Title
The potential for tropical restoration legume and non-legume trees to suppress the invasive C4 grass Saccharum spontaneum in Panama

Author
Cummings, Justin

Publication Date
2013

UNIVERSITY OF CALIFORNIA
SANTA CRUZ

THE POTENTIAL FOR TROPICAL RESTORATION LEGUME AND NON-LEGUME TREES TO SUPPRESS THE INVASIVE C_4 GRASS *SACCHARUM SPONTANEUM* IN PANAMA

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY
with a designated emphasis in ENVIRONMENTAL STUDIES

by

Justin A. Cummings

June 2013

The Dissertation of Justin A. Cummings is approved:

Professor Ingrid M. Parker, Chair

Professor Gregory S. Gilbert, Co-Chair

Professor Karen D. Holl

Professor Jarmila Pittermann

Tyrus Miller
Vice Provost and Dean of Graduate Studies

Table of Contents

List of Tables

Chapter 1 Page

Chapter 2

Chapter 3

List of Figures

The potential for tropical restoration legume and non-legume trees to suppress the invasive C4 grass *Saccharum spontaneum* in Panama

Justin A. Cummings

Abstract

Saccharum spontaneum L. subsp. *spontaneum* is a large, exotic C_4 grass species that invades abandoned agricultural fields in many tropical countries and can prevent natural forest regeneration. In Panama, studies on restoration of areas dominated by *S. spontaneum* have focused on identifying trees that establish readily after *S. spontaneum* removal. Few studies have evaluated how planted trees can suppress subsequent regeneration of *S. spontaneum*.

This dissertation focused on how legume and non-legume trees planted for restoration alter light availability and soil chemistry, and the influence of those effects on *S. spontaneum* suppression. In addition, I explored the potential use of agricultural legumes to suppress *S. spontaneum* through nutrient competition. Finally, I examined potential for using allelopahty as a tool for forest restoration and exotic species management both through a review of the literature and green house experiments.

I evaluated the response of *S. spontaneum* along a gradient of understory light conditions in single species plots of trees planted for restoration. *S. spontaneum* regeneration after planting restoration trees was most limited by light availability. Legume trees produce deeper shade than non-legume trees, and were more efficient at suppressing *S. spontaneum*. In addition, legume overstory species suppressed *S. spontaneum* abundance beyond the expected reductions associated with shade. *S. spontaneum* is nitrogen and phosphorus co-limited in our system in the absence of competition. However, although we find trends towards higher soil nitrogen and lower phosphorus under legume trees, greenhouse and field experiments provided weak evidence for both phosphorus competition and allelopathy to play roles in *S. spontaneum* suppression.

Overall, results emphasize the value of incorporating legume trees into restoration plantings where invasive *S. spontaneum* suppression is desired. Restoration practitioners should consider establishing mixed species stands dominated by legume tree species when reforesting areas dominated by *S. spontaneum* to expedite the reforestation process.

Acknowledgements

This thesis would not have been possible with out the help of many colleagues, friends, and family. To begin, I would first like to thank my advisers Ingrid M. Parker and Gregory S. Gilbert for their trust, guidance, attention, patience, and support throughout my thesis. Even during the most difficult times they both provided me with the guidance and support necessary to continue moving forward towards the completion of my thesis. Additionally they taught me what it means to be a professional in the field of ecology and I am forever indebted to them for all they have taught me. I would also like to thank my committee members Jarmila Pittermann and Karen D. Holl for their help with deciding how to best execute my dissertation work, and their insightful feedback. Additionally I would like to thank the NSF, CENTREAD, STRI, Jean Langenheim, and GAANN for providing funding during my dissertation.

I would also like to thank the many people at the Smithsonian Tropical Research Institute in Panama for their help with logistics, research, and intellectual support. First I must thank Jefferson Hall for allowing me to work in the PRORENA plots. Without access to these plots, none of this work would have been possible during the time frame of this dissertation. I would also like to

thank Joe Wright for serving as my STRI adviser and taking the time to discuss ideas relating to my dissertation, and Ben Turner for helping me with project development and allowing me to work in his lab. Additionally I must thank Marcela Paz for helping to make sure I always had a roof over my head in Panama, and a special thanks to Raineldo Urriola for always taking the time to help me with logistics. In addition, I wouldn't have been able to conduct any of my leaf nutrient extracts if it weren't for Laurel Fox, Dyke Andreasen, and Rob Franks at UC Santa Cruz.

In addition to having intellectual support, conducting the actual fieldwork would have been impossible without extra sets of hands. I feel very fortunate to have had some of the best, brightest, and toughest (mentally and physically) field assistants as a grad student and hope to have more in the future. This being said I would like to thank Emelie McKain, Oscar Muñoz, and Giancarlo Onorati for their excellent support as field and lab assistants.

I must also thank all of my lab mates from both the Gilbert Lab and the Parker Lab from 2007 – 2013, and the members of the EEB 3rd floor alliance. Thank you for always being available for tossing ideas around, and for all the support and encouragement throughout this dissertation. In addition, I must also thank the members of my Cohort Jimmy O'Donnell, Cyril Michel, Rodrigo Beas, Melinda Fowler, Justin Yeakel, and Erin McCreless for countless intellectual discussions and necessary distractions.

Finally I would like to thank all of my family and friends in Panama, California, Chicago, and all other parts of the world for their support and encouragement. I would especially like to thank my immediate family for always believing in me and exposing me to the natural world at a young age, and would finally like to dedicate this PhD to my mother who from day 1 has supported me in all of my endeavors and never lost faith. Thank you all for your love and support and enjoy!

Introduction

In the tropics many exotic C_4 grasses are noxious pests that colonize post agricultural sites and inhibit forest succession (D'Antonio and Vitousek 1992, Hooper et al. 2004, Gibson 2008). Because the mono-dominant stands of such grasses promote a fire cycle that then ensures the continued dominance of the invasive grass, grassland and forest can be viewed as alternative states for these systems. Restoration efforts aim to push the systems out of the grassland state and into a successional trajectory to forest cover.

How to best establish tree species in exotic grasslands is a major focus of restoration ecology in the tropics (Holl 1999, Loik and Holl 1999, Holl et al. 2000, Hooper et al. 2004, Jones et al. 2004). Numerous studies have compared growth, survival, canopy cover and native understory recruitment as a way of evaluating which tree species to plant when attempting to reforest degraded and invaded habitats (Hooper et al. 2002, Jones et al. 2004, Wishnie et al. 2007, Craven et al. 2009). In areas dominated by C_4 exotic grasses, the production of canopy cover is especially important because shade inhibits growth of C_4 grasses (Lambers et al. 2008**).** Understanding how grasses respond to different tree species planted for restoration is critical for informing tree species choice for grass suppression, and deciphering the mechanisms that govern grass responses.

S. spontaneum L. subsp. *spontaneum* is a large, exotic C_4 grass species that invades abandoned agricultural fields in many tropical countries. It is adapted to drought, burns frequently, and is difficult to eradicate, which is why it is considered one of the most serious weeds where it occurs (Holm et al. 1979, Hammond 1998, Hooper et al. 2004). In Panama, fires promoted by the presence of *S. spontaneum* reduced germination and species richness of native trees, and promoted *S. spontaneum* growth and reproduction (Hooper et al. 2004, Hooper et al. 2005, Saltonstall and Bonnett 2012). Such fires have resulted in the loss of hundred of hectares under restoration by removing planted trees and converting areas under reforestation back to *S. spontaneum* dominated grasslands (e.g. Ciudad del Arbol, PRORENA; personal communication).

Restoration efforts have focused on identifying trees that are able to establish and rapidly produce deep shade to inhibit *S. spontaneum* persistence (Hooper et al. 2002, Jones et al. 2004, Wishnie et al. 2007, Hall et al. 2011b). In particular, legume trees are often used in tropical forest restoration due to their ability to establish on degraded substrates and increase nitrogen availability over time (Vitousek and Sanford 1986, Franco and DeFaria 1997a, Chaer et al. 2011, Joslin et al. 2011). As a C_4 grass, shade strongly inhibits *S. spontaneum* growth by reducing ATP regeneration and carbon fixation. In Panama, numerous species of legume trees have been recommended for restoration due to their ability to establish rapidly, produce deep canopy cover, reduce *S. spontaneum* persistence, and enhance understory tree cover more than non-legume species (Jones et al. 2004, Wishnie et al. 2007).

Variation in *S. spontaneum* performance under different tree species with similar shade suggests that the mechanisms through which overstory trees inhibit understory *S. spontaneum* persistence are more complex than simple competition for light. Although nitrogen fixers are generally associated with increasing nitrogen in systems, they have also been shown to cause significant phosphorus depletion (e.g., Hooper and Vitousek 1998). Earlier studies have suggested that reduced P in can limit P_i recycling (Stitt 1986) and reduce ribulose-1,5-bisphosphate regeneration (Reich et al. 2009), which can have pronounced effects on *S. spontaneum* at low light. The potential for increased light and nutrient competition highlights the importance of understanding how trees suppress *S. spontaneum* regeneration and the extent to which competition is increased in the presence of legume trees.

This dissertation focused on two major topics of interest: (1) how the effects of legume trees on light and soils influence *S. spontaneum* abundance, and (2) the practical application of using leguminous agricultural species to inhibit *S. spontaneum* growth. To address these topics this thesis was divided into three chapters. In chapter one I evaluated the response of *S. spontaneum* along a gradient of understory light conditions in plots of mono-specific restoration trees. Reduced *S. spontaneum* density under legume vs. non-legume trees at canopy openness <18% suggests increased *S. spontaneum* suppression under legume trees. I conducted a number of field and greenhouse studies to determine whether above- or below-ground factors drove variation in *S. spontaneum* suppression, including stronger effects of legume trees. I compared *S. spontaneum* foliar N and P availability, along with soil N and P availability, across plots to determine whether variation in grass density could be explained by reduced soil and foliar phosphorus under legume trees.

