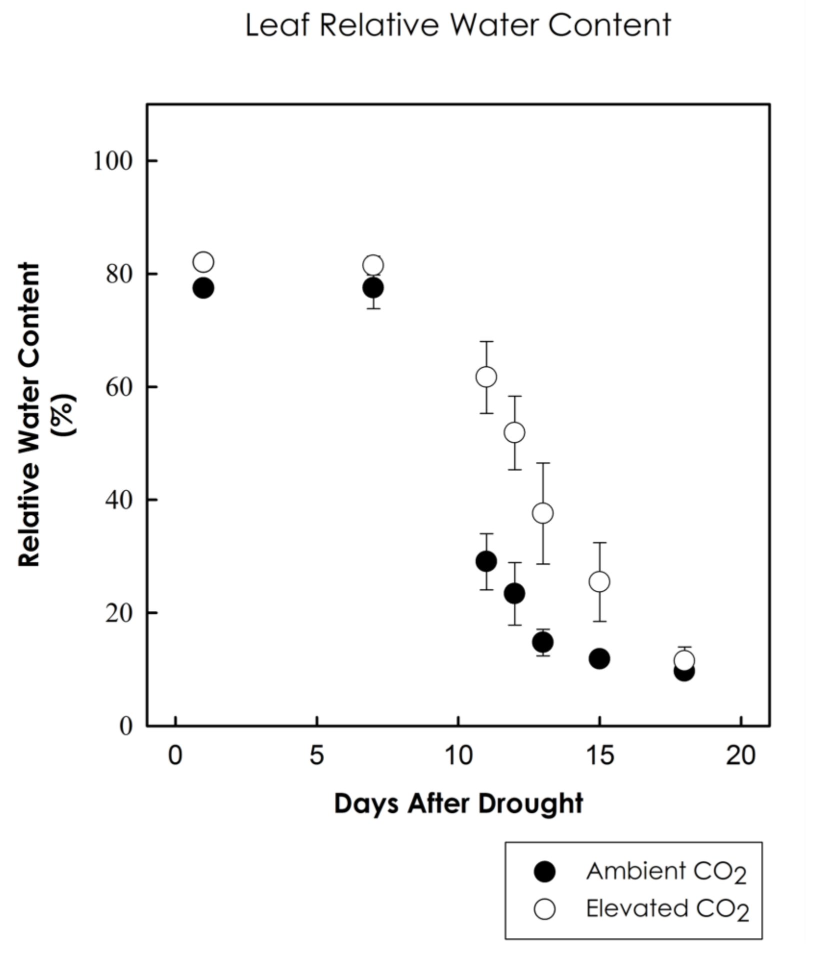


Figure 2-5: Mean values (n=8) with SE error bars representing leaf relative water content (RWC) as drought progressed. RWC is shown to be similar for the first week of the drought between the two CO_2 treatments, 400 mmol mol^{-1} and 800 mmol mol^{-1} for ambient and elevated respectively. After day seven the plants in the ambient chamber lose water content at a more rapid rate that the plants grown in the elevated CO_2 chamber. The difference between treatments is likely related to initially decreased stomatal conductance by the plants grown in elevated CO_2.

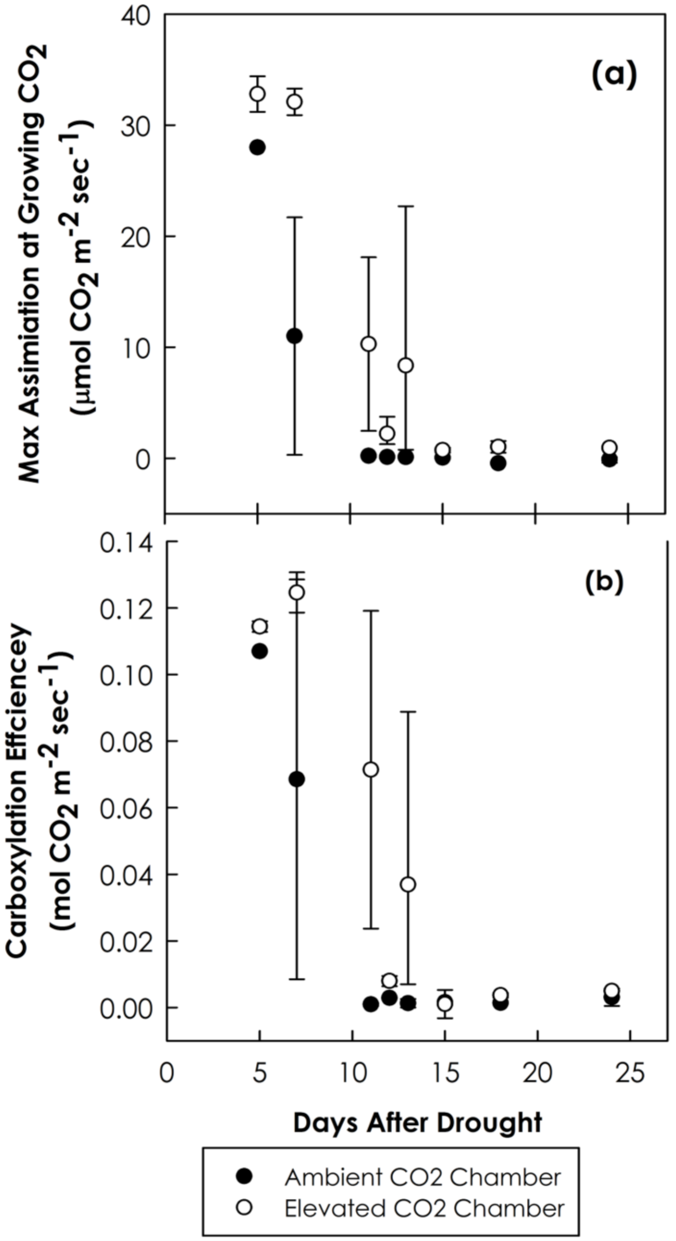


Figure 2-6: Leaf gas exchange responses from plants (n=2, error bars are max min) grown in ambient and elevated CO_2 chambers, 400 mmol mol^{-1} and 800 mmol mol^{-1} respectively. **2-6a** is the maximum assimilation (Amax) rates showing that drought more quickly decreases Amax for plants grown in ambient CO_2 compared with plants grown in elevated CO_2. **2-6b** is a plot of the carboxylation efficiency which is the increase in photosynthetic rate achieved per unit increase in CO_2at the site of CO_2 fixation. The values were quantified from the initial slopes of the *A/Ci* curves.

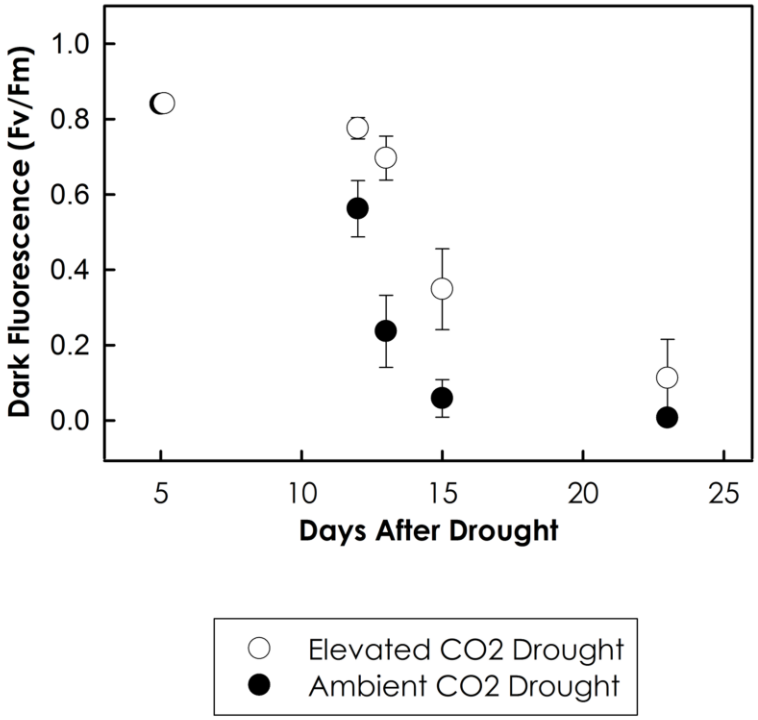


Figure 2-7: Mean values (n=8) with SE error bars of dark fluorescence ratios collected from both CO2 treatment groups, ambient 400 mmol mol^{-1} and elevated 800 mmol mol^{-1}. Plot illustrates the overall delay by both treatment groups, i.e. drought did not produce a decrease in fluorescence ratios until after day five. After that time the ambient CO_2 group was affected more rapidly by drought compared with the plants grown in the elevated CO_2 chamber.

Chapter III:

Growth and leaf physiological response to salt stress in *Arundo donax*

Abstract:

A. donax is promoted as is a promising bioenergy feed-stock continued commercial pressure from the bioenergy industry controversy about the ecological consequence of cropping *A. donax* remains. The contentious aspect of cropping *A. donax* relates to its long history of biological invasion in global riparian and estuarine ecosystems. One condition that will be of interest to both agronomists as well as ecologists is a quantification of *A. donax*'s ability to tolerate salt (NaCl) stress. Although the observations of *A. donax* growing in saline waters is a clear indicator that it has some halophytic tendencies, the relationship between growth and physiology would be better understood from an investigation of the response by *A. donax* to increasing soil salinity. Therefore, an experiment was conducted the University of Washington Botanic Gardens in Seattle, Washington, USA testing the effects of salt on growth and gas-exchange of a wild strain of *A. donax*. Biomass growth analysis revealed that net assimilation rate (NAR) was negatively correlated with increasing salinity levels and that NAR was positively correlated with relative growth rates (RGR). Additionally, salinity was shown to decrease specific leaf area (SLA), decreases in SLA were shown to decrease RGR. Leaf gas-exchange analysis identified stomatal conductance to decrease with increasing salinity. These decreases in conductance were shown to proportionally limit photosynthesis. Thus, while *A. donax* was able to persist and grow in salinized soil, this species growth was impaired even by lowest levels of salt.

Introduction:

Arundo donax is a rhizomatous, C_3, grass that has been grown and harvested by humans for thousands of years (Dudley, 2000b). Consequently it has naturalized around the world in environments it was transported for fodder, thatching or industrial purposes. *Arundo donax* has been declared an invasive plant pest or noxious weed by many governmental agencies because it aggressively spreads into and dominates in formerly indigenous plant communities outside of its natural range. In recent years the traits which favored its use by early civilizations, including exceptional biomass production on marginal land, has caught the interest of the nascent cellulosic ethanol industry. However, the development of *A. donax* as a feed-stock supply has been hindered by policies that prohibit the planting of invasive plants for bioenergy crops outside of their native range. Despite its classification as an invasive species *A. donax* continues to be a popular feedstock by some agronomists, because it provides a potentially productive revenue source. Although *A. donax* is promoted as is a promising bioenergy feed-stock (Lewandowski, Scurlock, Lindvall, & Christou, 2003) continued commercial pressure from the bioenergy industry (Electric, 2012) controversy about the ecological consequence of cropping *A. donax* remains (Groom, Gray, & Townsend, 2008).

The contentious aspect of cropping *A. donax* relates to its long history of biological invasion in global riparian and estuarine ecosystems. Bioenergy agronomists claim that this sterile species does not constitute an invasion risk, while ecologists point to the history of invasion as evidence that *A. donax* will naturalize in waterways outside of the agricultural fields. Surprisingly for a plant whose growth generates interest and controversy few studies have investigated how divergent environmental conditions will affect *A. donax*'s biomass production. One condition that will be of interest to both agronomists as well as ecologists is a quantification of *A. donax*'s ability to tolerate salt (NaCl) stress.

Soil salinity is a world-wide problem, it is estimated that >5% of the world's land and 30% of the world's irrigated areas already suffer from salinity problems (FAO, 2005). Alleviating soil salinity is typically untenable because it requires drenching with un-salinized water, and thus salinized lands have been traditionally left fallow. Yet rising demands for arable land is driving production into traditionally fallow fields. Moreover it has been predicted that expansion of agriculture to semi-arid and arid regions with the use of intensive irrigation will increase secondary salinization as a result of changes in the hydrologic balance of the soil between water applied and water used by crops (FAO, 2011). In addition, continuous sea-level rise in a warming world threatens increased salinity cultivated soils in coastal lowlands (Rozema & Flowers, 2008). Thus identifying salt tolerant crops or plants less impacted by salt will be an important task in agriculture if the demand for growing new energy crops can be satisfied. One of these new energy crops is *A. donax* which has some of the highest production rates found for any plants. Preliminary studies have shown that *A. donax* is able to be grown as a biomass crop under saline conditions (Williams, 2008). However these studies focused primarily on biomass growth and not on the physiological mechanisms related to the growth. Unlike agronomists who search for salt tolerant crops, ecologists are concerned about invasive species that are able to tolerate stresses greater than native plant communities.

In salinized ecosystems salt tolerance plays a key role in interspecific interactions and often dictates plant community assemblages. Clearly, an introducing a plant species with a greater capacity for salt tolerance than the native plant species would likely lead to a competitive advantage by the non-native species. This scenario has been documented for some salt tolerant invasive species (Daehler, 2003; Vasquez, Glenn, Guntenspergen, Brown, & Nelson, 2006; Zedler & Kercher, 2004) and is a potential scenario in regions in the western United States where management practices have curtailed floods over the past 30 years. Salt tolerance has been identified as a key reason why salt cedar, one of the prolific invasive trees in North America, has been able to dominate native plant communities (Glenn et al., 1998). *Arundo donax* currently invades similar habitats as salt cedar, e.g., riparian communities disturbed by agricultural manipulation of natural hydrology. Furthermore, on altered rivers in eastern Washington, cottonwoods and willow populations have decreased in response to increased water or salinity stress; mortality has increased during hot, dry summer periods of reduced streamflow, and lack of spring flooding has limited new cohort establishment (Wissmar, 2004). Anthropogenic changes to the riparian geophysical and biological structure have reduced native plant succession and will likely foster replacement by stress-tolerant species. Therefore *A. donax* may occupy expanded saline and xeric niches as the rivers become increasingly channelized, dammed and diverted. While the potential for *A. donax* to gain a competitive advantage from soil salinization is clear, the halophytic nature of *A. donax* remains unclear.

Presently, *A. donax* is not typically considered a halophyte because it primarily invades freshwater riparian habitats. However, the salt tolerance of this species is evident in the Californian and South African estuaries where it has established and persisted (Gary P. Bell, 1997; Rossa, et al., 1998). If sea levels continue to rise as predicted in many climate change scenarios (Rahmstorf, 2007) then *A. donax* already populating freshwater streams in coastal areas may have a distinct advantage over salt sensitive native plant communities. Although the observations of *A. donax* growing in saline waters is a clear indicator that it has some halophytic tendencies, the relationship between growth and physiology would be better understood from an investigation of the response by *A. donax* to increasing soil salinity. Therefore, an experiment was conducted the University of Washington Botanic Gardens in Seattle, Washington, USA testing the effects of salt on growth and gas-exchange of a wild strain of *A. donax*. The results of this study will have implications for both agronomists wishing to expand *A. donax* to fields dominated by saline soils, and ecologists are concerned about the concomitant spread of *A. donax* with altered stream hydrology and sea-level rise.

Materials and Methods

Research location

All of summarized research was conducted at the Douglas Research Conservatory (DRC), University of Washington, Seattle, Washington, USA.

Plant material

April 13, 2011, at the Douglas Research Conservatory (DRC), Seattle, WA, USA, cuttings were made from a potted *A. donax* plant that had been grown from rhizomes collected from *A. donax* growing wild in the San Francisco Bay Area, CA, USA. The cuttings were standardized by length (12 cm in length, ~1 cm in diameter) ensuring at least two stem nodes per cutting; and were planted, one cutting per pot, in sand filled Deepots™” (Model D27L: 0.45 liter, Stuewe and sons Inc., Tangent, Oregon, USA); and placed into a mist bench for germination. Seven weeks after planting the cuttings (June 1, 2011), 89 rooted cuttings were transferred into sand filled tree-pots (Model CP59R: 2.54 liter, Stuewe and sons Inc., Tangent, Oregon, USA), and moved onto a steel wire bench (5’ x 18’) in one of the DRC glasshouses. Daylight was supplemented in the glasshouse by high pressure sodium 400 watt single phase bulbs (Phillips Electronics North America Corp., Andover, Massachusetts, USA) during 0800 to 2200 hrs; and plants were watered once a week with 1/16 per gallon dilution of 17-17-17 (N-P-K) fertilizer. After seven weeks growth (July 21, 2011) with no experimental treatments, nine plants were randomly selected for destructive harvest to attain an initial pre-treatment biomass record.

Salt treatments

Soil salinity measurements made by solute electrical conductivity (EC) are classified into groups of saline soils by deci-Siemen/meter (dS m^{-1}): Non-Saline (<2 dS m^{-1}), Slightly Saline (2-4 dS m^{-1}), Moderately Saline (4-8 dS m^{-1}), Strongly Saline (8-16 dS m^{-1}), and Very Strongly Saline (>16 dS m^{-1}) (Rhoades, Chanduvi, Lesch, & Food and Agriculture Organization of the United Nations., 1999). Correspondingly, agricultural crops have been classified by their ability to grow and yield in saline soils. Crop classification are also based on soil solute EC: Sensitive (0-8 dS m^{-1}), Moderately Sensitive (8-16 dS m^{-1}), Moderately Tolerant (16-24 dS m^{-1}), Tolerant (24-32 dS m^{-1}), >32 dS m^{-1}causing unacceptable yields for most crops (Tanji, Kielen, & Food and Agriculture Organization of the United Nations., 2002). For this experiment, salinity was measured as soil solution conductivity in deci-Siemens per meter (dS m^{-1}). Therefore each of the ten blocks contained one of each of the eight salt treatments: non-saline (NS: 0-2 dS m^{-1}), slightly saline (SlS: 2-6 dS m^{-1}), moderately saline (MS: 6-10 dS m^{-1}), strongly saline (StS: 14-18 dS m^{-1}), very strongly saline$_1$ (VS1: 18-22 dS m^{-1}), very strongly saline$_2$ (VS2: 24-28 dS m^{-1}), very strongly saline$_3$ (VS3: 28-32 dS m^{-1}), very strongly saline$_4$ (VS4: 38-42 dS m^{-1}). Salinity was supplied as sodium chloride halite(CAS #: 7647-14-5) in solution. The sodium chloride crystals were dissolved in a tank containing 80 liters of ½-strength Hoaglands #2 mix (Epstein & Bloom, 2005). Adjacent to this salt/Hoaglands mix was another tank containing 80 liters of ½-strength Hoaglands #2 mix. The NS treatment received only the ½ strength Hoaglands with no additional salt. All other treatments received a combination of two solutions (salt/Hoaglands; regular Hoaglands) in proportions that matched the appropriate dS $m^{-1}$of the each salinity treatment. Treatment solutions were added to the five liter buckets when needed to prevent soil desiccation. Furthermore, the five liter buckets were emptied of leachate and refreshed weekly to prevent residual salt accumulation. As part of the treatment quality assurance, leachate conductivity and pH was tested before and after each solution application. Salt treatments were initiated on July 21, 2011 and terminated after two months (September 19, 2011).

Data collection

Leaf gas exchange

Physiological data was assessed with a leaf chlorophyll meter (SPAD-502, Konica Minolta, Ramsey, New Jersey, USA); a leaf chlorophyll fluorescence and gas exchange was measured with an infra-red gas analyzer (IRGA) with a leaf chamber fluorometer (LI-6400-40; LI-COR, Inc., Lincoln, Nebraska, USA). SPAD values were collected weekly, and gas-exchange measurements were initiated after three weeks of the salt-treatment when the visual differences in plant height were evident. The IRGA was used to record gas exchange during the morning hours (0700 hrs –1200 hrs) on young, fully expanded leaves.

The first sets of chlorophyll fluorescence and gas exchange measurements were the instantaneous (spot) measurements, in which the IRGA was set to mimic ambient conditions: 400 µmol mol^{-1} CO_2, light as photosynthetic photon flux density (PPFD) at 1500 µmol m^{-2} sec^{-1}, the flow rate at 300 µmol s^{-1}, and temperatures were kept at 25^o C. These readings were recorded on three different sampling dates (August 12, 25 and 26, 2011) for all plants and all treatment groups. For the spot measurements, the time allowed for the leaf to acclimate to the IRGA chamber was a minimum of five minutes and maximum of ten minutes.

The second chlorophyll fluorescence and gas exchange measurements involved using the using the auto-program 'fluorescence light curve (*A/Q*)' to quantify the leaf photosynthetic responses to changing light regimes for three plants from the NS and the VS_4 groups. The *A/Q* auto-program was set at a flow rate 300 µmol s^{-1}, temperature set at 25^o C, CO_2 levels at 400 µmol mol^{-1} CO_2; with variable light levels at 10 different levels increasing, in distinct steps, between 50 and 2500 µmol m^{-2} s^{-1}. These measurements were recorded on August 23 and 24, 2011. The time allowed for the leaf to acclimate to for the *A/Q* curve recordings was set to vary from 15-30 minutes.

Additionally, between August 29 and September 1, the auto-program 'fluorescence A/C_i curve' photosynthetic-CO_2 response (A/C_i) curve was used to monitor the photosynthetic responses to changing CO_2 for five plants from the NS group and five plants from the VS_4 groups. The A/C_i auto-program was set at flow rate of 300 µmol s^{-1}, a temperature at 25^o C, light levels at 1500 µmol m^{-2} s^{-1}; with CO_2 values at eight different levels that were programmed to increase from 0 to 1500 µmol mol^{-1}. Additional, "Low-light" A/C_i were created from September 8-10, 2011 to be used to estimate dark respiration (Rd) and CO_2 compensation point (Γ*) for three plants from the NS and three from the VS_4 groups. Again the A/C_i auto-program was used, however three curves were made for each plant at three different light levels (500 µmol m^{-2} s^{-1}, 120 µmol m^{-2} s^{-1}, and 30 µmol m^{-2} s^{-1}). The time allowed for the leaf to acclimate to for the A/C_i curve recordings was set to vary from 15-30 minutes.

On September 16, 2011, photosynthetic induction curves were created using the IRGA for all of the treatments and control. These measurements recorded photosynthetic response of four plants from the control group and four from the VS_4 treatment groups. Measurements were recorded from transitioned from total darkness to bright light (PPFD 1500 µmol m^{-2} s^{-1}). In this case, leaves that had been dark adapted for a minimum of two hours were exposed to light in the IRGA chamber, and the photosynthetic responses were recorded every 10 seconds for one hour.

Growth analysis

On September 19th, the 60th day after the treatments were initiated, all plants were deconstructed by plant organ (leaf, stem, and root) as part of a classical growth analysis. Classical growth analysis is an explanatory, holistic and integrative approach to interpreting plant form and function (Hunt, Causton, Shipley, & Askew, 2002). The leaves were scanned with a leaf-area meter (LI-3000; LI-COR, Inc., Lincoln, Nebraska, USA) and then placed in paper bags. The stems and roots were also bagged separately, and all the plant parts were dried for 48 hours in a forced air oven heated to 80^{o} C. The oven-dry biomass was weighed for relative growth calculations. The relative growth rate (RGR), and its components net assimilation rate (NAR), specific leaf area (SLA), and leaf weight fractions (LWF) where calculated as the means of the natural logarithm-transformed plant weights.

Experimental design and statistical analysis

The 80 pots were set in five liter buckets, and placed in a randomized complete block design. Randomization was performed with a computer generated algorithm for random numbers (random.org). Blocking was used to account for potential thermal and solar gradients along the 5.5m bench. A row of eight pots a row of eight pots perpendicular to the length of the bench constituted one block. There were ten blocks total with one replicate from each treatment group being assigned to each of ten blocks. The experiment represents a completely balanced, blocked, single factor with salt concentration as electrical conductivity of the fertilizer solution as the main effect. Therefore analysis of variance (ANOVA) was appropriately used to quantify the influence of the random effect of the blocks, the fixed effects of the salt treatment, and the interaction between blocks and salt. Additionally, Tukey's Honest Significant Difference tests were used for pair-wise testing between treatment group biomass responses. Furthermore, linear regressions were also used to compare the treatment effect for both growth and gas-exchange responses. Lastly, analysis of covariance (ANCOVA) was used to compare the heterogeneity of the curves generated by the gas-exchange *A/Ci* and *A/Q* auto-programs. The ANCOVA was used to determine if either CO_2 concentration (for *A/Ci*) or light-level (for *A/Q*) interacted as a covariate with the main effect (the salinity treatments) to influence the assimilation rates. All data analyses were calculated using R 2.12 statistical software (Team, 2011)and SigmaPlot 12.2 (Systat software, INC., San Jose, CA, USA).

Results:

Biomass

Increasing salinity decreased the biomass growth of *A. donax*, but there was no mortality recorded even at the highest (40 dS m^{-1}) saline levels. Plants in all groups and treatments had little change in the chlorophyll content of the leaves, i.e., SPAD values for all treatments were greater than 42.5 (SPAD units) (Table 1) which indicates chlorophyll A degradation (Spencer, et al., 2008) was not evidenced at the leaf level. The raw biomass values from the deconstructive harvests were natural-log (*ln*) transformed to stabilize variance and linearize the results (Hoffmann & Poorter, 2002; Hunt, et al., 2002). The *ln* transformed growth biomass will be hereafter simply referred to as 'biomass'. The growth inhibition with increasing salinity are clearly shown by the negative linear relationship (R^2=0.92) between mean biomass and the electrical conductivity of soil solution extract (Figure 3-1) (SE error bars; n=10). An analysis of variance (ANOVA) supported these results with a highly significant ($p < 0.001$) relationship between salinity and biomass (Table 2); and a highly significant ($p < 0.001$) relationship between experimental blocks. However, since there was not a significant block and salinity treatment interaction ($p > 0.99$) the two factors were considered independently. A Tukey's Honestly Significant Difference test (Tukey test) was used to compare differences between mean biomass of the salinity treatment groups (Table 3-2). The Tukey tests show that there were no significant differences in biomass between consecutive salt treatment groups for six out of the eight treatment levels. The two consecutive group pairings that did show significant differences in biomass were separated by an electrical conductivity ≥ 8 dS m^{-1} (MS-StS and VS2-VS3), whereas, the majority of consecutive group pairings (*e.g.* NS-SlS and SlS-MS *etc.*) were separated only by 4 dS m^{-1}. Yet eight dS m^{-1} cannot be cited as the deciding threshold because two groups that were also ≥8 dS m^{-1} apart (StS -VS2 and VS3-VS4) did not exhibit significantly different mean biomass values: p-values >0.08 and >0.052, respectively. Classical growth analysis was used to further understand the role of salt plays in biomass production.