Legumes consistently suppressed *S. spontaneum* survival when grown in low light understories, and in the absence of below ground competition. Leaf N:P ratios were significantly higher for *S. spontaneum* occurring under legumes. Although not statistically significant, nutrient concentrations followed our predicted trend towards higher soil N, lower soil P and *S. spontaneum* leaf P, which suggests that legume overstory trees may be competing for P with *S. spontaneum*.

Chapter two evaluates the potential for agricultural legumes to suppress *S. spontaneum* through nutrient competition. Local Panamanian farmers have historically used the woody agricultural legume species *Cajanus cajan* (aka guandu, pigeon pea) to suppress *S. spontaneum* growth. This experiment was designed to test the potential for *C. cajan* to out-compete *S. spontaneum* for nutrients, and to evaluate nutrient limitation on the growth and density of *S. spontaneum*. I evaluated the response of *S. spontaneum* above ground biomass and density to nitrogen, phosphorus, and nitrogen+phosphorus addition in the presence and absence of *C. cajan*. I was unable to evaluate interspecific competition between *C. cajan* and *S. spontaneum* due late sowing and low growth of *C. cajan*. However, temporal variation in *S. spontaneum* biomass in response to N and P, and density to N, indicates that *S. spontaneum* is nutrient limited in this system, and that growth allocation varies temporally.

In addition to direct competitive interactions for nutrients, reduced *S. spontaneum* density under legumes may be due to suppression through allelopathy. In chapter three I explore the potential use of allelopathy as a tool in forest restoration and exotic species management. I begin by reviewing literature from agroforestry, summarizing the approaches taken and evidence provided for allelopathic effects of trees on herbaceous weeds and crops. I then focus on allelopathy in the context of invasive species, examining situations where allelopathic species and response species do not share an evolutionary history. Finally, I present data from two greenhouse studies that test the allelopathic potential of leaves from trees used for forest restoration to suppress the growth of the exotic invasive grass *S. spontaneum* L. We found fairly weak evidence for allelopathic effects of trees on *S. spontaneum* in our study; in some cases addition of tree litter even enhanced growth and survival of the grass. These results suggest that caution and particular attention be given to species-specific effects when recommending particular species for restoration.

The overall objective of these studies is to determine whether legume overstory species are altering the understory abiotic environment in such a way that legumes reduce *S. spontaneum* abundance below the expected reductions associated with shade. These experiments specifically focus on the influence of light, nutrients, and allelopathy on *S. spontaneum* performance. Overall, the results from these studies suggest that legumes are reducing understory *S. spontaneum* density through more rapid canopy cover closure when compared to non-legumes. Although phosphorus competition and allelopathy may indirectly contribute to understory *S. spontaneum* suppression, it is still unclear how important these mechanisms are for suppression of *S. spontaneum.* Future studies will need to evaluate the physiological response of *S. spontaneum* and overstory trees to different N and P concentrations, and isolate, identify, and test the effects of potential allelochemical produced by trees on *S. spontaneum* persistence.

Works Cited

Chaer, G. M., A. S. Resende, E. F. C. Campello, S. M. de Faria, and R. M. Boddey. 2011. Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. Tree Physiology **31**:139-149.

Craven, D., J. Hall, and J. M. Verjans. 2009. Impacts of herbicide application and mechanical cleanings on growth and mortality of two timber species in *Saccharum spontaneum* grasslands of the Panama Canal Watershed. Restoration Ecology **17**:751-761.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Franco, A. A. and S. M. DeFaria. 1997. The contribution of N-2 fixing tree legumes to land reclamation and sustainability in the tropics. Soil Biology & Biochemistry **29**:897-903.

Gibson, D. J. 2008. Grasses and grassland ecology. Oxford University Press, USA.

Hall, J. S., B. E. Love, E. J. Garen, J. L. Slusser, K. Saltonstall, S. Mathias, M. van Breugel, D. Ibarra, E. W. Bork, D. Spaner, M. H. Wishnie, and M. S. Ashton.

2011. Tree plantations on farms: Evaluating growth and potential for success. Forest Ecology and Management **261**:1675-1683.

Hammond, B. W. 1998. *Saccharum spontaneum* (Gramineae) in Panama. Journal of Sustainable Forestry **8**:23-38.

Holl, K. D. 1999. Factors limiting tropical rain forest regeneration in abandoned pasture: seed rain, seed germination, microclimate, and soil. Biotropica **31**:229-242.

Holl, K. D., M. E. Loik, E. H. V. Lin, and I. A. Samuels. 2000. Tropical montane forest restoration in Costa Rica: overcoming barriers to dispersal and establishment. Restoration Ecology **8**:339-349.

Holm, L., J. V. Pancho, J. P. Herberger, and D. L. Plucknett. 1979. A geographical atlas of world weeds. New York.

Hooper, E., R. Condit, and P. Legendre. 2002. Responses of 20 native tree species to reforestation strategies for abandoned farmland in Panama. Ecological Applications **12**:1626-1641.

Hooper, E., P. Legendre, and R. Condit. 2005. Barriers to forest regeneration of deforested and abandoned land in Panama. Journal of Applied Ecology **42**:1165-1174.

Hooper, E. R., P. Legendre, and R. Condit. 2004. Factors affecting community composition of forest regeneration in deforested, abandoned land in Panama. Ecology **85**:3313-3326.

Jones, E. R., M. H. Wishnie, J. Deago, A. Sautu, and A. Cerezo. 2004. Facilitating natural regeneration in *Saccharum spontaneum* (L.) grasslands within the Panama Canal Watershed: effects of tree species and tree structure on vegetation recruitment patterns. Forest Ecology and Management **191**:171-183.

Joslin, A. H., D. Markewitz, L. A. Morris, F. D. Oliveira, R. O. Figueiredo, and O. R. Kato. 2011. Five native tree species and manioc under slash-and-mulch agroforestry in the eastern Amazon of Brazil: plant growth and soil responses. Agroforestry Systems **81**:1-14.

Lambers, H., F. S. Chapin, and T. L. Pons. 2008. Plant physiological ecology. Springer Verlag.

Loik, M. E. and K. D. Holl. 1999. Photosynthetic responses to light for rainforest seedlings planted in abandoned pasture, Costa Rica. Restoration Ecology **7**:382-391.

Reich, P. B., J. Oleksyn, and I. J. Wright. 2009. Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia **160**:207-212.

Saltonstall, K. and G. D. Bonnett. 2012. Fire promotes growth and reproduction of *Saccharum spontaneum* (L.) in Panama. Biological Invasions **14**:2479-2488.

Stitt, M. 1986. Limitation of photosynthesis by carbon metabolism. 1. Evidence for excess electron-transport capacity in leaves carrying out photosynthesis in saturating light and CO2 Plant Physiology **81**:1115-1122.

Vitousek, P. M. and R. L. Sanford. 1986. Nutrtient cycling in moist tropical forest Annual Review of Ecology and Systematics **17**:137-167.

Wishnie, M. H., D. H. Dent, E. Mariscal, J. Deago, N. Cedeno, D. Ibarra, R. Condit, and P. M. S. Ashton. 2007. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. Forest Ecology and Management **243**:39-49.

CHAPTER 1

Evaluating the suppressive effects of restoration trees on the persistence of the exotic grass *Saccharum spontaneum* L. in the Panama Canal Watershed

Abstract

A major focus of restoration ecology is identifying and understanding why some species are better candidates than others in habitat rehabilitation efforts. In Panama, habitats invaded by *Saccharum spontaneum* are a target for such restoration and this study identifies the characteristics of trees that may serve to inhibit the growth of this aggressive C_4 grass. Recent restoration efforts have attempted to suppress the regeneration of *S. spontaneum* through increasing understory shade. We found reduced *S. spontaneum* performance under legume trees at low light levels when compared to non-legume trees, which suggests that legumes may further suppress *S. spontaneum* persistence through resource competition. We conducted a number of field and greenhouse studies to determine whether above- or below-ground competition resulted in increased *S. spontaneum* suppression, and whether competition was strongest under legume trees. Our results suggest that legume trees are creating darker understory conditions, which strongly inhibits the below ground biomass production of *S. spontaneum* and reduces persistence. We also found increased *S. spontaneum* leaf N:P ratio in the presence of legumes, suggesting that legumes may be competing with *S. spontaneum* for phosphorus. We recommend that future

studies determine how increased phosphorus competition at low light between *S. spontaneum* and legume trees influences *S. spontaneum* photosynthesis and resource allocation. We also recommend the incorporation of legume trees in forest restoration models in areas where *S. spontaneum* occurs to increase competition and suppression of *S. spontaneum* when it occurs.

Introduction

When agricultural lands are abandoned in the moist tropics, natural succession will often lead to the regeneration of forests (Aide et al. 1995, Aide et al. 1996, Aide et al. 2000, Marcano-Vega et al. 2002, Letcher and Chazdon 2009). In many cases, high light conditions, coupled with the low nutrient availability that follows intense agriculture practices, can hinder natural succession and create an opportunity for exotic species to colonize (Aide and Cavelier 1994, Rivera et al. 2000, Zimmerman et al. 2000, Cubina and Aide 2001, Chazdon 2003, 2008). In particular, many exotic C_4 grasses that are considered noxious pests readily colonize post agricultural sites in the tropics and inhibit subsequent forest succession (Aide et al. 1995, Hooper et al. 2004, Gibson 2008).