The two-harvest classical growth analysis method was used to decompose total biomass by component parts to analyze relative growth rates across the treatments. Plant relative growth rate (RGR) is the rate of increase in a plant mass per unit of plant mass already present (Evans, 1972). Linear regression of RGR as a function of EC (Figure 3-2) reveals a strongly negative relationship, with RGR decreasing as EC increases. Further, RGR is a factor of two components: net assimilation rate (NAR) and leaf area ratio (LAR). NAR represents the grams biomass per cm^2 of leaf area per day; whereas LAR explains the amount of leaf area per total mass. LAR can be further subdivided as a factor of two components: specific leaf area (SLA) and leaf mass ratio (LMR). SLA accounts for the amount of leaf area per leaf mass; and LMR explains leaf mass per total plant mass. Regression analysis of influence salinity played on each of these components (Figures 3-3a-d) showed that salinity most strongly correlated with changes in NAR (R^2=0.98) (Fig. 3-3a) and SLA (R^2=0.87) (Fig. 3-3c), but was weakly correlated to LAR (R^2=0.46) (Fig. 3-3b) and not linearly related to LMR (R^2=0.02) (Fig.3c). Thus, if RGR is a product of NAR*LAR, salinity growth inhibition was primarily related decreases in NAR (physiological capacity to gain carbon per unit leaf area) and to a lesser extent decreases in LAR (morphological plasticity to allocate gained carbon to photosynthetic organs for light capture). Analysis of biomass presents holistic results of the summed metabolic processes required for plant growth. Contrarily infrared gas-exchange analysis investigates a much smaller, albeit crucial, subset of plant growth: the diffusion of carbon and water vapor into and out of plant leaves.

Leaf gas-exchange

A/Q

The light response curve *(A/Q)* (Figure 3-4) suggests a difference in overall photosynthetic capacity at saturating light conditions between the NS and VS_4. The VS_4 group max assimilation rate (A_{max}) of 10.7 ± 1 µmols m^{-2} sec^{-1} was reached when light levels were only 700 PPFD; A_{max} began to decrease at the highest light levels. On the other hand, the NS treatment group A_{max} 31.73 ± 1.54 µmols m^{-2} sec^{-1} was not attained until the maximum light level (2000 PPFD) (Table 3). Further evidence suggesting photosynthetic inhibition was evidenced by the divergent quantum yield (Φ), evidenced by the initial slope, which like A_{max} were also different between the two treatment groups. An analysis of covariance (ANCOVA) was used to determine the heterogeneity of Φ for NS and VS_4; the summary showed a significant relationship for the interaction between light (PPFD) and salinity (EC) (p–value <0.05). This suggests that Φ is affected by salt. The dark respiration (Rd) recorded by the LI-COR at PPFD 0 was -2.05 and -0.238 µmols m^{-2} sec^{-1} for the NS and VS4 treatments, respectively (Table 3).

A/C$_i$

Like the *A/Q* plot, the *A/C$_i$* curve (Figure 3-5) shows a distinct separation in photosynthetic assimilation for the NS and VS_4 groups at a variety of CO_2 levels. First the difference is apparent in the carboxylation efficiency represented by the initial slope of the *A/Ci* curve. An ANCOVA was used again to determine the heterogeneity of carboxylation efficiency for NS and VS_4 *A/Ci*; the summary showed a strong interaction between Ci and salinity (EC) (p-value <0.001). The reduced initial slope of the *A/Ci* curve for the VS_4 treatment group suggests that CO_2 supplies are limiting the rate of functioning of Rubisco. From the *A/Ci* plot (Figure 3-4) the maximum assimilation rates (A_{max}) at saturating CO_2 levels also differ between the two treatment groups: 47.94 µmols m^{-2} sec^{-1} and 28.86 µmols m^{-2} sec^{-1} for NS and VS_4 groups (Table 3-3). Additionally *A/C$_i$* curves were completed at three sub-saturating light levels (Figure 3-6) and were used to determine Γ* the CO_2 compensation point which is the CO_2 concentration where photorespiration (oxygenation of RuBP and subsequent metabolism) produces as much CO_2 as is consumed by photosynthesis (carboxylation of RuBP). The Γ* values were 24.61 µmol mol^{-1} of CO_2 and 30.5 µmol mol^{-1} of CO_2 for the NS and VS_4 groups, respectively (Table 3-3).

Spot measurements of A_{max}

While A/C_i and A/Q curves can be used to determine photosynthetic limitations related to CO_2 supply and demand, spot measurements provide information about the plants' photosynthetic behavior under typical growing conditions. Spot measurements of A_{max} are also a useful metric because the decreased time to collect each data point allows for measurements across all treatment groups rather than just the two extreme groups (NS and VS_4). A plot of the net photosynthetic assimilation of the spot measurements (Figure 3-7a) show negative relationships between photosynthesis and salinity. The linear regression of the mean net assimilation rates as a function of EC exhibits the similar negative relationship (R^2=0.99) that were illustrated by biomass and NAR with EC (Figures 1 and 3b, respectively). Additionally, the net assimilation spot measurements for the NS and SlS treatment groups were on average more than twice the assimilation rates recorded for the VS_3 or VS_4 groups. The light adapted variable fluorescence ratio (Fv'/Fm')—a proxy measurement for apparent PSII efficiency— is another metric yielded from the spot measurements. The Fv'/Fm' ratio plot also illustrates an inverse linear relationship to increasing salt (R^2=0.94) (Figure 3-7b). Interestingly however, the effects of salt do not seem to be differentiated in the Fv'/Fm' between in the lower salt groups as much as in net assimilation by the same groups. A Tukey test of net assimilation and Fv'/Fm' detected no statistically significant difference between any of the low EC groups (NS, SlS, and MS) (Table 3-4). However, the order of magnitude difference between p-values for NS-MS photo and Fv'/Fm' are an indication that net assimilation was more strongly affected than Fv'/Fm' at the lower salt concentrations. It is known that salt stress can cause osmotic imbalances that mimic water stress and trigger stomatal closure; therefore it was important to investigate the role of stomatal limitations in photosynthesis.

By plotting stomatal conductance against net assimilation (Figure 3-8a) and then against salinity concentration the patterns show that increasing net assimilation is exponentially proportional to stomatal conductance while the decay of stomatal conductance is exponentially proportional to increasing salinity (Figure 3-8b). Correlation between stomatal conductance and photosynthetic inhibition at higher salt levels are evident in the comparison of induction curves for the NS (n=3) and VS_4 (n=4) groups (Figure 3-9). The relationships between stomatal conductance and net assimilation are expressed by the similar timing of A with g_S. In figure 9b A saturates for VS_4

between minutes 10-20 corresponding with the timing of the greatest conductance rate for VS_4 (Figure 3-9a). Similarly, the NS group attains greatest conductance around minute 20, the timing of which corresponds with A_{max} for this treatment group. Additionally, the rate of change also differs between the NS and VS_4 groups. This is evidenced by the rapid increase in gS in the NS group, i.e., in less than a minute conductance is >0.2 mol H2O m-2 s-1, in contrast the VS_4 group which never reached gS values as high as recorded for the NS group. For the NS group, the slopes for both gS and *A* are steeper than the gradual slopes detected for the VS_4 group. An ANCOVA revealed highly significant interactions (p-value <0.001) between EC and time for both the net assimilation and stomatal conductance, confirming that slopes are different and thus that EC affected photosynthetic induction.

Discussion

The focus of this research was to increase our understanding of the effect of salinity on the growth and productivity of *A. donax* since this grass is considered to be one of the most promising plants to grow for biofuel feedstock production because of its high growth rates and biomass production. There are two primary mechanisms by which high salt levels stress plants: (i) water stress is imposed by the increase in osmotic potential of the rooting medium as a result of high solute content, and (ii) high concentration of nonessential ions can be toxic to plants and reduce their CO_2 uptake due to diffusion limitations or cause oxidative stress (Chaves, Flexas, & Pinheiro, 2009). Since extensive planting of this grass is being proposed on former agricultural lands that are now highly degraded and where agricultural crops are uneconomical to grow, it is critical to understand the implications of extending the planting of this grass into areas that it is not naturally found. Considering that this grass has been classified as an invasive species, prior to expanding the area planted in *A. donax*, it is critical to better understand the ecophysiological basis of dominance in habitats that is beyond its natural range. Despite the interest in increasing the planting of *A. donax* as a biofuels feedstock, few ecophysiological studies have investigated how divergent environmental conditions affect its biomass production and whether the higher salinity levels found in the degraded areas will alter its growth rates and its ability to outcompete native plants. Anecdotal evidence has suggested that *A. donax* growth rates would not be impacted by saline soils. This research contributes to the literature that supports the salt tolerance of *A. donax* but also suggests that higher saline conditions will decrease its biomass production.

A comparison of the relative growth of *A. donax* with literature values for other species (Table 3-5) reveals that *A. donax* is more salt tolerant than most native species it co-habitates with; and also more salt tolerant than some biomass crops. Compared to other riparian plant species that may co-occur with *A. donax*, all these plants experiences initial yield declines at with very small increases in salinity. Yet, unlike the native cottonwood, willow and *Baccharis* species—all which experience greater that 10% decrease in growth increase per dS m^{-1} increase in salinity—*A. donax* decrease in growth is only 2%. Compared with the notoriously salt tolerant invasive species salt cedar, *A. donax* is less tolerant of salt. Salt tolerance is a function of the relative growth at increasing salinity levels. For *A. donax* the relative growth is most limited by NAR. Although NAR has not been correlated with RGR in studies of unstressed plants (Poorter & Remkes, 1990), other salinity studies has shown it to be correlated with RGR (Ruiz, Martinez, & Cerda, 1997). Since NAR is related to the balance of carbon gain in photosynthesis and carbon use in whole-plant respiration, variations in NAR are therefore due to reduced photosynthesis, increased respiration or a combination of both (Lambers, Chapin, & Pons, 2008). The gas exchange results give an indication that reduced photosynthesis was the primary component influencing NAR (and growth) and also a key strategy that *A. donax* used to tolerate the high soil salinity. While both NAR and LAR responded to salt stress, LAR was less strongly related to RGR despite a strong response of SLA to salt. This response however was masked by a quadratic response of LMR when combined in LAR. Studies have shown that salinity stress can reduce expansive growth and leaf area development which may result in the alteration of the morphological features of the plants (P. Curtis & Lauchli, 1986; R Munns & Termaat, 1986). Reduction of SLA can be understood as a strategy for the plant to maintain fitness under environmental stress. By decreasing SLA the plant may be attempting to increase WUE because thicker leaves have a higher density of chlorophyll and proteins per unit leaf area; minimizing leaf area reduces overall stomata thus maximizing photosynthesis while minimizing transpiration.

Salinity tolerance and A. donax growth rates

Plant tolerance to salinity can be evaluated in one of three ways: the ability of a plant to survive on saline soils, the absolute plant growth (or yield), and the relative growth (or yield) on saline soil compared to growth (or yield) on non-saline soils (Maas, 1986). The results from this experiment provide information that heretofore was unavailable for *A. donax* regarding all three metrics, and the potential mechanisms limiting growth.

First, the results confirm anecdotal evidence of *A. donax's* ability to survive on saline soils. The biomass growth of *A. donax* (Figure 3-1) suggests that this grass is able to survive in saline soils. The research also suggests that there is a threshold above which the *A. donax* growth rates is reduced but not where mortality starts to occur. The VS_4 treatment group plants persisted albeit with limited growth at salinity concentrations (EC 40 dS m^{-1}) equivalent to two-thirds the concentration found in ocean water (EC~60 dS m^{-1}). Survival in saline soils supports claims made by ecologists that *A. donax* may replace salt intolerant natives as freshwater systems become more saline from hydrological alterations or sea level rise. Conversely, the ability to tolerate saline soils supports agronomists hopes that *A. donax* can be cropped in soils salinized naturally or from poor irrigation management. Yet, survival alone is a limited metric considering that growth under saline conditions is the more important for both ecologists and agronomists since the consequences of growth are relevant for ecological competition or harvest economics. As previously stated growth responses to salt can be considered in two ways: relative growth and absolute growth.

Absolute growth can be limited in its utility because it may not present a spatially or temporally comparable result. For instance, in this study conditions (other than soil salinity) were optimized for plant growth. The absolute growth values reported reflect these conditions and may not be indicative of agricultural fields or unmanaged estuaries where *A. donax* is often found growing. Alternatively, relative yields are a translatable value. Relative yield is expressed as a fraction of the yield obtained under non-saline conditions. On a relative basis one can compare plant growth within species or between species at different locations. Understanding the relative salt tolerance of *A. donax* compared with other native, invasive, and agricultural plant species along salinity gradients will be the key for managing *A. donax* invasions or plantations.

Physiological strategies for salt tolerance

Net photosynthetic assimilation rates (*A*) are a proxies for growth. All of the measurements of *A* at different light and CO_2 levels (Figures 3-4—3-9) show that *A* was significantly reduced by increasing salinity. Significant reductions in photosynthesis were strongly correlated with stomatal conductance (Figures 3-8, 3-9).