Many C_4 grasses were introduced to Central America, South America, and the Caribbean for pastoral grazing because of their high initial productivity and their ability to spread rapidly (Parsons 1972, D'Antonio and Vitousek 1992, Aide and Cavelier 1994). Neotropical regions have been widely invaded by exotic grasses including *Panicum maximum* Jacq. (Guinea Grass), *Brachiaria mutica* (Forsk.) Stapf (Pará Grass), *Melinis minutiflora* Beauv. (Molasses Grass), *Hyparrhenia rufa* (Nees) Staph (jaraguá), *Pennisetum clandestinum* Holcht. (Kikuyu Grass), and *Digitaria decumbens* Stent (Pangola Grass) (Parsons 1972). Through seed dispersal by wind and animals, as well as through vegetative reproduction and accidental dispersal by humans, these grasses are able to disperse widely and colonize disturbed areas away from where they were planted, initiating an anthropogenic savannization process (Hooper et al. 2005). For example, in a lowland Bolivian forest, invasion of disturbed sites by grasses is facilitated by dispersal of seeds on logging vehicles (Veldman and Putz 2010). In another study, between 1986 and 2005, 1420 km^2 of removed forest was replaced by derived savannahs comprised of non-native and non-savannah grass species that were floristically distinct from natural savannahs (Veldman and Putz 2011).

Exotic C_4 grasses have several physiological advantages that allow them to form mono-dominant stands under the abiotic conditions that follow abandonment of tropical agricultural lands. Under high light and temperature, and low nutrient conditions, C_4 species are able to maintain high levels of resource use efficiency, maximizing net primary production when compared to most C_3 species (Parrotta 1992, Sage and Monson 1999, Niu et al. 2006, Funk and Vitousek 2007, Cristiano et al. 2012).

Loss of soil nutrients due to intensive agriculture can reduce growth, germination, and natural succession by native species after agricultural abandonment (Aide and Cavelier 1994, Moran et al. 2000, Lu et al. 2002, Lawrence et al. 2007). A reduction in the rate of succession can allow for the establishment and dominance by exotic grasses. The resulting high biomass of grasses can increase fire frequency and accentuate the disturbance regime, creating a stable state where the presence of the exotic species inhibits succession to forest (Parrotta 1992, Aide et al. 1995, Nepstad et al. 1996, Hooper et al. 2002, Hooper et al. 2004, Jones et al. 2004, Gilbert and Lechowicz 2005, Craven et al. 2009, Hobbs et al. 2009).

Restoration efforts aim to push the systems out of the grassland state and into a successional trajectory to forest cover. Developing effective approaches to establish trees in exotic grasslands is a major challenge to restoration ecology in the tropics (Holl 1999, Loik and Holl 1999, Holl et al. 2000, Hooper et al. 2004, Jones et al. 2004). Numerous studies have compared growth, survival, canopy cover, and wood density of a range of tree species to identify the best species for reforesting degraded and invaded habitats (Hooper et al. 2002, Jones et al. 2004, Wishnie et al. 2007, Craven et al. 2009). Eliminating grass competition through mechanical or chemical above-ground removal or trenching can increase restoration tree establishment (Holl 1998, Wishnie et al. 2007, van Breugel et al. 2011). Once established, native overstory tree species have a variety of impacts on the surrounding abiotic environment. Shade produced by overstory species limits the growth of C_4 grasses and shade-intolerant species, and creates conditions that favor shade-tolerant species (Somarriba 1988, Hooper et al. 2002, Jones et al. 2004, Cole and Weltzin 2005, Joo Kim et al. 2008, Lambers et al. 2008, Perry et al. 2009). Below-ground competition can further suppress grasses. For example, understory grass productivity in savannahs increased when trenching removed below-ground competition with the tree *Acacia tortilis* (Belsky 1994, Ludwig et al. 2004a). Such limitations on grass productivity were driven by resource competition for phosphorus, and water (Ludwig et al. 2001). Restoration projects that include a variety of tree species planted into grasslands permit evaluation of which tree species are most effective at reducing grass productivity and persistence, and the possibility of understanding which mechanisms are most important in reclaiming anthropogenic savannahs.

Nitrogen fixing trees, in particular, may have unique effects on below-ground processes during forest restoration. Legume trees are often recommended for restoration because they can establish on highly degraded soils, and over time, increase nutrient availability through nitrogen fixation (Wang et al. 2010, Chaer et al. 2011). The ability of legumes to fix nitrogen can increase tropical soil fertility and facilitate colonization of species that would have been otherwise nitrogen limited (Franco and DeFaria 1997b). Although nitrogen fixers are usually thought to increase nitrogen in systems, they have high demands for both nitrogen and phosphorus and can be strong competitors for limiting resources during the early stages of restoration (Ledgard and Steele 1992, Siddique et al. 2008). Legumes can cause significant soil phosphorus depletion, and even decrease the density of other plant species through competition for phosphorus (Hooper and Vitousek 1998, Ludwig et al. 2001). For example, in a tropical savannah, phosphorus additions relieved nutrient limitation of grasses growing in the understory of *Acacia* trees (Ludwig et al. 2001). The potential for phosphorus competition creates an opportunity to test whether legume trees may be effective at reducing phosphorus availability to a level that suppresses *S. spontaneum* growth.

Saccharum spontaneum

Saccharum spontaneum L. subsp. *spontaneum* is a large, exotic grass species that invades abandoned agricultural fields in many tropical countries. It inhibits forest regeneration and slows reforestation in the areas where it occurs (Hooper et al. 2004, Jones et al. 2004, Hooper et al. 2005). *S. spontaneum* is adapted to drought, burns frequently, and is difficult to eradicate. These attributes make it one of the most serious weeds in many tropical areas (Holm et al. 1979, Hooper et al. 2004). In Panama, *S. spontaneum* is a major agricultural and environmental weed (Hammond 1998). Although numerous explanations have been offered for how *S. spontaneum* arrived to Panama, evidence suggests that it was introduced to the Canal Zone Experimental Gardens for sugarcane breeding in 1928. It was not until the 1960s or 1970s, however, that it was found around the Panama Canal Watershed in disturbed areas (Craven et al. 2009).

In Panama, reforestation of abandoned agricultural lands necessarily includes active control of *S. spontaneum*, until planted trees grow to produce enough shade to effectively outcompete *S. spontaneum* (Wishnie et al. 2007). In addition, anecdotal accounts from local farmers report that planting woody legumes crops (such as pigeon pea, *Cajanus cajun (L.)* Millsp.), helps suppress growth of *S. spontaneum.* A shade cloth experiment showed that growth of *S. spontaneum* was reduced at light intensities ≤ 25% (Joo Kim et al. 2008), but there have been no empirical field tests of the effects of shade or legumes on *S. spontaneum* growth in the field.

Variation in growth, survival, and canopy structure among the different tree species used for restoration creates an excellent opportunity to examine how *S. spontaneum* performs across a gradient of naturally occurring light conditions. It also allows for a test of the hypothesis that legumes have special effects on *S. spontaneum* density and growth. We conducted a number of field and greenhouse studies to determine how light and below-ground factors influence *S. spontaneum* suppression under legume and non-legume trees.

We compared *S. spontaneum* density and biomass across a gradient of understory light under legume and non-legume trees. We also compared soil nutrient availability and *S. spontaneum* foliar nutrient concentrations between legume and non-legume plots. Finally we separated above ground and below ground interactions by growing *S. spontaneum* tillers soil from beneath legume and non-legume overstory trees under a homogenous shade environment in the greenhouse, and by growing *S. spontaneum* tillers in nursery soil under legume and non-legume trees.

We expected *S. spontaneum* density and biomass to decrease with decreasing understory light. We predicted that under light limiting conditions, legume overstory trees would suppress *S. spontaneum* growth more than non-legumes due to increased below ground competition. Reduced density would be the result of increased phosphorus competition as revealed by lower soil and *S. spontaneum* leaf P concentrations, and by reduced *S. spontaneum* growth in soils collected beneath legumes. We did not expect to observe differences in *S. spontaneum* growth in the understory of legume and non-legume trees when understory below ground competition is removed.

Methods

Field Site

Field studies were carried out in a subset of mono-specific plots located in Parque Nacional Soberanía in the Panama Canal Watershed established by the Native Species Reforestation Project (PRORENA). PRORENA is a collaborative program in Panama between the Smithsonian Tropical Research Institute, the Center for Tropical Forest Science, and the Tropical Resources Institute at the Yale School of Forestry and Environmental Studies. The goal of PRORENA is to develop viable strategies for restoring diverse, native tropical forests (http://prorena.research.yale.edu).

The PRORENA Native Species Reforestation site experiences a mean rainfall of 2226 mm and 4.1 dry months annually (defined as months with < 100 mm rainfall) (Wishnie et al. 2007). Soils at this site are predominantly clay and silty clay loams, and are classified as ultisols (Park et al. 2010). The forest at this site was cleared by 1960, and the area underwent small-scale grazing and agriculture until its abandonment around 1993. From 1993-2003 *S. spontaneum* was the dominant species at this site. Mono-specific, 9 x 12m plots plots of native and commercially important tree species were established by PRORENA in 2003, and *S. spontaneum* was controlled to ensure tree establishment until 2007. In this study we used a total of 42, for 19 tree species with three replicate plots per species when possible (Table 1). Tree species choice attempted to capture the full gradient of light intensities experienced by *S. spontaneum* under both legume and non-legume overstory trees. Plots and species where no trees survived, or were inaccessible, were excluded from the study.

S. spontaneum suppression under shade

To quantify the response of *S. spontaneum* to increasing shade, we measured *S. spontaneum* density and biomass, along with canopy openness, in 40 plots under 17 tree species (Table 1). Measurements were taken during the rainy season from August – November of 2009. In each plot we randomly selected three points within the plot, excluding a 1m buffer zone from the plot edge (n=120). At each point, we took hemispherical canopy photos using a Nikon Coolpix 955 digital camera with a fisheye lens. Photos were analyzed using the Gap Light Analyzer 2.0 program (Simon Fraser University, Burnaby, British Columbia, CANADA; Institute of Ecosystem Studies, Millbrook, New York, USA) to quantify mean canopy openness for each plot. At each point we also counted the number of stems of *S. spontaneum* in 1-m radius circles, and we measured basal stem diameter, height to the tallest leaf, height to the tallest leaf node, and number of live leaves of five randomly selected individuals. In a subset of 30 circles, we collected, dried, and weighed the above ground biomass of the five randomly selected individual stems, as well as the total above ground biomass. We were unable to remove *S. spontaneum* from plots that were shared with other investigators. From the 30 circles, where biomass was collected we constructed the following allometric equation to estimate *S. spontaneum* biomass (dry weight, in kg) from density (stems per m^2) and mean basal diameter (in mm) within a 1-m radius circle ($R^2 = 0.68$, $p < 0.001$):

Log_{10} (biomass (kg)) = -1.2677+(0.8567*mean basal diameter (mm))+(0.0327* density (m^2)).

We then used density and basal diameter data to estimate biomass in plots where we were unable to physically remove *S. spontaneum.*

Nutrient comparisons: Soil nutrient availability and leaf nutrient concentrations

We evaluated nutrient availability under 18 tree species in 42 plots (6 legume species with 17 plots; 12 non-legume species with 25 plots), to determine whether legume overstory species cause nutrient limitation in *S. spontaneum* (Table 1). We took 10 soil cores (1 cm diameter and 6 cm deep) haphazardly in each plot. Soil cores were homogenized within each plot, and a subsample was used to determine available nitrogen and phosphorus concentrations. We followed standardized KCl (Maynard DG 1993) and Mehlich (Mehlich 1984) protocols for the extraction of soil nitrogen and phosphorus. Samples were run on a Lachat Quickchem 8500 Flow Injection Analyzer (Lachat Insturments, Milwaukee, WI USA) to determine ammonium (NH_4^+), nitrate (NO_3^-), total N ($NH_4^+ + NO_3^-$), and phosphorus HPO_4^{-2} concentrations (Baldeck et al. 2013). Soil nutrient concentrations are reported as mg/kg.

Leaf N:P ratios have been used in a number of systems to determine nutrient limitation (Koerselman and Meuleman 1996, Ludwig et al. 2004b, James 2008). We used the concentration of nutrients in leaves of *S. spontaneum* to compare nutrient limitation across tree species. We collected leaves from 15 randomly selected *S. spontaneum* individuals from each of the 40 plots used in the understory density/biomass study (Table 1). On each plant, the third mature, non-senescent leaf from the base was collected. In plots where there were 15 individuals or fewer, one leaf was taken from every individual. From the 15 leaves collected for each plot, five leaves were randomly selected for N and P extraction. Nitrogen and phosphorus were extracted from leaves following the Kjeldahl acid digestion protocol (Novozamsky et al. 1983)using the Lachat BD 46 block digester (Lachat Instruments, Milwaukee, WI USA). Samples were run on a Lachat Quickchem 8000 Flow Injection Analyzer (Lachat Insturments, Milwaukee, WI USA) and nitrogen and phosphorus concentrations were reported as mg/g leaf material. We compared foliar nutrient concentrations across legume and non-legume plots using a nested ANOVA with species nested within functional group.

Effects of soil on S. spontaneum growth in the greenhouse

To determine whether overstory species change the soil environment in such a way that it discourages the persistence of *S. spontaneum*, we conducted a greenhouse experiment in which tillers of *S. spontaneum* were grown in soils from beneath different tree species.

For 17 tree species (Table 1), we randomly selected five trees from a single plot and collected roughly equal amounts of soil 1m away from each tree to a depth of 10cm. Soils were taken to the greenhouse and homogenized by species, and tillers of *S. spontaneum* were planted in 1L nursery bags (n=15/species). Before planting, we measured the height of the tallest leaf node and basal stem diameter of each tiller. Individuals were randomized in three blocks (n = 255) by location in the greenhouse, and grown for 57 days under 80% shade cloth. At harvest, we measured survival, height, and diameter. We separated and collected above- and below-ground biomass, dried the biomass material for four days at 60^{o}C, and obtained dry weights.

S. spontaneum plantation bioassay

To isolate the influence of light on *S. spontaneum* under different overstory species, we did a bioassay in the field with plants in flats. We planted tillers of *S. spontaneum* in nursery bags filled with gardening soil, placed five bags in flats, and placed three flats at random locations in 18 plots (nine plots for three legume species, nine plots for three non-legume species). Gardening soil was used to equalize soil nutrient concentrations in the field. Initial height of highest leaf node and basal diameter were measured for each individual. We quantified canopy cover above each flat using hemispherical photos, as above. This project was replicated in two successive years at the same points in the same plots, and canopy cover was quantified in each year at the end of the rainy season. In 2010 plants were left in the field for one month from November to December. In 2011, plants were left in the field for 1.5 months from mid October to December. At the end of each study we measured final height and diameter, counted the number of new tillers, and collected, dried, and measured above- and below-ground biomass.

Data analysis

We conducted an ANCOVA to look at the response of *S. spontaneum* density and biomass to increasing understory shade, and to compare the response between legume and non-legume trees. In this system, one goal of restoration is to establish closed canopies that shade out exotic grasses. Kim et al. (Joo Kim et al. 2008) found that *S. spontaneum* biomass significantly reduced when artificial shade reduces light below <25%. Because we observed that in deep shade *S. spontaneum* density varied strikingly, we also examined how *S. spontaneum* responded to canopy openness and legume/non-legume canopies in only those plots with the densest shade ($\leq$ 18% canopy openness).

We tested whether soil and leaf nutrient concentration differed between legume and non-legume canopy species using a nested analysis of variance with tree species nested within legume/non-legume groups. Because we observed a strong effect of shade on *S. spontaneum* density, we removed the effect of shade with a residual analysis to ask whether variation in nutrient conditions among plantations of different tree species could explain the remaining variation in *S. spontaneum* density. To do so, we calculated the residuals from a linear regression of grass density on canopy openness (hereafter, density residuals).

We then performed a linear regression of these residuals against each of the soil and leaf nutrients we measured.

In our greenhouse experiment, we used a nested ANOVA to compare below ground biomass, above ground biomass, total biomass, change in height, and change in diameter between legume and non-legume species with species as our nested effect. *S. spontaneum* tiller survival was analyzed using a general linear model with binomial errors.

In the outplanting experiment, we conducted a nested ANOVA to compare canopy openness between legume and non-legume plots. A logistic regression was used to test the effects of canopy openness and legume/non-legume overstory trees on outplant survival. We also performed an analysis of covariance (ANCOVA) to evaluate the effects of legume/non-legume and shade on height, diameter, above ground biomass, below ground biomass and total biomass of outplanted tillers. We conducted an AIC comparison of models to determine whether the interaction term (functional group X canopy openness) should be included in the analyses. *S. spontaneum* survival was analyzed using a logistic regression to compare the proportion survived as a function of canopy openness, and legume/non-legume trees. All analyses were done using the R statistical software program (version 2.14.0, The R Foundation for Statistical

Computing). Note that the canopy*functional group interaction terms was never significant ($p > 0.05$ for all) and was removed from all ANCOVA models.

Results

S. spontaneum density and biomass

Both *S. spontaneum* density (R^2=0.57, $F_{(2,37)}$=26.44, p $\leq$ 0.001, fig. 1A) and biomass (R^2 = 0.33; $F_{(2,36)}$=10.27; t=4.509; p$\leq$0.001; fig 1C) showed a significant positive relationship with canopy openness. Neither *S. spontaneum* density (t=0.71; $F_{(2,37)}$=26.44; p= 0.48, fig 1A) nor biomass (t=0.26; $F_{(2,36)}$=10.27, p= 0.80, fig 1C) differed between legume and non-legume overstory species.

For the subset of plots at the lowest canopy openness (≤18% openness), both density ($F_{(2,22)}$=12.93; R^2=0.50, p $\leq$ 0.001; Fig 1B) and biomass ($F_{(2,21)}$=2.76; R^2=0.13; p $\leq$ 0.03; Fig 1D;) increased with canopy openness. At these low light levels, *Saccharum* density was significantly lower under legume trees ($F_{(2,22)}$ =12.93; t=3.77; p $\leq$ 0.001; fig 1B). Biomass of *S. spontaneum,* however, was not significantly different between legume and non-legume groups ($F_{(2,21)}$=2.76; t=-0.05; p =0.96; fig1D).

Nutrient comparisons: Soil nutrient availability and leaf nutrient concentrations

We found no differences in soil or leaf nutrient concentration between legume and non-legume plots (Table 3). However, the leaf N:P ratio was significantly higher under legumes ($F_{(1,14)}=6.27$; $p<0.02$; Table 3). None of the nutrients measured significantly explained variation in the residuals from the linear regression of density on canopy openness (Table 4). However, there was a marginally significant effect of soil P on residual *S. spontaneum* density, with greater residual density in plots with higher phosphorus (df=1,23; t=1.90; p=0.07; Table 4).

Results: Greenhouse soil isolation experiment

We found a significant effect of species on below ground biomass ($F_{(1,15)}=0.21$; $p<0.03$); Tale 2) with marginally significant trend towards more below ground biomass in soils from under legumes ($F_{(1,15)}=3.64$; $p=0.06$; Table 2).