Photosynthetic induction results (Figure 3-9) are responses to (i) changes in cell metabolism involving the light activation of enzymes, particularly ribulose-1, 5-bisphosphte carboxylase (Rubisco) and(ii) increase in stomatal conductance (gs) (Allen & Pearcy, 2000). The induction curves revealed an early asymptote in stomatal conductance in the VS_4 corresponding with the asymptote in *A* which is a strong indication limitation of photosynthesis was primarily stomatal. Stomatal closure in response to increasing salinity is a very typical response (Hale, Orcutt, & Thompson, 1987). Stomata close in response to root generated chemical signals, such as abscisic acid, that are transferred from root through the shoot to the stomata in the leaves (R. Munns, 2002). When stomata close the supply of CO_2 to Rubisco is impaired causing increased energy dissipation through fluorescence and down-regulation of photosynthesis. Stomatal closure may serve multiple purposes for *A. donax*. The stomatal closure may be in part a form of osmotic regulation causing a buildup of carbohydrates, amino acids, or organic acids in the leaves. Osmatic balance is achieved by accumulating non-inhibiting solutes, known as compatible osmolytes, in the cytoplasm to maintain turgor as the soil water potential becomes more negative. Osmolytes are synthesized from basic metabolism of amino acids (Hasegawa, Bressan, Zhu, & Bohnert, 2000). Conversely, as a protective measure stomatal regulation is an important mechanism for reducing the Na^+ ions delivered to the leaves via transpiration. Since the transport of salt occurs mainly via the transpiration stream, regulating of transpiration prevents the entry of salt and is a form of salt exclusion.

While there are a variety of species-specific responses to salt there are two general categories of salt tolerance known for grasses: exclusion and compartmentalization (Flowers, Troke, & Yeo, 1977). Salinity levels greater than 10dS m^{-1} appear to severely inhibit many enzymes (including photosynthetic enzymes such as Rubisco) when exclusion or compartmentalization processes do not exist or are insufficient (R. Munns, James, & Lauchli, 2006). In this research, degradation of enzymes of photosynthetic enzymes was not evident from SPAD values (Table 1). Considering that SPAD values have been strongly correlated with chlorophyll A content in *A. donax* (Spencer, et al., 2008) the results indicate that reduced CO2 assimilation rates were not caused by reduction in chlorophyll A content. Additionally, although the chlorophyll A fluorescence values, Fv'/Fm', (Figure 7b) do show a strong relative decrease in response to salinity, indicating an increase in energy dissipation through fluorescence, the absolute values for Fv'/Fm'are not indicative of a plant under great stress (*i.e.* values from the drought stress fluorescence). Other studies of salt stress graminoid crops have also shown a relative decrease in quantum efficiency to salinity while maintaining high absolute values (Abdeshahian, Nabipour, & Meskarbashee, 2010). For less salt tolerant rice species chlorophyll A and quantum efficiency has declined with increasing salinity (Moradi & Ismail, 2007).

Although grasses are characterized as either excluders or accumulators even the accumulators exclude 95% of the Na+, the key distinction between the groups is that the accumulators have efficient mechanisms to sequester Na+ in cell vacuoles and excrete excess NaCl onto leaf surfaces(Vasquez, et al., 2006). During this experiment salt excretions were not found on the leaf surfaces. Additionally, if leaf vacuoles were expanding to concentrate greater amounts of solutes in the higher salinity groups this mechanism should be evidenced by relatively greater leaf masses in the higher salinity groups. Such a relationship was not found in leaf mass ratio. It has yet to be determined if *A. donax* was accumulating excess salts in other organ vacuoles or other tissues. Without conclusive evidence of accumulation I hypothesize that *A. donax* is salt excluder rather than an accumulator. Future studies on leaf anatomy and internal structure should be conducted to validate or repudiate my salt exclusion hypothesis. The mechanism for exclusion is likely related to the low stomata conductance associated with increasing salinity.

Stomatal mediated exclusion via reduced transpiration may not confer the same degree of salt tolerances as other halophytic invasive species (*i.e. Tamarisk spp.* or *Spartina alterniflora*) that are able to accumulate sodium ions in their shoots and excrete excess salts through salt glands onto the leaf surfaces. Thus, at a competitive level within either estuarine or salinized southwestern riparian corridors *A. donax* may be outcompeted by other more salt tolerant invasive species. Alternatively, comparisons with literature values for native species found in the Western riparian systems shows that *A. donax* does have greater salt tolerance that native species and thus may out compete them. Moreover, *A. donax* has greater salt tolerance than many agricultural crops, thus in salinized regions where this species is indigenous it may be a suitable biomass feedstock.

Although this study reveals a lot about the growth and photosynthetic responses to drought many questions remain regarding the mechanisms regulating salt tolerance. Much recent work has shown that alternations in the expression of a multitude of genes are directly related to the mechanisms for controlling salt tolerance (Zhu, 2001). Therefore additional work should investigate the genetic mechanism *A. donax* for its salt tolerance. Correspondingly, additional work into the anatomy and morphology of this species could reveal more about the exclusion/accumulation strategy. Lastly, although growth and yield declined during this experiment, mortality did not occur. Therefore future studies should have higher salt levels, equal to or greater than sea-water (60 EC dS m^{-1}) and should be conducted for longer periods of time.

Figures

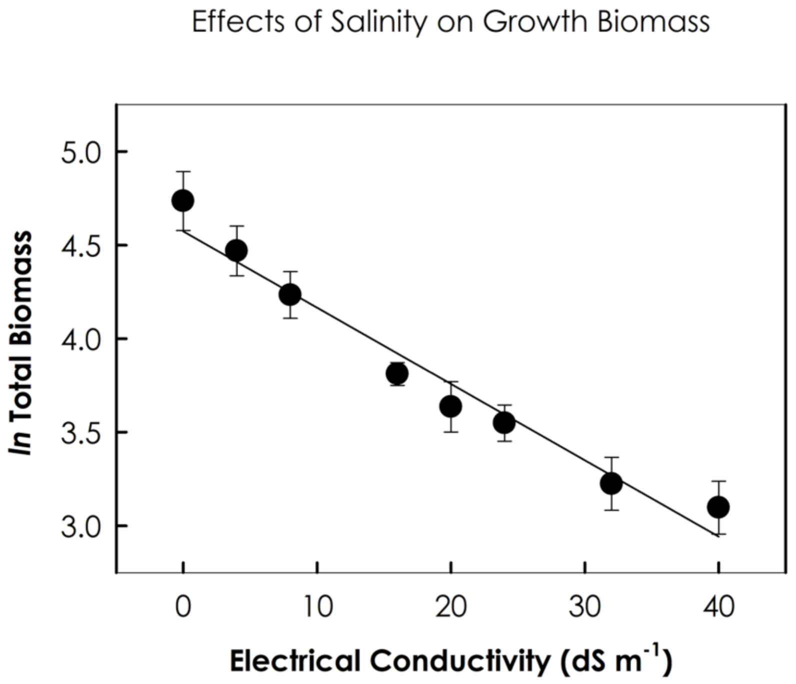


Figure 3-1: Natural log transformed mean biomass per plant (n=10, SE error bars). Linear regression (R^2=0.92) indicates a strong negative correlation between increasing salinity (NaCl measured in dS m^{-1}) and biomass accumulation.

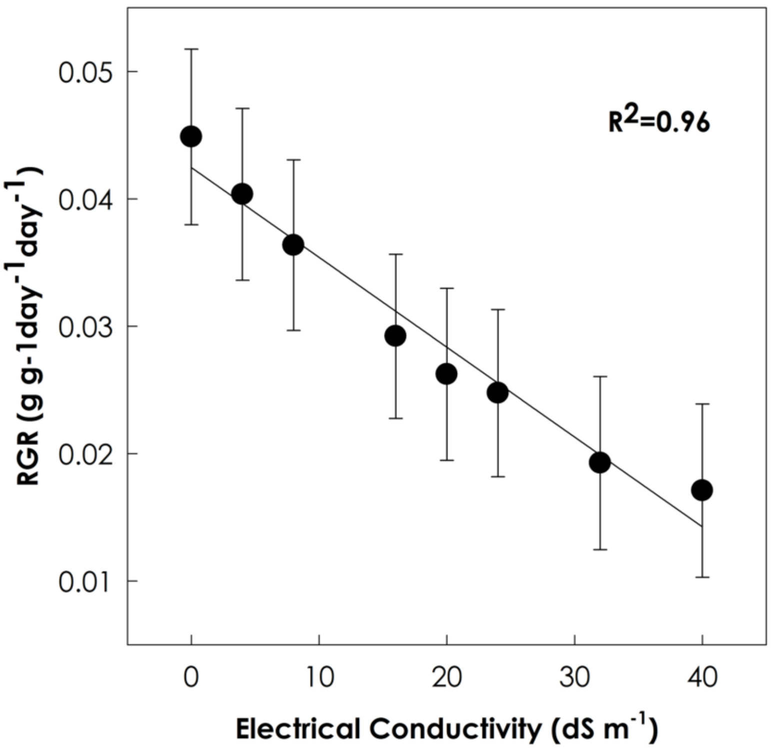


Figure 3-2: Linear regression of mean relative growth rates (n=10, SE error bars) illustrates strong negative relationship between increasing salinity (measured as electrical conductivity of pot leachate) and growth rates. Greatest growth rates were for plants grown without NaCl and lowest growth rates for plants grown with greatest NaCl concentrations.

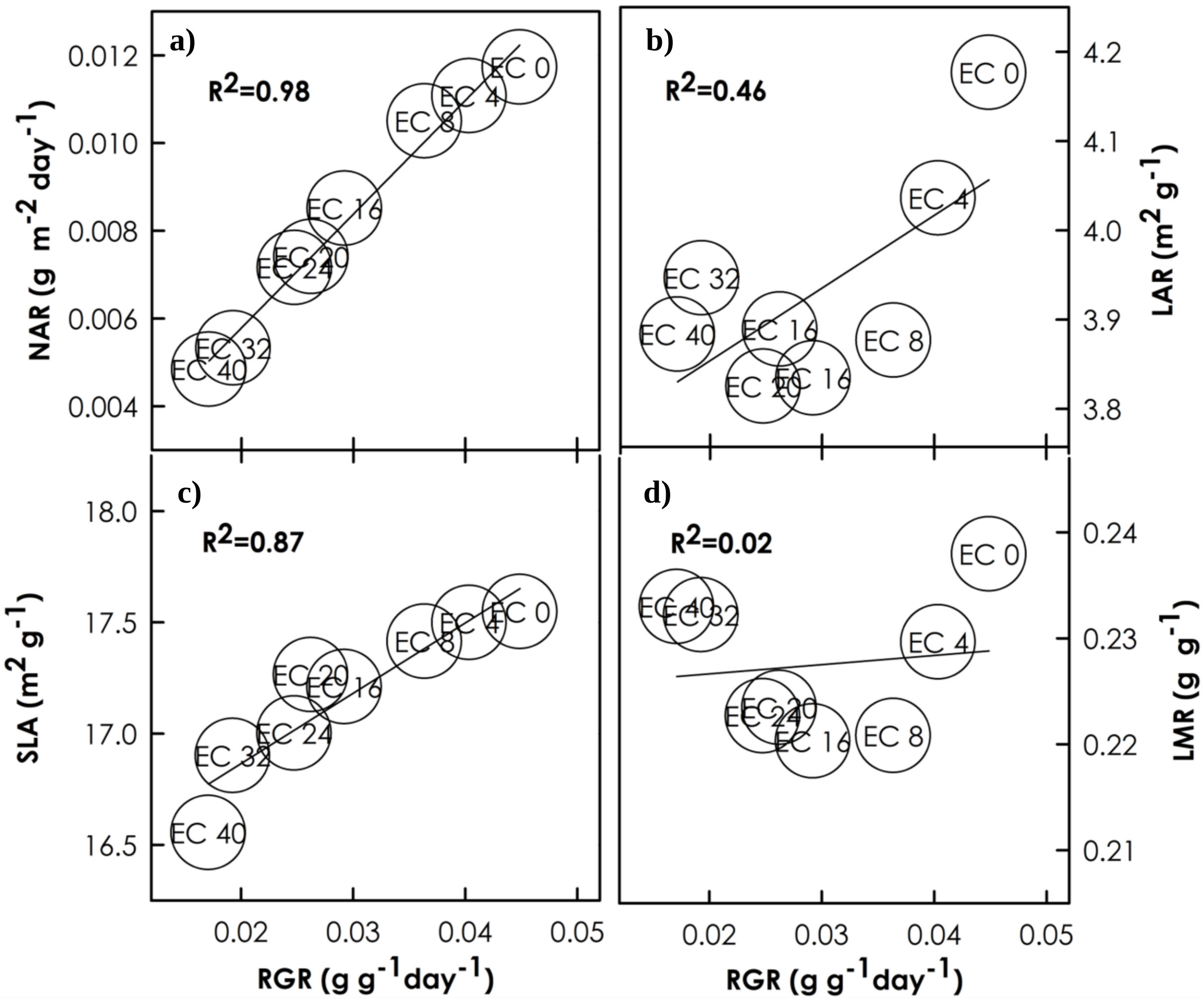


Figure 3-3: Compositions of relative growth rate (RGR). RGR is equal to the product of net assimilation ratio (NAR: **3-3a**) and leaf area ratio (LAR: **3-3b**). LAR is product of specific leaf area (SLA: **3-3c**) and leaf mass ratio (LMR: **3-3d**). **Figure 3-3** show strongest relationships between NAR and RGR(**3-3a)**; SLA and RGR(**3-3c)**. These findings suggest that RGR was primarily determined by NAR and SLA. The quadratic trend in leaf mass ratio (**3-3d**) somewhat indicates that leaves have greatest masses at low and high salinity