S. spontaneum field plot bioassay

For *S. spontaneum* plants set out into plantations in bags of greenhouse soil, we found that canopy openness significantly increased survival in the first year of our study, but not in the second year (year 1: df=15, $p \leq 0.007$, fig 2A; year 2: df=15, p=0.11, fig 2B; Table 5). Survival of *S. spontaneum* was significantly lower under legume trees than non-legumes in both years (year 1: df=15, $p \leq 0.001$, fig

2A; year 2: df = 15, $p \leq 0.001$, fig 2B; Table 5). However, legume plots were also significantly darker than non-legume plots across both years (year 1: $F_{(1,4)}=20.08$, $p \leq 0.001$; year 2: $F_{(1,4)}=22.13$; $p \leq 0.001$) with a significant species effect in the first year but not in the second (year 1: $F_{(1,4)}=8.43$, $P \leq 0.001$; year 2: $F_{(1,4)}=1.37$, $p=0.30$). We found no effect of canopy openness or legume overstory species on the change in height (Table 5), diameter (Table 5), or above ground biomass (fig. 3; Table 5). There was a significant increase in below-ground biomass with increasing canopy openness in year 1 (df=15, $p \leq 0.001$, fig 4A; Table 5) but not in year 2 (df = 13, p = 0.20, fig 4B; Table 5). Below ground biomass was also marginally significantly reduced in legume plots in both years (year 1: df = 15, p = 0.06, fig 4A; year 2: df = 13, p = 0.08, fig 4B; Table 5).

Discussion

Plantation tree species that generated deeper shade were more effective in suppressing *S. spontaneum*. This is consistent with shade cloth experiments showing that shade suppresses growth of *Saccharum spontaneum* (Joo Kim et al. 2008). Since C_4 plants require an additional molecule of ATP for CO_2 fixation, reduced ATP synthesis under shade conditions likely decreases ATP availability and significantly diminishes CO_2 fixation in *S. spontaneum* (Ehleringer 1978, Sage and Monson 1999).

Legume tree species in particular created understories with low light and were especially effective at suppressing *S. spontaneum.* In Panama, several species of legume trees have been recommended for restoration due to their ability to produce large dense crowns and attain canopy closure after two years (Wishnie et al. 2007). In our study, understory *S. spontaneum* density decreased with increasing canopy cover, but under legume trees, density was also significantly lower than expected at low light. Biomass decreased with increasing canopy cover at low light, biomass was not lower under legume overstory species. Biomass when light was <18% was only weakly associated with degree of light. This finding was consistent with the findings of Joo Kim et al. 2008, where below 25% light intensity there was no difference in *S. spontaneum* biomass across light levels. This suggests that at low light, *S. spontaneum* establishment of new individuals declines more quickly than the size of persistent individuals.

When we evaluated the response of *S. spontaneum* to low light in the absence of below ground competition, we found that survival of *S. spontaneum* was consistently lower under legume overstory trees. Although light intensities overlapped between legume and non-legume plots in the first year of study, legume plots were darker than non-legume across both years of study. A more narrow range of canopy openness under legume trees suggests that legume understories were becoming darker more rapidly than non-legumes, leading to a greater reduction in understory *S. spontaneum* survival. We also observed a significant reduction of below ground biomass accumulation in the understory of legume trees when *S. spontaneum* was grown in the absence of below ground competition. This is similar to demonstrated inhibition of rhizome production by *Cynodon dactylon* (Bermuda grass) under low light and low nutrient conditions (Dong and Dekroon 1994). Reduced rhizome and stolon production can reduce the ability of persistent understory grasses to forage for available water and nutrients, and inhibit vegetative reproduction. Reduced below ground biomass production could impede the ability of *S. spontaneum* to thrive in the dry season, especially in the understory of evergreen trees. Future studies in our system may evaluate the effect of reduced below ground biomass on water competition during the dry season*.*

One reason for the observed differences in the physiological response of *S. spontaneum* under legumes might be differences in the distribution of light. At low light, the reduced density of *S. spontaneum* under legumes may be explained by differences in the distribution of sunflecks*.* In one study, invasion by the invasive grass *Microstegium vimineum* (Trin) into the understory of *Asimina triloba* was reduced by the lack of sunflecks to promote the invasion (Cole and Weltzin 2005). This is the result of the interaction between overstory and midstory canopies reducing the amount of sunflecks at the ground level.

Increased density of secondary understory species under legumes can contribute to reducing sun flecks and light availability. In previous studies, legumes not only recruit more tree seedlings (Jones et al. 2004), but seedling survival is also higher under legume trees (Schweizer et al. 2013). In another study, light availability 0.75m above the forest floor decreased with increasing sapling and shrub density (Montgomery and Chazdon 2001). Thus, legumes can help to further increase shade by facilitating the establishment of secondary understory species.

We hypothesized that differences in nutrient availability in legume and non-legume plots may have contributed to the reduced performance of *S. spontaneum* under legumes. Although legumes are associated with increasing nitrogen and phosphorus over long times scales (Vitousek et al. 2002, Ludwig et al. 2004b, Wang et al. 2010), they may be strong nutrient competitors during the early stages of restoration and succession in disturbed systems. Assuming that the only cost of fixation to the plant is through the supply of carbon to the symbiont compared to the cost of nitrogen uptake, nitrogen fixation can be expected to occur in soils when the cost of uptake exceeds that of fixation (Vitousek et al. 2002). In this system, the presence of pink nodules suggests active N-fixation by legumes (Cummings personal observation); high associated demand for P could then lead to increased competition for available P (Israel 1987, Vitousek et al. 2002, Siddique et al. 2008). Soil and leaf nutrients, although not significantly different, followed our predicted trends of higher soil N, lower soil P, lower *S. spontaneum* leaf P, and higher soil N:P and significantly higher leaf N:P ratios under legumes than non-legumes. Earlier studies have suggested that reduced P can limit photosynthesis and ribulose-1,5-bisphosphate regeneration (Reich et al. 2009), which could have pronounced effects on electron transport and carbon fixation in *S. spontaneum* at low light. Thus, higher competition for P may be contributing to the suppression of *S. spontaneum* under legume trees.

Our results are consistent with previous studies that show that although legumes can increase understory nutrient availability, legume trees can impose P limitation on co-occurring C_4 grasses (Ludwig et al. 2001). This was reflected by an increase in the leaf P concentration of grasses in P addition plots(Ludwig et al. 2001). In another study, C_4 grass N:P ratios were higher under large legume trees when compared to small trees and shrubs, and increased with decreasing distance to large trees (Ludwig et al. 2004b). Reduced foliar P in light-limited conditions can have physiological implications leading to reduced *S. spontaneum* performance. Leaf N and P concentrations can interact such that as leaf P increases there are increasingly positive responses of photosynthetic capacity (A_{max}) for a given leaf N (Reich et al. 2009). Thus, reduced leaf P concentrations under light limiting conditions can further reduce C_4 plant performance through reduced photosynthetic capacity.

Nutrient addition studies would be ideal to determine whether higher *S. spontaneum* leaf N:P ratios imply greater P limitation under legume trees in our system (Ludwig et al. 2001). In another study (chapter 3), both N and P limited *S.*

spontaneum growth in plots with full sun. Although we were unable to evaluate the *in-situ* response of understory grasses to increased nutrient availability in our plots, when grasses were grown in the greenhouse on soils from beneath legume and non-legume trees in the absence of below ground competition, there was no difference in *S. spontaneum* survival and above ground growth in soils from beneath legume and non-legume trees. However, increased below ground biomass of *S. spontaneum* in legume soils may be the result of nutrient release through decomposition of legume tissue in the absence of competition. A better understanding of nutrient uptake and cycling could help decipher the competitive interactions that influence the understory persistence of *S. spontaneum.*

In conclusion, legume trees appear to be both good at establishing in highly degraded systems, and are also able to produce deep shade rapidly, reducing the persistence of weedy exotic grasses better than non-legumes. Although legume trees appear to be increasing soil nitrogen in our system, lower *S. spontaneum* leaf P and higher leaf N:P ratios suggest that legume overstory trees may be competing for P with *S. spontaneum*. Light and nutrient limitation can act synergistically to inhibit physiological functions leading to a reduction in *S. spontaneum* persistence. Future studies should evaluate the physiological response of *S. spontaneum* to nutrient limitation in the presence of legume overstory species that are imposing light limitation to determine whether increased resource competition in the presence of legume overstory trees is leading to a reduction in *S. spontaneum* persistence. This study further highlights the benefits of incorporating legume trees into restoration models where exotic grasses inhibit forest succession and complicate restoration effort.

Table 1. List of species from PRORENA plots planted in 2003 that were used in the various studies. For each study the number of plots used for each species is listed.

Species	Family	Legume/Non-legume	Density/ Biomass	Leaf Nutrients	Soil Nutrients	Out-planting	*Saccharum* response to soils
Acacia mangium (Willd.)	Fabaceae	Legume	3	3	3	0	1
Albizia adinocephala (Donn.Sm.) Rose ex Record	Fabaceae	Legume	0	0	0	0	1
Albizia guachapele (Kunth) Dugand	Fabaceae	Legume	2	2	2	0	1
Diphysa americana (Mill.)	Fabaceae	Legume	3	3	3	3	1
Enterolobium cyclocarpum (Jacq.) Griseb.	Fabaceae	Legume	3	3	3	0	1
Gliricidia sepium (Jacq.) Kunth ex. Walp.	Fabaceae	Legume	3	3	3	3	1
Inga punctata (Willd)	Fabaceae	Legume	3	2	3	3	1
Calycophyllum candidissimum (Vahl.) DC.	Rubiaceae	Non-legume	1	1	1	0	1
Cedrela odorata	Meliaceae	Non-legume	1	1	1	0	0

(L.)							
Colubrina glandulosa (Perkins)	Rhamnaceae	Non-legume	2	2	3	0	1
Guazuma ulmifolia (Lam.)	Sterculiaceae	Non-legume	2	2	2	0	1
Luehea seemannii (Planch. & Triana)	Tiliaceae	Non-legume	3	3	3	0	1
Ochroma pyramidale (Cav. ex Lam.) Urb.	Malvaceae	Non-legume	3	3	3	0	1
Pachira quinata (Jacq.) W.S. Alverson	Bombacaeae	Non-legume	3	3	3	3	1
Spondias mombin (Jacq.)	Anacardiaceae	Non-legume	0	0	1	0	0
Tabebuia guayacan (Hemsl)	Bignoniaceae	Non-legume	1	1	1	0	1
Tabebuia rosea (DC.)	Bignoniaceae	Non-legume	1	1	1	0	1
Tectona grandis (L.f.)	Lamiaceae	Non-legume	3	3	3	3	1
Terminalia amazonia (J.F.Gmel) Exell	Combretaceae	Non-legume	3	2	3	3	1

Table 2. *S. spontaneum* performance in soils from beneath legume and non-legume overstory trees. All test statistics are nested ANOVA's, except for survival, which is a χ^2. Test statistics represent z values for χ^2 tests and F values for the nested ANOVA effects of legumes and species. Means (±1 standard deviation) are reported.

Response variable	Legume mean	Non-legume mean	Test statistic	df	Legume P – Value	Species P - value
Survival (%)	53(±21)	56(±15)	0.42	15	0.67	na
Change in height (cm)	21.27(±16.48)	17.28(±17.16)	1.46(0.94)	1,15	0.23	0.52
Change in diameter (mm)	-0.67(±0.91)	-0.72(±1.05)	0.07(0.71)	1,15	0.80	0.77

Above ground biomass (g)	1.44(±0.89)	1.24(±0.79)	2.20(1.20)	1,15	0.14	0.28
Below ground biomass (g)	0.56(±0.4)	0.45(±0.31)	3.64(1.88)	1,15	0.06	0.03
Total biomass (g)	2(±1.19)	1.71(±0.98)	2.61(1.52)	1,15	0.11	0.11

Table. 3. Results from a nested analysis of variance comparing soil nutrient availability and *S. spontaneum* leaf nutrient concentrations between legume and non-legume plots. Means (+/- 1 standard deviation) are reported for legume and non-legume groups.

Response variable	Legume mean (± 1 sd)	Non-legume mean (± 1sd)	F-value	df	p-value
Leaf N (mg/g)	14.44 (3.00)	14.23 (3.08)	0.06	1,15	0.81
Leaf P (mg/g)	1.52 (0.34)	1.71 (0.45)	1.93	1,15	0.18
Leaf N:P	9.84 (1.32)	8.68 (1.32)	6.21	1,15	0.02
Soil NH_4^+ (mg/kg)	15.80 (17.53)	9.96 (4.76)	1.93	1,16	0.18

Soil NO_3^- (mg/kg)	7.69 (19.44)	1.56 (2.45)	2.01	1,16	0.17
Total Soil N (mg/kg)	23.48 (36.65)	11.52 (6.27)	2.03	1,16	0.17
Soil HPO_4^- (mg/kg)	1.42 (0.44)	2.19 (2.77)	1.16	1,16	0.29
Soil N:P	15.41 (19.76)	8.51 (6.81)	1.99	1,16	0.17

Table 4. Linear regression of the *S. spontaneum* density residual values on soil and leaf nutrients for plots where canopy openness is <19% and for all plots. *S. spontaneum* density residual values are from a regression of *S. spontaneum* density on light levels.

Response	<19% Canopy Openness			All plots		
variable	t-value	df	p-value	t-value	df	p-value
Leaf N	0.59	1,21	0.56	0.45	1,36	0.66
Leaf P	0.38	1,21	0.71	0.07	1,36	0.94
Leaf N:P	-0.22	1,21	0.83	0.21	1,36	0.84
Soil NH_4^+	-0.74	1,23	0.47	0.95	1,38	0.35
Soil NO_3^-	-0.63	1,23	0.53	1.02	1,38	0.31
Total soil N	-0.72	1,23	0.48	1.00	1,38	0.32
Soil HPO_4^-	1.90	1,23	0.07	0.62	1,38	0.54
Soil NP	0.51	1,23	0.62	-0.15	1,38	0.89

Table 5. ANCOVA table for the understory *S. spontaneum* response to canopy openness under legume and non-legume overstory tree species across 2 years of study. Test values represent t-values for ANCOVA analyses and Z values for the survival logistic regression. Degrees of freedom for survival analyses represent the degrees of freedom for the entire model.

Response variable	Year 1			Year 2		
	Test value	df	p-value	Test value	df	p-value
Canopy openness		1, 4			1,4	
Legume	20.08		<0.001	22.13		≤0.001
Species	8.43		≤0.002	1.37		0.30
Survival		15			15	
Canopy	2.68		0.007	1.57		0.11
Legume/non-legume	3.49		≤ 0.001	4.08		≤0.001
Change in Height (cm)		2,15			2,13	
Canopy	0.32		0.75	0.66		0.52
Legume/non-legume	0.56		0.59	-0.79		0.44

Change in diameter		2,15			2,13	
(mm)	-0.43		0.67	1.09		0.30
Canopy	0.50		0.63	-0.45		0.66
Legume/non-legume						
Above ground biomass		2,15			2,13	
Canopy	0.94		0.36	0.21		0.84
Legume/non-legume	0.64		0.53	1.62		0.13
Below ground biomass		2,15			2,13	
Canopy	3.08		0.008	1.36		0.20
Legume/non-legume	1.99		0.07	1.93		0.08
Total biomass		2,15			2,13	
Canopy	1.36		0.20	0.48		0.64
Legume/non-legume	0.96		0.35	1.52		0.15

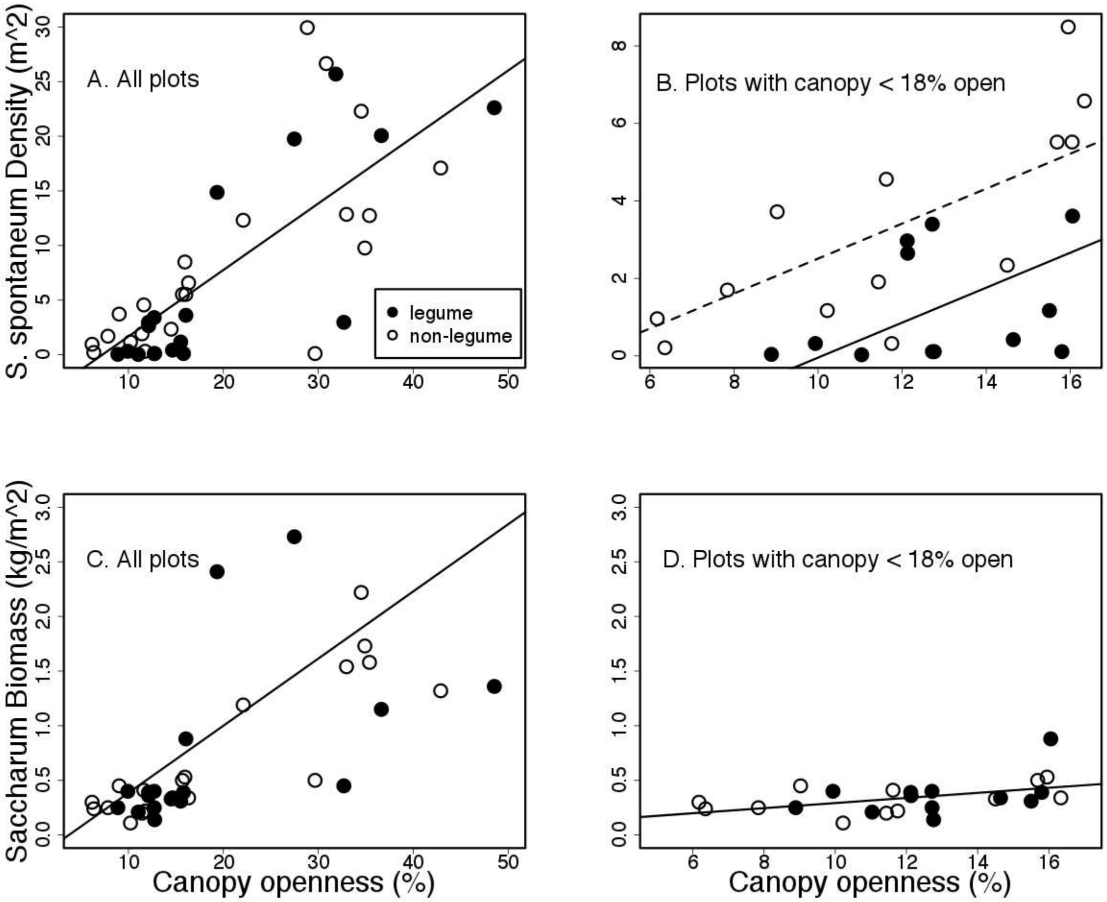

Figure 1. Mean *S. spontaneum* density and biomass as a function of canopy openness. Points represent mean *S. spontaneum* density and biomass for each plot. Open circles represent non-legume overstory species plots and filled circles represent legume overstory species plots. Solid lines represent the regression for all plots in the study in figures 1A, C, and D. In figure 1B the dotted regression line is fitted to the points representing non-legume plots, and the solid line is fitted to the points representing legume plots. A. Density for all plots in the study. B. Density for plots where canopy openness <18%. C. Biomass for all plots in the study. D. Biomass for plots where canopy openness <18%.