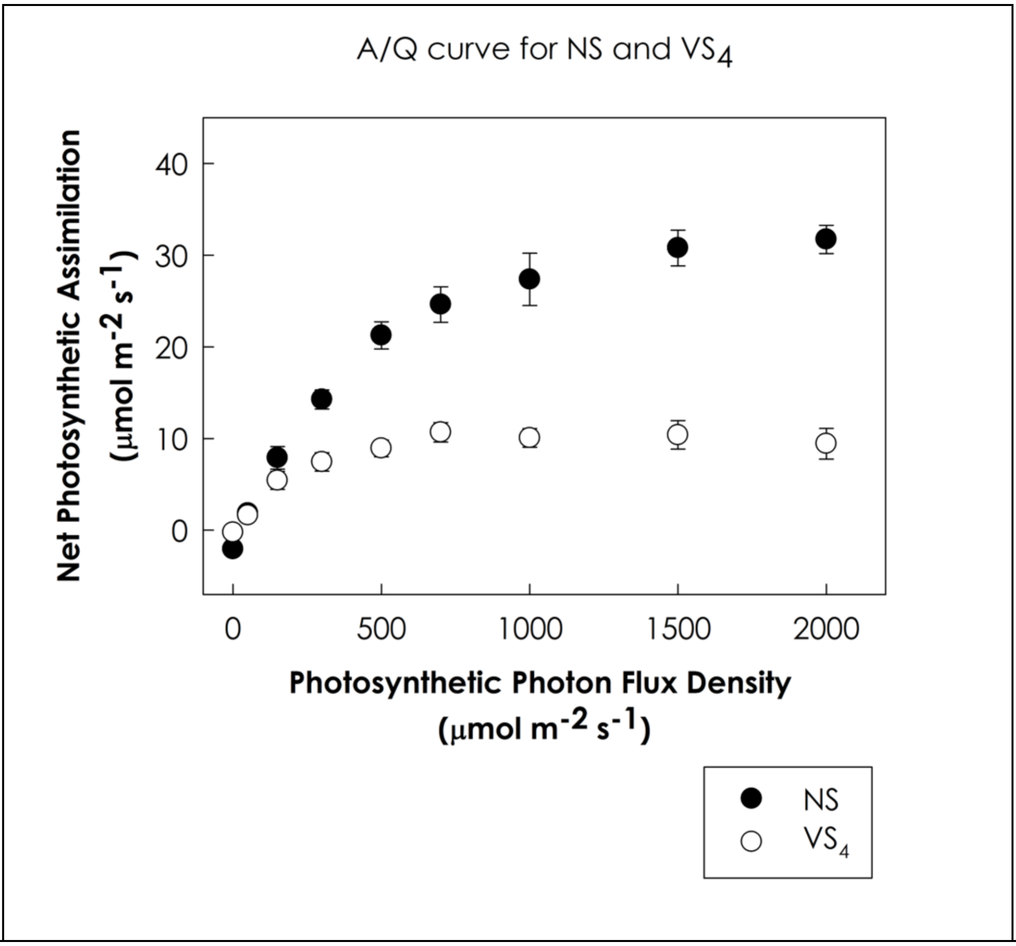


Figure 3-4: The light response curve *(A/Q)* suggests a difference in overall photosynthetic capacity at saturating light conditions between the NS and VS_4. The VS_4 group max assimilation rate (A_{max}) of 10.7 ± 1 µmols m^{-2} sec^{-1} was reached when light levels were only 700 PPFD; A_{max} began to decrease at the highest light levels. On the other hand, the NS treatment group A_{max} 31.73 ± 1.54 µmols m^{-2} sec^{-1} was not attained until the maximum light level (2000 PPFD). Further evidence suggesting photosynthetic inhibition was evidenced by the divergent quantum yield (Φ), evidenced by the initial slope, which like A_{max} were also different between the two treatment groups. An analysis of covariance (ANCOVA) was used to determine the heterogeneity of Φ for NS and VS_4; the summary showed a significant relationship for the interaction between light (PPFD) and salinity (EC) (p–value <0.05).

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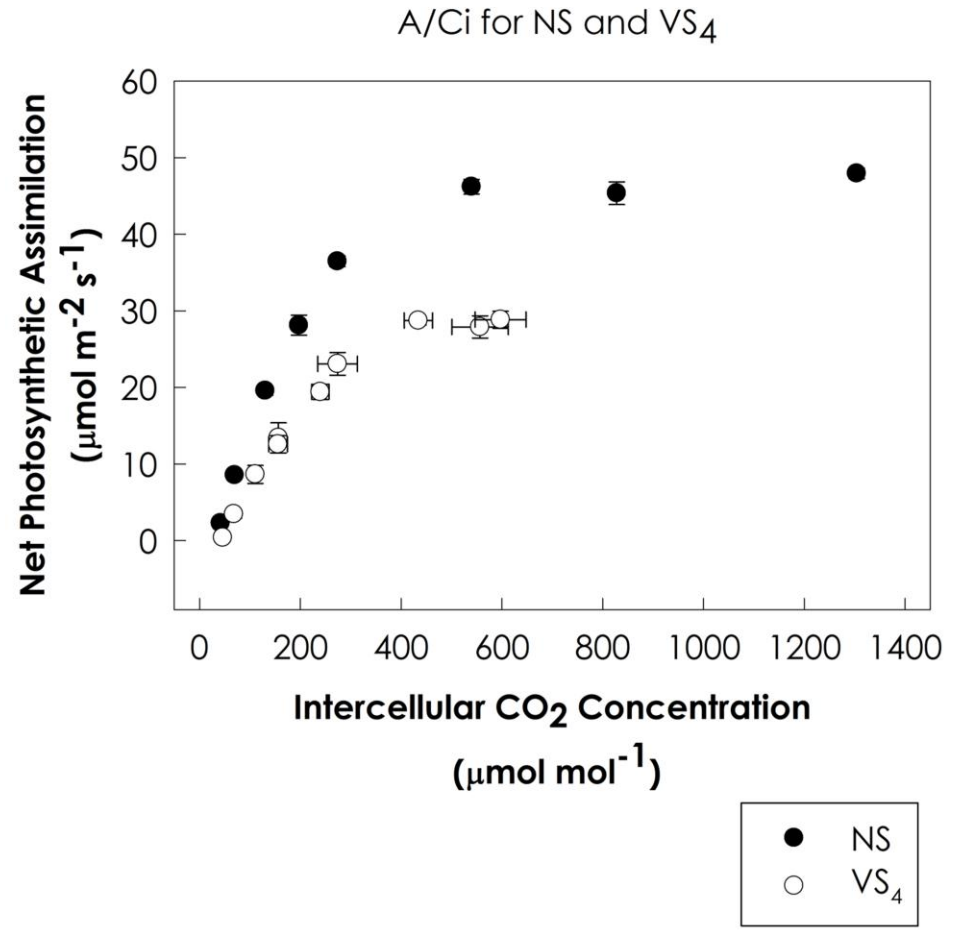


Figure 3-5: The A/C_i curve shows a distinct separation in photosynthetic assimilation for the NS and VS_4 groups at a variety of CO_2 levels. First the difference is apparent in the carboxylation efficiency represented by the initial slope of the *A/Ci* curve. An ANCOVA showed a strong interaction between Ci and salinity treatment group (P=0.001). The reduced initial slope of the *A/Ci* curve for the VS_4 treatment group suggests that CO_2 supplies are limiting the rate of functioning of Rubisco. Additionally, the maximum assimilation rates at saturating CO_2 levels also differ between the two treatment groups: 47.94 µmols m^{-2} sec^{-1} and 28.86 µmols m^{-2} sec^{-1} for NS and VS_4 groups.

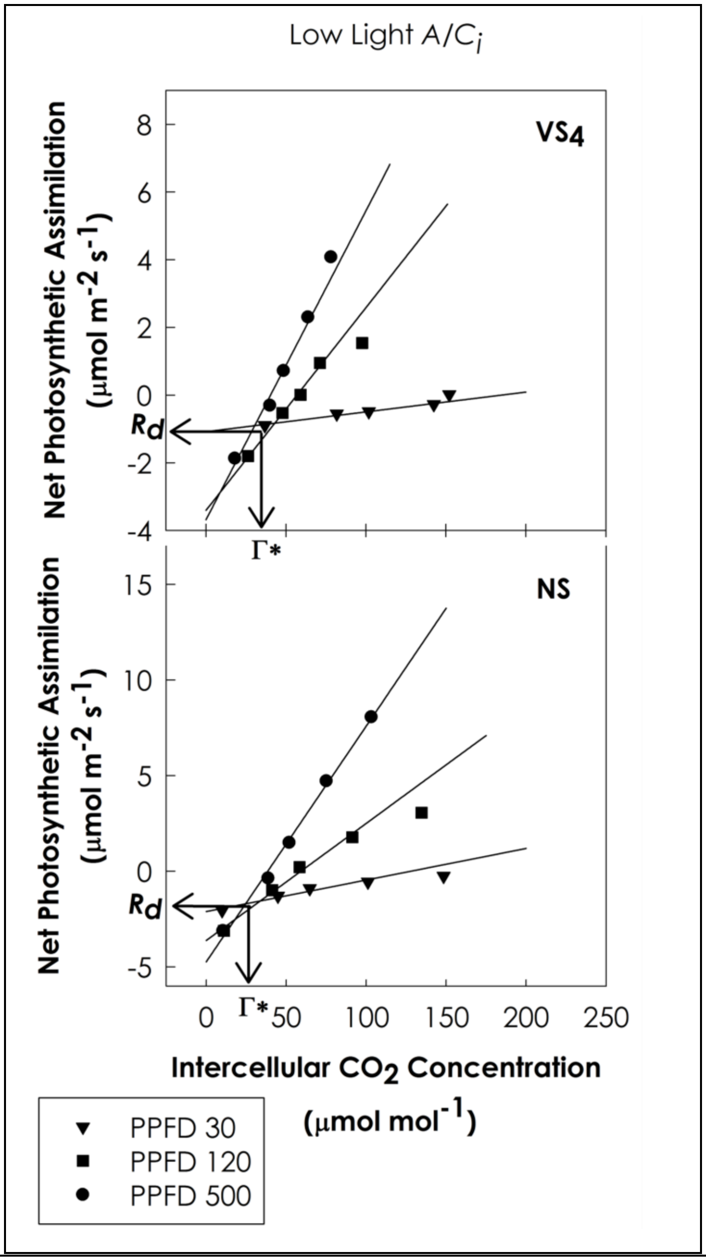


Figure 3-6: *A/C$_i$* curves were completed at three sub-saturating light levels and were used to determine Γ*, the CO_2 compensation point, which is the CO_2 concentration where photorespiration (oxygenation of RuBP and subsequent metabolism) produces as much CO_2 as is consumed by photosynthesis (carboxylation of RuBP). The Γ* values were 24.61 µmol mol^{-1} of CO_2 and 30.5 µmol mol^{-1} of CO_2 for the NS and VS_4 groups, respectively.

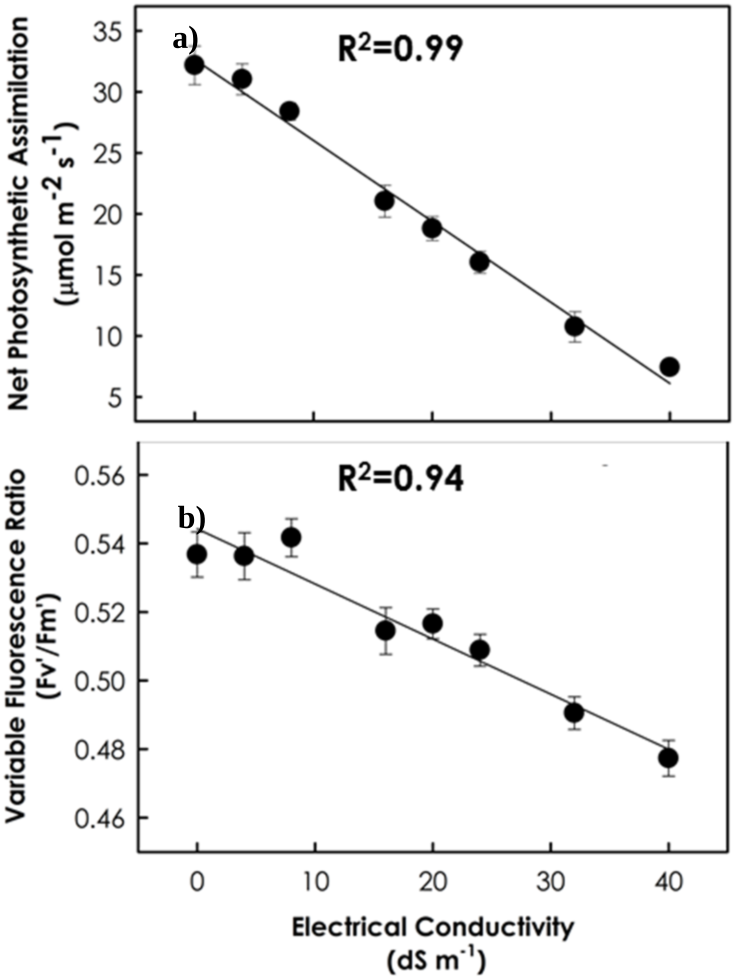


Figure 3-7: A plot of the net photosynthetic assimilation of the mean value (n=10, SE error bars) (**7a**) shows a negative relationship (R^2=0.99) between photosynthesis and salinity (EC). Additionally, the light adapted variable fluorescence ratio (Fv'/Fm') (**7b**)—a proxy measurement for apparent PSII efficiency—also illustrates an inverse linear relationship to increasing salt (R^2=0.94).