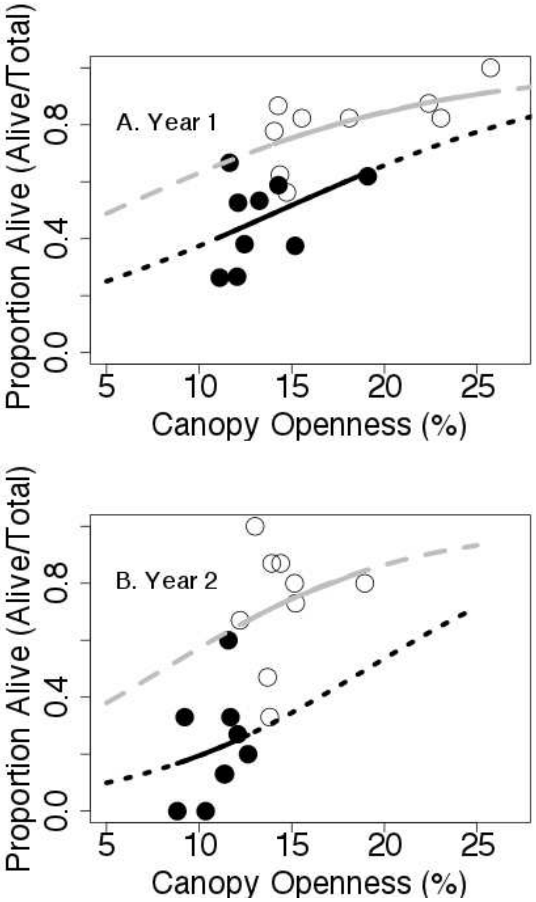

Figure 2. *S. spontaneum* survival in the absence of below ground competition in the first (A) and second (B) year of the study. Open circles (grey dashed lines) represent plots where overstory species were non-legume and filled circles (black dotted lines) represent plots where overstory species are legumes. Dotted sections of the lines represent the expected survival for a given canopy openness, extended beyond observed data for that functional group of canopy trees.

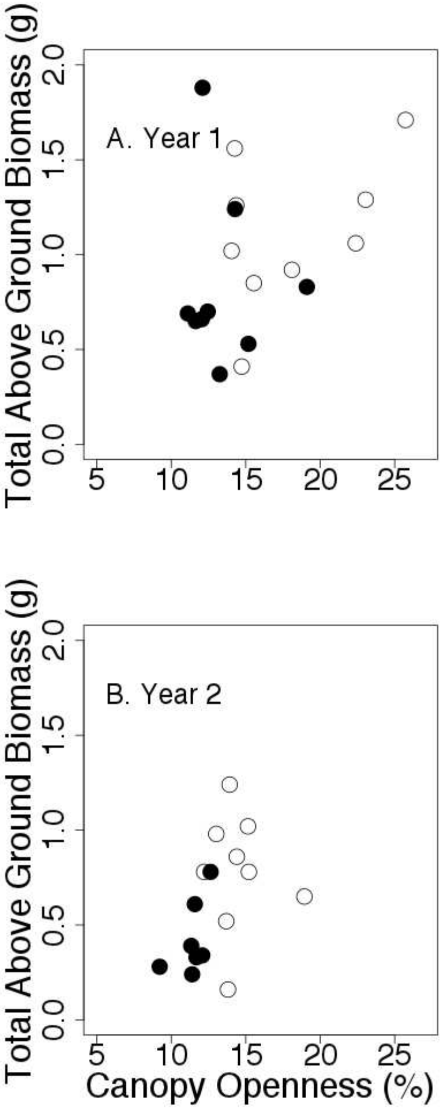

Figure 3. Average *S. spontaneum* above ground biomass in response to canopy openness in the first (A) and second (B) year of the study. Open circles (grey dashed lines) represent plots where overstory species were non-legume and filled circles (black dotted lines) represent plots where overstory species are legumes. There was no significant effect of canopy openness on total *S. spontaneum* above ground biomass in either year.

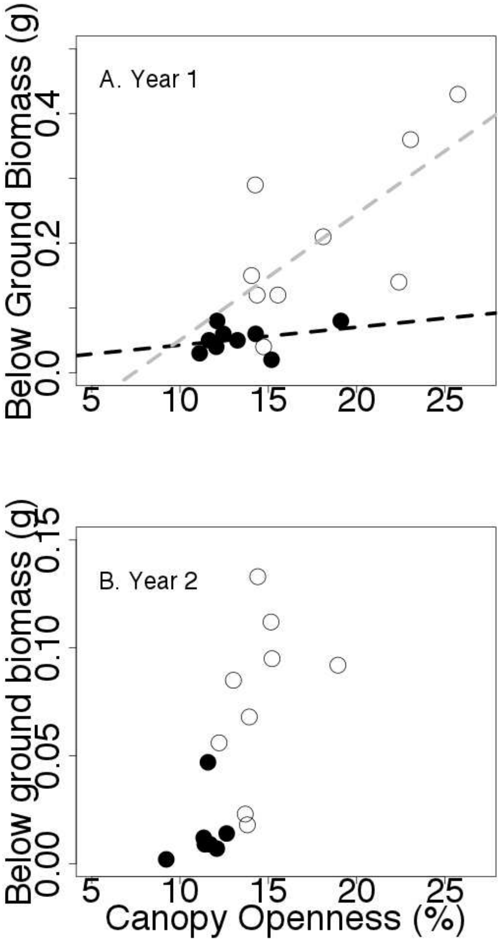

Figure 4. Average *S. spontaneum* below ground biomass in response to canopy openness in the first (A) and second (B) year of the study. Open circles (grey dashed lines) represent plots where overstory species were non-legume and filled circles (black dotted lines) represent plots where overstory species are legumes. Lines in the first year of the study represent linear regressions for the response of below ground biomass to different levels of canopy openness

Works Cited

Aide, T. M. and J. Cavelier. 1994. Barriers to lowland tropical forest restoration in the Sierra Nevada de Santa Marta, Colombia. Restoration Ecology **2**:219-229.

Aide, T. M., J. K. Zimmerman, L. Herrera, M. Rosario, and M. Serrano. 1995. Forest recovery in abandoned tropical pastures in Puerto Rico. Forest Ecology and Management **77**:77-86.

Aide, T. M., J. K. Zimmerman, J. B. Pascarella, L. Rivera, and H. Marcano-Vega. 2000. Forest regeneration in a chronosequence of tropical abandoned pastures: Implications for restoration ecology. Restoration Ecology **8**:328-338.

Aide, T. M., J. K. Zimmerman, M. Rosario, and H. Marcano. 1996. Forest recovery in abandoned cattle pastures along an elevational gradient in northeastern Puerto Rico. Biotropica **28**:537-548.

Baldeck, C. A., K. E. Harms, J. B. Yavitt, R. John, B. L. Turner, R. Valencia, H. Navarrete, S. J. Davies, G. B. Chuyong, D. Kenfack, D. W. Thomas, S. Madawala, N. Gunatilleke, S. Bunyavejchewin, S. Kiratiprayoon, A. Yaacob, M. N. N. Supardi, and J. W. Dalling. 2013. Soil resources and topography shape local tree community structure in tropical forests. Proceedings of the Royal Society B-Biological Sciences **280**.

Belsky, A. J. 1994. Influences of trees on ssavanna productivity- Tests of shade, nutrients and tree-grass competition Ecology **75**:922-932.

Chaer, G. M., A. S. Resende, E. F. C. Campello, S. M. de Faria, and R. M. Boddey. 2011. Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. Tree Physiology **31**:139-149.

Chazdon, R. L. 2003. Tropical forest recovery: legacies of human impact and natural disturbances. Perspectives in Plant Ecology Evolution and Systematics **6**:51-71.

Chazdon, R. L. 2008. Beyond deforestation: Restoring forests and ecosystem services on degraded lands. Science **320**:1458-1460.

Cole, P. G. and J. F. Weltzin. 2005. Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. Biological Invasions **7**:477-488.

Craven, D., J. Hall, and J. M. Verjans. 2009. Impacts of herbicide application and mechanical cleanings on growth and mortality of two timber species in

Saccharum spontaneum grasslands of the Panama Canal Watershed. Restoration Ecology **17**:751-761.

Cristiano, P. M., G. Posse, C. M. Di Bella, and T. Boca. 2012. Influence of contrasting availabilities of water and nutrients on the radiation use efficiency in C3 and C4 grasses. Austral Ecology **37**:323-329.

Cubina, A. and T. M. Aide. 2001. The effect of distance from forest edge on seed rain and soil seed bank in a tropical pasture. Biotropica **33**:260-267.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Dong, M. and H. Dekroon. 1994. Plasticity in morphology and biomass allocation in Cynodon dactylon, a grass species forming stolons and rhizomes Oikos **70**:99-106.

Ehleringer, J. R. 1978. Implications of quantum yield differences on distributions of C3 and C4 grasses Oecologia **31**:255-267.

Franco, A. A. and S. M. DeFaria. 1997. The contribution of N-2-fixing tree legumes to land reclamation and sustainability in the tropics. Soil Biology & Biochemistry **29**:897-903.

Funk, J. L. and P. M. Vitousek. 2007. Resource-use efficiency and plant invasion in low-resource systems. Nature **446**:1079-1081.

Gibson, D. J. 2008. Grasses and grassland ecology. Oxford University Press, USA.

Gilbert, B. and M. J. Lechowicz. 2005. Invasibility and abiotic gradients: The positive correlation between native and exotic plant diversity. Ecology **86**:1848-1855.

Hammond, B. W. 1998. *Saccharum spontaneum* (Gramineae) in Panama. Journal of Sustainable Forestry **8**:23-38.

Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution **24**:599-605.

Holl, K. D. 1998. Effects of above- and below-ground competition of shrubs and grass on Calophyllum brasiliense (Camb.) seedling growth in abandoned tropical pasture. Forest Ecology and Management **109**:187-195.

Holl, K. D. 1999. Factors limiting tropical rain forest regeneration in abandoned pasture: seed rain, seed germination, microclimate, and soil. Biotropica **31**:229-242.

Holl, K. D., M. E. Loik, E. H. V. Lin, and I. A. Samuels. 2000. Tropical montane forest restoration in Costa Rica: overcoming barriers to dispersal and establishment. Restoration Ecology **8**:339-349.