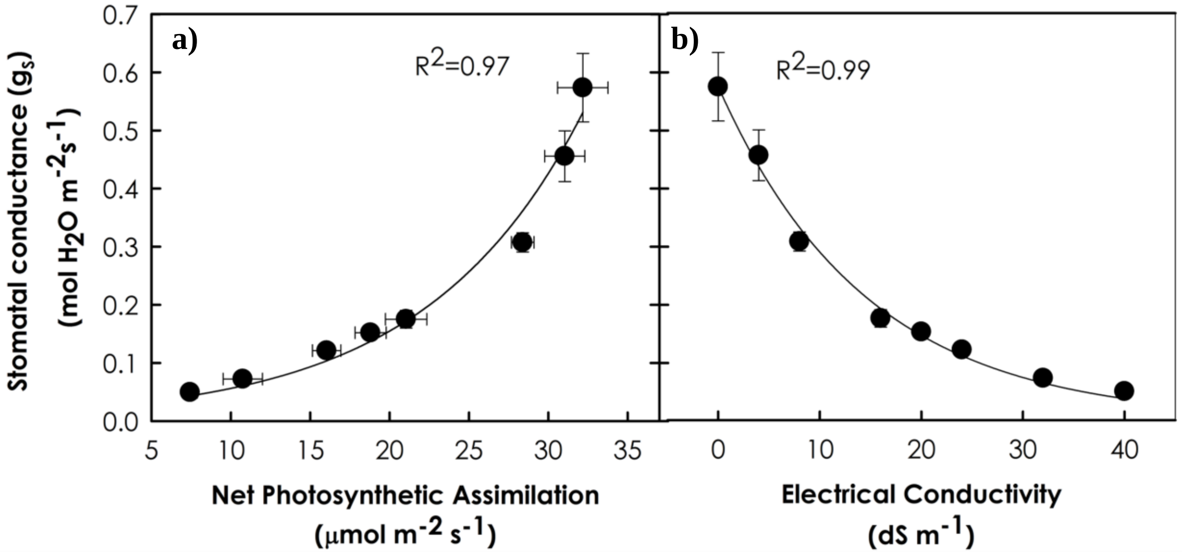


Figure 3-8: Plotting stomatal conductance against net assimilation (**3-8a**) and then against salinity concentration (**3-8b**) the mirrored patterns show that increasing net assimilation is exponentially proportional to stomatal conductance while the decay of stomatal conductance is exponentially proportional to increasing salinity.

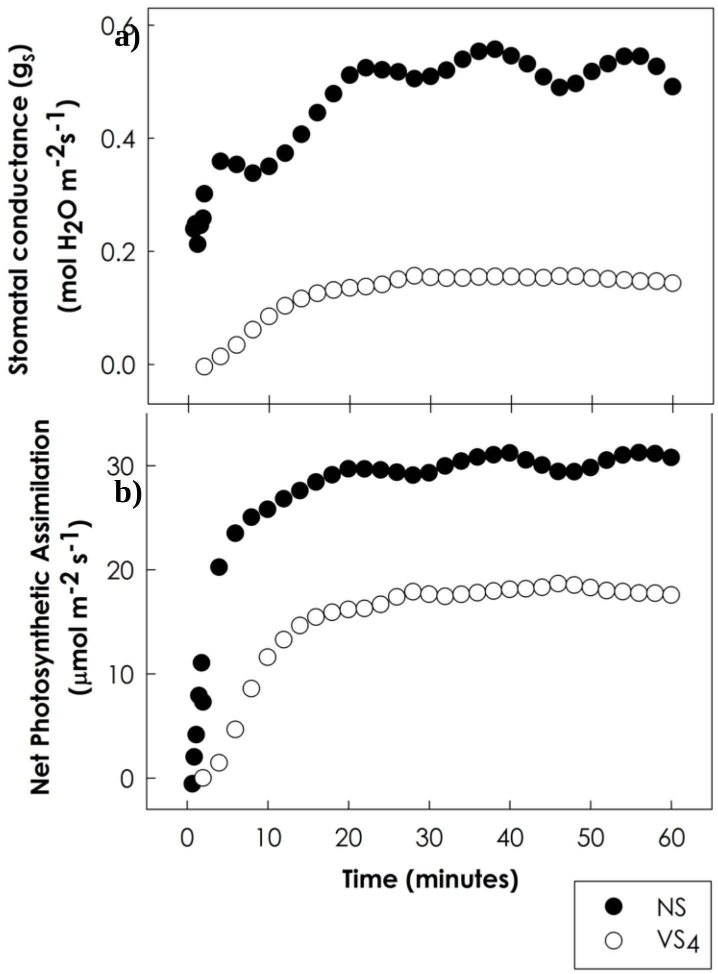


Figure 3-9: Correlation between stomatal conductance and photosynthetic inhibition at higher salt levels are evident in the comparison of induction curves for the non-salinity (NS; n=3) and highest salinity (VS_4 ; n=4) groups. The mean stomatal conductance is markedly greater for the NS groupd compared to the VS_4 group (**9a**). Likewise assimilation rates are also much greater for both NS compared to VS_4 (**9b**). An analsysis of covariance revealed an interaction (P=0.001) between salinity and time for both the net assimilation and stomatal conductance, confirming that slopes for each treatment group in either plot (**9a** and **9b**) different and thus that EC affected photosynthetic induction.

Tables:

Table 3-1: Chlorophyll A content unaffected by salt concentration. The mean SPAD values across all salinity (EC) treatments show that even at the highest sodium concentrations (i.e. 40 dS m^{-1}) that SPAD does not decrease below 42. SPAD units greater than 40 are correlated with high chlorophyll A content. These results suggest that during this experiment NaCl did not decrease chlorophyll A. Chlorophyll degradation can be an

EC (dS m-1)	n	SPAD mean	SE
0	**10**	**44.2**	**0.619**
4	**10**	**45.1**	**0.921**
8	**10**	**45.1**	**0.872**
16	**10**	**47.1**	**1.238**
20	**10**	**45.4**	**0.664**
24	**10**	**45.6**	**0.724**
32	**10**	**44.4**	**0.438**
40	**10**	**42.6**	**0.632**

Table 3-2: The Tukey tests show that there were no significant differences in biomass between consecutive salt treatment groups for six out of the eight treatment levels. The two consecutive group pairings that did show significant differences in biomass were separated by an electrical conductivity ≥ 8 dS m^{-1} (MS-StS and VS2-VS3), whereas, the majority of consecutive group pairings (*e.g.* NS-SlS and SlS-MS *etc.*) were separated only by 4 dS m^{-1}. Yet eight dS m^{-1} cannot be cited as the deciding threshold because two groups that were also ≥8 dS m^{-1} apart (StS -VS2 and VS3-VS4) did not exhibit significantly different mean biomass values: p-values >0.08 and >0.052, respectively.

Treatment Effect	**Pr(>F)**	**Non-Significant Tukey Groups**	**Adjusted P-value**	**EC difference (dS/m)**
Block	0.0003 ***	NS-SlS	0.116	4
Salinity	0.0002 ***	SlS-MS	0.171	4
Interaction	NS	StS-VS1	0.46	4
		StS-VS2	0.052	8
		VS1-VS2	0.969	4
		VS3-VS4	0.804	8

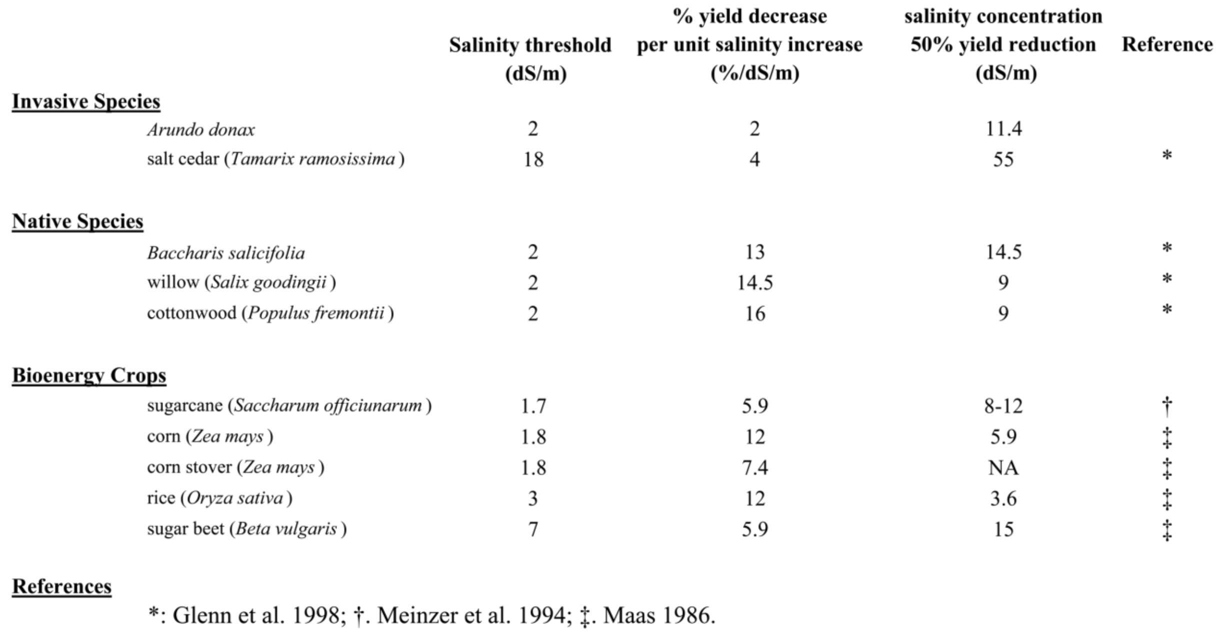
Table 3-3: Summary table of photosynthetic responses collected from various leaf gas-exchange experiments with the Li-6400.

	EC (dS/m)	Carboxylation Efficiency	*ACi* Amax	SE	*ACi Ci/Ca* 400 ppm	*AQ* Amax	SE	*AQ Ci/Ca* 400 ppm	Φ	Γ*	Measured Rd when PAR 0	LCP
NS	0	0.146	47.94	0.685	0.683	31.73	1.54	0.706	0.0457	24.61	-2.05	10.83
VS_4	40	0.0961	28.86	1.114	0.389	10.7	1.06	0.392	0.0378	30.5	-0.238	6.376

Table 3-4: A Tukey test of net assimilation and Fv’/Fm’ detected no statistically significant difference between any of the low EC groups (NS, SlS, and MS). However, the order of magnitude difference between p-values for NS-MS photo and Fv’/Fm’ are an indication that net assimilation was more strongly affected than Fv’/Fm’ at the lower salt concentrations.

Group Comparison	Photo p-value	Fv'Fm' p-value	significance
NS-SlS	0.79	0.99	NS
SlS-MS	0.26	0.81	NS
NS-MS	0.09	0.86	NS

Table 3-5: A comparison of the relative growth of *A. donax* with literature values for other species (Table 5) reveals that *A. donax* is more salt tolerant than most native species it co-habitates with; and also more salt tolerant than some biomass crops.



	Salinity threshold (dS/m)	% yield decrease per unit salinity increase (%/dS/m)	salinity concentration 50% yield reduction (dS/m)	Reference
Invasive Species				
Arundo donax	2	2	11.4	
salt cedar (*Tamarix ramosissima*)	18	4	55	*
Native Species				
Baccharis salicifolia	2	13	14.5	*
willow (*Salix goodingii*)	2	14.5	9	*
cottonwood (*Populus fremontii*)	2	16	9	*
Bioenergy Crops				
sugarcane (*Saccharum officiunarum*)	1.7	5.9	8-12	†
corn (*Zea mays*)	1.8	12	5.9	‡
corn stover (*Zea mays*)	1.8	7.4	NA	‡
rice (*Oryza sativa*)	3	12	3.6	‡
sugar beet (*Beta vulgaris*)	7	5.9	15	‡

References

*: Glenn et al. 1998; †. Meinzer et al. 1994; ‡. Maas 1986.

Chapter 4:

Bioenergy that Supports Ecological Restoration

Abstract

Expanding lignocellulosic bioenergy infrastructure in central Washington State, U.S.A., will provide economic incentives to expand regional wetland restoration by utilizing low-cost restoration wood-wastes as a fuel source for bioenergy. Additionally, we show that the most common invasive trees in the western United States—Russian olive (*Eleagnus angustifolia*) and salt cedar (*Tamarix* spp.) — have aberrant physicochemical properties compared to the regional biomass supply. Aberrant physicochemical properties place natural demand-side constraints on invasive tree residues. Thus, having an affordable supply of invasive tree residues, with physicochemical constraints on total demand, means restoration will expand while there is little risk that the facility will become reliant on sustained harvest of invasive tree residues. By addressing the regional nature of biomass markets and their complex interactions with regional landscape ecology goals, this study raises key questions on the appropriate use of scale when evaluating economic and environmental impacts of lignocellulosic bioenergy.

Introduction:

Bioenergy is often promoted as a low-carbon, domestic energy source that also provides development opportunities for rural communities (Farrell et al., 2006; Powlson, et al., 2005). Yet, critiques of ‘first generation’ bioenergy feedstocks have highlighted complex subsidy dependencies, dubious carbon gains, and potentially detrimental impacts on land and water resources (Hill, Nelson, Tilman, Polasky, & Tiffany, 2006; Naylor, et al., 2007; Searchinger et al., 2008). These shortcomings provided the impetus to develop ‘second generation’ lignocellulosic feedstocks from biomass residues and non-food crops grown in marginal conditions. Additionally, an emerging literature has reported planning strategies for incorporating lignocellulosic biomass synergistically with waste reduction, ecological restoration, and biodiversity development (Jakubowski, Casler, & Jackson, 2010; Kim & Dale, 2004; Wiens, Fargione, & Hill, 2011). However, while most of the reports have focused on national or global implications of bioenergy technologies the fact remains that lignocellulosic biomass markets are largely regional owing to two major constraints: (i) the limited distance low value biomass can be economically transported (Polagye, Hodgson, & Malte, 2007; J. J. Richardson et al., 2011; Walsh, 1998), and (ii) the need to tailor bioenergy infrastructure to the physicochemical traits of the dominant lignocellulosic resources (Van Loo & Koppejan, 2008). The combination of these two major constraints dictates the complex economic, ecologic, and technologic interactions that define the lignocellulosic “fuelshed”.

Analysis of fuelsheds almost always focuses on supply-side issues raised in item (i) while overlooking the technological demand-side limitations of item (ii). Yet, technological constraints of bioenergy infrastructure cannot be disregarded in the demand-side analysis of the fuelsheds. For even the most robust technologies, such as biomass-fired boilers, are sensitive to biomass feedstock traits such as alkali (ash), nitrogen, silica, and sulfur (Baxter et al., 1998; Giuntoli et al., 2010). Moreover, since harmful emissions (*e.g.* NO_x gasses) are proportional to the fuelwood elemental content (Giuntoli, et al., 2010; Van Loo & Koppejan, 2008)environmental permitting of biomass-fired boilers is also directly affected by feedstock traits. In this paper, we combine supply-side and demand-side frameworks for considering the economic, ecological, and technological potential for integrating restoration debris from two invasive trees, salt cedar (*Tamarix* spp.) and Russian olive (*Eleagnus angustifolia*) into a regional fuelshed created by the proposed construction of a biomass-fired boiler.

These invasive trees were selected for our study because of their prevalence in Western watersheds (Friedman et al., 2005). Furthermore ecological restoration of areas invaded by Russian olive and salt cedar has been deemed necessary to improve conditions for wildlife, reduce the threat of unnatural wildfire, and enhance natural hydrology of invaded watersheds (Coalition, 2008); additionally the restoration activities for either tree typically generates large quantities of wood-waste. Moreover, eradicating these tree species is expensive (Coalition, 2008), with funding for ecological restoration being generally supplied either from mitigation contracts or subsidized governments works projects (U.S. House 2006). Thus, generating revenues by selling invasive plant residues into regional bioenergy has tremendous potential for offsetting the ecological restoration costs, so long as 1. there are sufficient regional biomass resources to support lignocellulosic bioenergy infrastructure, and 2. the physicochemical traits of the invasive plants' are compatible with the regional bioenergy infrastructure.

Probing the economic and ecologic implications of introducing invasive plant residues into a bioenergy fuelshed requires an understanding of both the regional supply and demand traits of the built infrastructure. Herein, we develop a scenario centered on the reservation of the Confederated Tribes and Bands of the Yakama Nation and five surrounding Washington State counties. This region is an ideal model, because of the abundance of invasive trees and the Tribe's interest in developing bioenergy based on the timber and agricultural biomass resources (J. J. Richardson, et al., 2011). Additionally, the Yakama Department of Natural Resources (Yakama DNR) conducts on-going Russian olive eradication projects to minimize the impact of this tree, which has already invaded most watersheds on the reservation. The eradication projects are costly to implement, and generate substantial wood-waste that incurs additional disposal costs. We hypothesized that Russian olive residues—a waste product from ecological restoration—could generate revenues by being sold as biomass feedstock.

Methods and Materials:

Determining the economic, ecological, and technological potential for integrating the eradication wastes of invasive trees with regional bioenergy systems requires 1. Identifying the distribution and quantity of the invasive plant source material, 2. Estimating extraction and delivery costs, and 3. Describing the invasive plant biomass' physicochemical traits relevant to the operation of the bioenergy facility.

Identifying the distribution and quantity of the invasive plant source material — the study area, south-central Washington State (Figure 1A, gray region), is heavily infested by Russian olive. Vegetation surveys conducted by the Yakama DNR used global positioning system (GPS) software to designate areas invaded by Russian olive near the Toppenish National Wildlife Refuge and the Sunnyside State Wildlife Recreation Area in southeastern Yakima County, Washington, USA. We established an experimental restoration site on 52.5 hectares within the survey region that was identified as densely infested with Russian olive. To generate a source of restoration debris, crew members from Yakama DNR cut and piled the Russian olive trees with chainsaws in combination with a feller/buncher harvester. The piled debris was then chipped and loaded into a semi-trailer truck (Figure 1B). Landscape level evidence of both the spread of Russian olive trees—at Horseshoe Lake in the Toppenish National Wildlife Refuge — prior to and during the restoration were captured by aerial photographs taken in 2006 and 2009 (Figure 4-1C and D). The total volume of woody debris removed from this site was quantified by weight and volume, allowing for the estimation of biomass residue generated per hectare of restored land.

Estimating extraction and delivery costs — Variability in restoration costs are associated with naturally variable plant densities; access, terrain, and weather restrictions; and chemical and mechanical treatments (*i.e.* herbicide spraying or mechanically extracting stumps). To present a more generalized, yet representative restoration cost we used the cost algorithm for "mechanical extraction using large excavator, without herbicide application or re-vegetation" developed for salt cedar and Russian olive removal by the Tamarisk Coalition (TamCo) (Coalition, 2008).

Costs are also incurred by delivering the debris from the restoration site to a bioenergy facility. To quantify these delivery costs a transportation model was constructed from a network analysis of the trucking routes. Road layers were imported into ArcGIS 9.2 from public datasets; road segments were classified by truck speed. The transportation model combines time-based ($29/hr) and distance-based ($0.75/km) transportation costs assuming operation of a 28 Mg chip van. Roadside biomass is assumed to be chipped and loaded onto the 28 Mg truck, transported, and delivered to the bioenergy facility. Details for assumed costs for chipping and loading are provided in Richardson *et al*. 2011.

Describing the invasive plants' biomass physicochemical traits — Russian olive, and salt cedar biomass were tested for thermochemical properties. The source material was derived from cut woody stems, approximately 2 kg mass, 10-15 cm diameter, and 30-38 cm long. These samples were chipped (approx. 7 cm) and dried for 8 days at 40^{o} C. The air-dry samples were successively pulverized: first coarse-ground (< 6 mm); then finely ground (< 1mm) until a 200 g sample could be processed in triplicate according to the American Society for Testing and Materials (ASTM) standards for biomass testing (E870-82, E873-82, ASTM D6721-01).

Results:

The residues produced from our 52.5 hectare study site were found to range from as little as 17 Mg/ha to as great as 58 Mg/ha (Mg herein are always reported as bone dry mass). Therefore, to generalize our findings across the environmental and dispersal gradients we estimated costs based on a moderate canopy cover density—between twenty to fifty percent of the total area—with an average yield of 34 Mg/ha. The actual Russian olive restoration costs ranged from $217-$1406/ per ha. Using the TamCo model the costs were estimated at $988 per ha treated. According to the 2011 US Department of Energy, Multi-Year Biomass Program Plan, woody biomass feedstock supply price is expected to equal or exceed $38.5/ Mg (DOE 2011). At this price 34 Mg/ha would be valued at $1309/ha. These theoretical revenues would exceed the costs of restoration by $321/ha. The $321 would be used as payment for transportation of the material.

The estimated round-trip costs associated with transporting chipped Russian olive between any given restoration site and a biomass-fired boiler in White Swan, WA, are shown on the transportation cost map (Figure 4-2). According to our transportation model revenues generated from the sale of the biomass could offset all costs for 34 Mg/ha where delivery costs to the power facility ≤$9.5/Mg. These calculations define the economically feasible fuelshed. The profitable fuelshed covers a large portion of the study area—all of the green colored areas (Figure 2) — which includes the entire Yakama Reservation. Moreover, Russian olive residues removed from sites in nearest proximity to the bioenergy facility (*i.e.* <$4/Mg) could subsidize the acquisition of some material located were transportation costs exceed $9.5/Mg. These results show that there are cost effective supplies of invasive plant wood-waste available for the biomass-fired facility. However, having an affordable supply of residues does not ensure the facility will seek to purchase all the material. Demand within the fuelshed also depends on the compatibility of the woody material with the technology used for converting biomass to energy.

The results of the thermochemical analysis (Table 4-1) compares some of the properties of the measurements made for Russian olive and salt cedar with reported values for the regional biomass fuel mix. We have estimated the regional supply of conventional woody biomass for the study area in earlier work (Richardson *et al.* 2011). The regional woody biomass residues are predominately agricultural residues including, but not limited to, timber harvest slash, fruit and nut tree trimmings, and grape vine pruning. The mix is roughly half from timber and half from trimming/pruning. The higher heating values (HHV) of Russian olive and salt cedar, 20.40 and 18.58 MJ/kg, respectively, are comparable to the regional feedstock fuel mix HHV, 20.70 MJ/kg. However, in key components related to the boiler operation—nitrogen, sulfur, and ash—Russian olive or salt cedar have much higher levels than the regional biomass mix.

Discussion

Will there be demand for invasive plant material?

Bioenergy infrastructure will always be designed to utilize the largest economically available supply of biomass materials. At a purchase price of $38.5/Mg delivered, the White Swan biopower facility has an estimated 231,485 Mg/year of timber harvest slash, fruit and nut tree trimmings, and grape vine pruning (J. J. Richardson, et al., 2011). Even though Russian olive has colonized many acres within the landscape, it represents an insignificant biomass resource compared to the regional supply of conventional woody biomass residues. The regional bioenergy infrastructure will therefore be designed to accommodate the physicochemical properties of the timber and trimming/pruning materials within the fuelshed, rather than Russian olive. For Russian olive, or any invasive plant material, to have significant demand within the biopower facility's fuelshed, its physicochemical properties must be compatible with the built infrastructure. However, in key components related to the boiler operation—nitrogen, sulfur, and ash—Russian olive and salt cedar have much higher levels than the regional biomass mix; and this places a technological constraint on demand by a facility designed to use the timber and trimming/pruning regional fuel mix. For example, Russian olive has three times more nitrogen than the baseline regional woody biomass fuel mix. Nitrogen content of the fuel source is a primary source of NO_x emissions for a biomass boiler (Giuntoli, et al., 2010), thus Russian olive residues can only be added to the regional-mix up to the levels set by the biomass boiler NO_x emission permits. Similarly, salt cedar biomass also exhibits divergent characteristics that act as physicochemical limitations.

For salt cedar, these include sulfur content two orders of magnitude greater and ash content three times greater, than the baseline regional biomass feedstock mix. Introducing large quantities of salt cedar residues into the regional fuel mix would increase rates of slag formation, boiler fouling, and particulate matter emissions. In short, despite being an economic source of lignocellulosic biomass the unusual physicochemical traits of the two invasive trees analyzed will likely limit their bioenergy demand. At the same time, the limited supply of invasive tree residues (relative to other sustainable sources of forestry and trimming/pruning residues) is also beneficial, since successful eradication of invasive trees will not significantly impact the fuelshed economics.

Conclusion

The challenge of restoring natural systems in face of the pervasive plant invasions, in a highly altered ecosystem, such as central Washington, demands innovative and pragmatic approaches.

Our study site suggests that building a biomass-fired boiler designed for conventional woody biomass sources (*e.g.* forestry and agriculture supplies) has the potential to stimulate a market in which restoration biomass could generate revenues to greatly subsidize the regional eradication of invasive trees. Our study also shows that Russian olive and salt cedar have inherent technological constraints from sub-optimal physicochemical characteristics (*e.g.* excessive N, S, and ash contents) that will limit their unchecked demand in a conventional fuelshed, reducing economic incentives to expand acreage of the invasive trees. And yet, even if these technological constraints limited demand for invasive tree biomass to 5% of the total regional supply, that would still enable sales of 9,525 Mg/year of residue at $38.5/Mg. Based on our results, the revenues generated from these sales could fund restoration of roughly 280 hectares per year.

We believe integrative calculations tied to both lignocellulosic biomass supply and the demands of the built infrastructure can help assess the risks associated with placing economic value on invasive plants. There has been concern that using invasive plants as feedstocks will foster cultivation of such species (Barney & Ditomaso, 2008). These ecological concerns assume unchecked demand if the material is economically viable. However technological demand limitations (*i.e* NO_x gas emissions) will likely temper incentives to cultivate invasive trees. Our study creates an important and unique template illustrating unrealized synergies between ecological restoration and bioenergy, and presents an opportunity for additional, similar partnerships in different ecologies. Although we have shown that lignocellulosic biomass markets are largely regional our case-study approach has nationally relevance when extended to other regional lignocellulosic feedstock markets. For instance, in the Midwest where stover, switchgrass, and *Miscanthus* dominate lignocellulosic development, bioenergy could partner with the ecological restoration of invasive grasses (*e.g. Phalaris arundiacea*). Additionally, in timber driven infrastructures like the Southeast, bioenergy could coordinate with the removal of invasive trees like *Sapium sebiferum*, *Schinus terebinthifolius* or *Melaleuca quinquenervia*. Should these or any of the invasive species not exhibit self-limiting physicochemical traits like the ones we found with Russian olive and salt cedar then permitting or certification policies would be needed to ensure there is no incentive to cultivate the invasive species.

Figures:

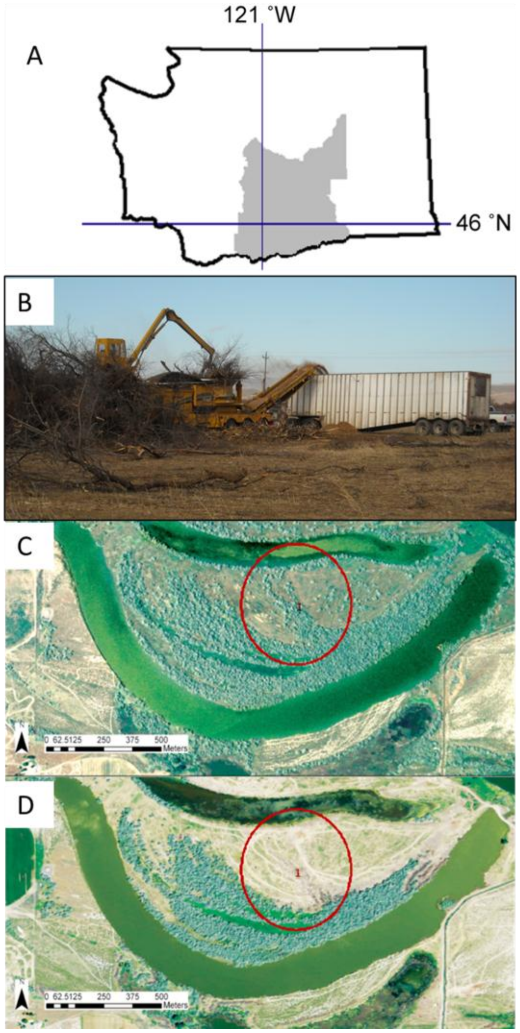


Figure 4-1 A: The study area, in gray, is shown in relationship to the State of Washington. **4-B:** chipping and loading of Russian olive wood debris into a semi-truck trailer. **4-C & 4-D**: Aerial photographs of at Horseshoe Lake, WA (Lat. 46.24, Long. -120.02). **4-C:** Image taken in 2006, before the eradication. **4-D**: Image taken in 2009, during the clearing process. The grey-green mottling are the Russian olive trees/groves. The red circles, labeled 1, are used to show density variability, increasing with distance from water. Source: NAIP Inventory (USDA, FSA, APFO: Aerial Photography Field Office, Salt Lake City, UT, 2006, 2009).

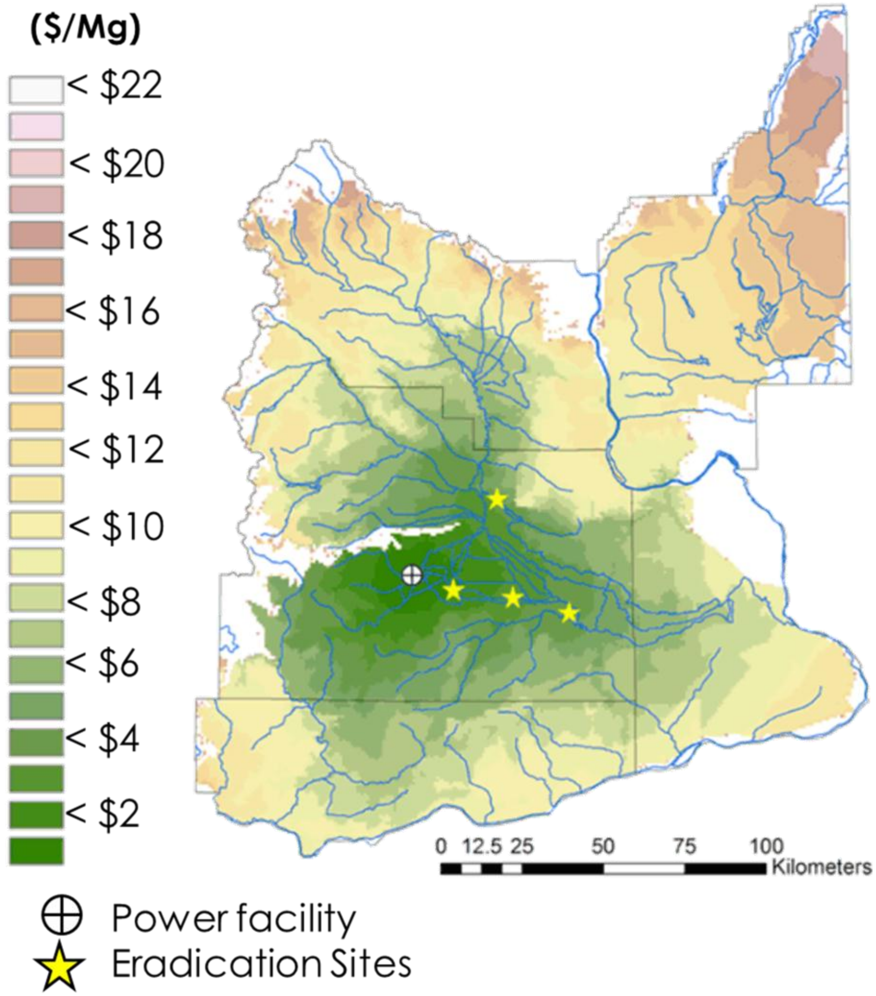


Figure 4-2: The map illustrates results from transportation model, which estimated biomass transportation costs to a biomass-fired boiler (circle with cross-hair) located in White Swan, WA. The four stars denote the experimental Russian olive eradication sites described in this work. Rivers in the study area are also shown as blue lines.

Table 4-1. Comparison of the physicochemical properties for two invasive trees and the regional biomass feedstock available for a delivered cost of $35/BDT.

		Invasive Trees		**White Swan feedstock***
		R. Olive	*Salt Cedar*	*(timber and orchard wood mix)*
	HHV (MJ/kg)	20.40	18.58	20.70
Ultimate (%)	**C**	51.18	47.75	52.92
	H	5.28	5.54	5.43
	O	41.54	41.77	39.64
	N	1.08	0.39	0.37
	S	0.04	1.08	0.01
Proxi	**Ash**	0.51	5.06	1.30

* Feedstock composition were calculated based on 36 % nut tree trimmings, 16 % grape vine pruning, and 48% timber slash (assumed Ponderosa pine) at $35/BDT supply composition (Richardson *et al.* 2011); physicochemical property values for agricultural species from (Gaur and Reed 1998)

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CURRICULUM VITAE

Education:

Ph.D—Candidate, *magna cum laude* University of Washington Botanic Gardens, School of Environmental and Forest Sciences, College of the Environment, University of Washington, August 2012.

Dissertation: Ecophysiology as a tool for evaluating invasive-plant based bioenergies: physiological and ecological case-studies of *Arundo donax* and *Elaeagnus angustifolia*

B.S. Environmental Horticultural Science

cum laude California Polytechnic State University San Luis Obispo, June 2003

Minor: Land Rehabilitation

Thesis: Ecological Restoration of James Creek

International Exp. Studied plant physiology; Aboriginal cultural ecology at University of Western Sydney, Richmond, Australia

Research Assistantships:

Bioenergy and woody invasive trees: Department of Chemical Engineering, University of Washington, Seattle, WA. March-June 2012.

Garlic Crop Modeling: impacts of temperature and carbon dioxide enrichment on the phenology, growth, and metabolism of *Allium sativum*. Korean Department of Agriculture. March 2010—January 2012

Bio-resource Based Energy for Sustainable Societies: Developing sustainable bioenergy power with the Confederated Tribes and Bands of the Yakama Nation. Integrative Graduate Education and Research Traineeship (IGERT), National Science Foundation 2008-2010

Honored Scholar: Graduate Office of Minority Opportunities Program, University of Washington, Seattle 2006-2007

Teaching Assistantships:

Plant Ecophysiology, ESRM 478. School of Environmental and Forest Resources, University of Washington. Seattle. January -March 2012; 2011; 2010.

Restoration Ecology, ESRM 374. School of Environmental and Forest Resources, University of Washington. Seattle. Sept-Dec. 2011

Native Plant Production, ESRM 412. School of Environmental and Forest Resources, University of Washington. Seattle. March 2011-June 2011

Plant Propagation, ESRM 411. School of Environmental and Forest Resources, University of Washington. Seattle. March 2011-June 2011

Pacific Northwest Ecosystems, ESRM 201. School of Environmental and Forest Resources, University of Washington. Seattle. Sept-Dec 2010.

Teaching Assistantships (cont.):

Forests and Society, ESRM 101. School of Environmental and Forest Resources, University of Washington. Seattle. March 2010-June 2010.

Environmental Sociology, ESRM 371. School of Environmental and Forest Resources, University of Washington. Seattle. June 2007-Sept 2007.

Field Trip Leader:

Biodiversity and Ecology, BIOL 180. Department of Biology, University Washington, Seattle. 2008; 2011

Related Work Experiences:

Graduate Plant-Research Assistant. Facilitated multi-user experiments at Douglas Research Conservatory, University of Washington Botanic Gardens, Seattle. 2011--current

Restoration Ecologist. Created and managed native plants nursery to provide plant materials for ecological restoration work in oak woodlands and coastal plant communities in San Francisco Bay Area. Shelterbelt Builders. 2003-2006

Publications:

Richardson, JJ., KA. Spies, S. Rigdon, S. York, V. Lieu, **LL. Nackley,** *et al.*2011. Uncertainty in biomass supply estimates: Lessons from a Yakama case study. *Biomass and Bioenergy*, **35**, pp 3698-3707

Vogt, KA, JJ Scullion, **LL. Nackley** and M Shelton. 2011. Conservation Efforts, Contemporary. *In*: (S. Levin, ed.) Encyclopedia of Biodiversity. 2nd Edition. Academic Press

Nackley, LL. 2010 Water Use by the Giant Reed (*Arundo donax*) *in* (KA Vogt *et al.*) Sustainability Unpacked: Food, Energy and Water for Resilient Environments and Societies. Earthscan. Publishers

Vogt, KA., DJ. Vogt, M. Shelton, R. Cawston, **LL. Nackley**, and J. Scullion *et al.* 2010. Bio-resource Based Energy for Sustainable Societies Handbook. Nova Science Publishers Inc., Hauppauge, NY.

Nackley, LL. 2010 A Lighter Footprint on Mother Earth. First Prize, Tribal Essay Contest Bonneville Power Authority, Portland, OR

Nackley, LL., 2009 Washington Park Arboretum Conservation Policy, University of Washington Botanic Gardens, Seattle, Washington

Publications in preparation:

Nackley, LL., V. Lieu, B. Battalia Garcia, J. Richardson, E. Isaac, K. Spies, S. Rigdon, and D.T. Schwartz. ***Submitted.*** Bioenergy that Supports Ecological Restoration.

Nackley, LL., SH. Reichard, and S-H. Kim. ***In Preparation***. Ecophysiology of an Invader: Water and Nitrogen Use Efficiencies of *Arundo donax* in present and future climate conditions. Target Journal: *Journal of Arid Environments*

Nackley, LL., NW. Hough-Snee. ***In Preparation.*** Restoration Ecophysiology: a Synthesis for Advancing Restoration Project Design. Target Journal: *Restoration Ecology*

Nackley, LL., J-H, Jeong, U. Cheung, and S-H. Kim. ***In Preparation.*** A Coupled Model of Photosynthesis, Conductance and Transpiration for Garlic (*Allium sativum*) Target Journal: *Agriculture and Forest Meterology*

Professional Presentations:

Physiological mechanisms of salt tolerance by invasive grass Arundo donax. **Impending.** September, 2012. Regional meeting, Pacific Northwest Chapter of the Society of Wetland Scientists. Boise, ID, USA.

Ecophysiology of Arundo donax, an invasive energy feedstock. August 2012, National Conference, Ecological Society of America, Portland, OR, USA

Bioenergy that supports ecological restoration. June 2012. IGERT Principal Investigators Meeting, National Science Foundation, Washington D.C., USA.

The Future of BioEnergy: Challenges, Trends and Opportunities. July, 2010. World Congress of Industrial Biotechnology, Dalian, China.

Codevelopment of Bioenergy and Biodiversity. February 2010. Society for Ecological Restoration Northwest Chapter Conference, Seattle, WA, USA.

Bioenergy Power Generation for the Yakama Nation: An Economic Assessment of Regional Biomass Feedstocks. November 2009, Presented to the Natural Resource Management Advisory Committee of the Confederated Bands and Tribes of the Yakama Nation, Toppenish, WA, USA.

Professional Societies:

Ecological Society of America, 2012--current

Society for Ecological Restoration, University of Washington Student Guild **President,** 2009—2011; **Vice President,** 2008. Member-current

Graduate and Professional Student Senate, University of Washington, **Senator,** 2007-2010; **Judicial Committee,** 2008

Xi Sigma Pi, Alpha chapter, Forestry Honors Society. University of Washington, **Ranger** 2008-2009

Curation Committee, University of Washington Botanic Garden **Representative Student**: 2008-2009

Society of American Foresters. **Member**: 2007-current

Technologies:

Hardware:
Infrared Gas Analysis: Li-Cor 6400, 6200; CIRAS-1.
Chlorophyll fluorometry: Handy Plant Effciency Analyzer, Hansatech.
Plant Water Potential: Scholander Pressure Chamber; Watermark water sensors; Carbon Isotope Analysis.
Leaf and Canopy Area Analysis: Li-Cor 2200 & 3000.
Chlorophyll Measurement: SPAD-502, gas chromatography.
Remote Data Collection: Trimble GeoExplorer, Hobo Sensors, Nikon Hypsometer, CR- 1000 data logger.
Proximate Analysis: Wiley Mini-Mill; Thermogravimetry; Bomb Calorimetry

Software:
Statsistical Analysis: R: univariate and multivariate statistical analyses; SAS; Sigma plot
Graphic Design: Adobe Design Premiere
Geospatial Analysis: ArcGIS
General: MS Office