Holm, L., J. V. Pancho, J. P. Herberger, and D. L. Plucknett. 1979. A geographical atlas of world weeds. New York.

Hooper, D. U. and P. M. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs **68**:121-149.

Hooper, E., R. Condit, and P. Legendre. 2002. Responses of 20 native tree species to reforestation strategies for abandoned farmland in Panama. Ecological Applications **12**:1626-1641.

Hooper, E., P. Legendre, and R. Condit. 2005. Barriers to forest regeneration of deforested and abandoned land in Panama. Journal of Applied Ecology **42**:1165-1174.

Hooper, E. R., P. Legendre, and R. Condit. 2004. Factors affecting community composition of forest regeneration in deforested, abandoned land in Panama. Ecology **85**:3313-3326.

Israel, D. W. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation   Plant Physiology **84**:835-840.

James, J. J. 2008. Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. Journal of Arid Environments **72**:1775-1784.

Jones, E. R., M. H. Wishnie, J. Deago, A. Sautu, and A. Cerezo. 2004. Facilitating natural regeneration in *Saccharum spontaneum* (L.) grasslands within the Panama Canal Watershed: effects of tree species and tree structure on vegetation recruitment patterns. Forest Ecology and Management **191**:171-183.

Joo Kim, T., F. Montagnini, and D. Dent. 2008. Rehabilitating abandoned pastures in Panama: Control of the invasive exotic grass, *Saccharum spontaneum* L., using artificial shade treatments. Journal of Sustainable Forestry **26**:192-203.

Koerselman, W. and A. F. M. Meuleman. 1996. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. Journal of Applied Ecology **33**:1441-1450.

Lambers, H., F. S. Chapin, and T. L. Pons. 2008. Plant physiological ecology. Springer Verlag.

Lawrence, D., P. D'Odorico, L. Diekmann, M. DeLonge, R. Das, and J. Eaton. 2007. Ecological feedbacks following deforestation create the potential for a catastrophic ecosystem shift in tropical dry forest. Proceedings of the National Academy of Sciences of the United States of America **104**:20696-20701.

Ledgard, S. F. and K. W. Steele. 1992. Biological nitrogen-fixation in mixed legume grass pastures   Plant and Soil **141**:137-153.

Letcher, S. G. and R. L. Chazdon. 2009. Rapid Recovery of Biomass, Species Richness, and Species Composition in a Forest Chronosequence in Northeastern Costa Rica. Biotropica **41**:608-617.

Loik, M. E. and K. D. Holl. 1999. Photosynthetic responses to light for rainforest seedlings planted in abandoned pasture, Costa Rica. Restoration Ecology **7**:382-391.

Lu, D., E. Moran, and P. Mausel. 2002. Linking amazonian secondary succession forest growth to soil properties. Land Degradation & Development **13**:331-343.

Ludwig, F., T. E. Dawson, H. H. T. Prins, F. Berendse, and H. de Kroon. 2004a. Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. Ecology Letters **7**:623-631.

Ludwig, F., H. de Kroon, F. Berendse, and H. H. T. Prins. 2004b. The influence of savanna trees on nutrient, water and light availability and the understorey vegetation. Plant Ecology **170**:93-105.

Ludwig, F., H. de Kroon, H. H. T. Prins, and F. Berendse. 2001. Effects of nutrients and shade on tree-grass interactions in an East African savanna. Journal of Vegetation Science **12**:579-588.

Marcano-Vega, H., T. M. Aide, and D. Baez. 2002. Forest regeneration in abandoned coffee plantations and pastures in the Cordillera Central of Puerto Rico. Plant Ecology **161**:75-87.

Maynard DG, K. Y. 1993. Nitrate and exchangeable amonium nitrogen Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Fla.

Mehlich, A. 1984. Mehlich-3 soil test extractant- A modification of Mehlich-2 extractant Communications in Soil Science and Plant Analysis **15**:1409-1416.

Montgomery, R. A. and R. L. Chazdon. 2001. Forest structure, canopy architecture, and light transmittance in tropical wet forests. Ecology **82**:2707-2718.

Moran, E. F., E. S. Brondizio, J. M. Tucker, M. C. da Silva-Forsberg, S. McCracken, and I. Falesi. 2000. Effects of soil fertility and land-use on forest succession in Amazonia. Forest Ecology and Management **139**:93-108.

Nepstad, D. C., C. Uhl, C. A. Pereira, and J. M. C. da Silva. 1996. A comparative study of tree establishment in abandoned pasture and mature forest of eastern Amazonia. Oikos **76**:25-39.

Niu, S. L., Y. F. Zhang, Z. Y. Yuan, W. X. Liu, J. H. Huang, and S. Q. Wan. 2006. Effects of interspecific competition and nitrogen seasonality on the photosynthetic characteristics of C-3 and C-4 grasses. Environmental and Experimental Botany **57**:270-277.

Novozamsky, I., V. J. G. Houba, R. Vaneck, and W. Vanvark. 1983. A novel digestion technique for multi-element plant analysis Communications in Soil Science and Plant Analysis **14**:239-248.

Park, A., M. van Breugel, M. S. Ashton, M. Wishnie, E. Mariscal, J. Deago, D. Ibarra, N. Cedeno, and J. S. Hall. 2010. Local and regional environmental variation influences the growth of tropical trees in selection trials in the Republic of Panama. Forest Ecology and Management **260**:12-21.

Parrotta, J. A. 1992. The role of plantation forests in rehabilitating degraded tropical ecosystems. Agriculture, Ecosystems and Environment **41**:115-133.

Parsons, J. J. 1972. Spread of African pasture grasses to the American tropics. Journal of Range Management **25**:12-17.

Perry, M. E. L., W. H. Schacht, G. A. Ruark, and J. R. Brandle. 2009. Tree canopy effect on grass and grass/legume mixtures in eastern Nebraska. Agroforestry Systems **77**:23-35.

Reich, P. B., J. Oleksyn, and I. J. Wright. 2009. Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia **160**:207-212.

Rivera, L. W., J. K. Zimmerman, and T. M. Aide. 2000. Forest recovery in abandoned agricultural lands in a karst region of the Dominican Republic. Plant Ecology **148**:115-125.

Sage, R. F. and R. K. Monson. 1999. C4 plant biology. Academic Pr.

Schweizer, D., G. S. Gilbert, and K. D. Holl. 2013. Phylogenetic ecology applied to enrichment planting of tropical native tree species. Forest Ecology and Management **297**:57-66.

Siddique, I., V. L. Engel, J. A. Parrotta, D. Lamb, G. B. Nardoto, J. P. H. B. Ometto, L. A. Martinelli, and S. Schmidt. 2008. Dominance of legume trees alters nutrient relations in mixed species forest restoration plantings within seven years. Biogeochemistry **88**:89-101.

Somarriba, E. 1988. Pasture growth and floristic composition under the shade of Guava (Psidium guajava L.) trees in Costa Rica   Agroforestry Systems **6**:153-162.

van Breugel, M., J. S. Hall, D. J. Craven, T. G. Gregoire, A. Park, D. H. Dent, M. H. Wishnie, E. Mariscal, J. Deago, D. Ibarra, N. Cedeno, and M. S. Ashton. 2011. Early growth and survival of 49 tropical tree species across sites differing in soil fertility and rainfall in Panama. Forest Ecology and Management **261**:1580-1589.

Veldman, J. W. and F. E. Putz. 2010. Long-distance Dispersal of Invasive Grasses by Logging Vehicles in a Tropical Dry Forest. Biotropica **42**:697-703.

Veldman, J. W. and F. E. Putz. 2011. Grass-dominated vegetation, not species-diverse natural savanna, replaces degraded tropical forests on the southern edge of the Amazon Basin. Biological Conservation **144**:1419-1429.

Vitousek, P. M., K. Cassman, C. Cleveland, T. Crews, C. B. Field, N. B. Grimm, R. W. Howarth, R. Marino, L. Martinelli, E. B. Rastetter, and J. I. Sprent. 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry **57**:1-45.

Wang, F., Z. Li, H. Xia, B. Zou, N. Li, J. Liu, and W. Zhu. 2010. Effects of nitrogen-fixing and non-nitrogen-fixing tree species on soil properties and nitrogen transformation during forest restoration in southern China. Soil Science and Plant Nutrition **56**:297-306.

Wishnie, M. H., D. H. Dent, E. Mariscal, J. Deago, N. Cedeno, D. Ibarra, R. Condit, and P. M. S. Ashton. 2007. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. Forest Ecology and Management **243**:39-49.

Zimmerman, J. K., J. B. Pascarella, and T. M. Aide. 2000. Barriers to forest regeneration in an abandoned pasture in Puerto Rico. Restoration Ecology **8**:350-360.

CHAPTER 2

Influence of interspecific competition and nutrient additions on the growth and regeneration of the invasive C_4 grass *Saccharum spontaneum*

Abstract

Invasive C_4 grasses, such as *Saccharum spontaneum,* inhibit the regeneration of secondary forest and create a state of arrested succession in previously disturbed tropical areas. Planting native trees for restoration can inhibit grasses through rapid establishment of a closed canopy; legume trees may be particularly effective. Legumes have demands for phosphorus, which suggests that in addition to competing for light, legumes may also inhibit grasses via competition for nutrients. In Panama, local farmers have historically used the woody agricultural species *Cajanus cajan* (guandu, pigeon pea) to suppress *S. spontaneum* growth. We tested the potential for *C. cajan* to compete with *S. spontaneum* for nutrients. We evaluated the response of *S. spontaneum* above ground biomass and density to nitrogen and phosphorus addition in the presence and absence of *C. cajan.* Unfortunately, *C. cajan* establishment was not sufficient to measure interspecific competition with *S. spontaneum*. However, the experiment was useful to evaluate nitrogen and phosphorus limitation in *S. spontaneum.*

S*. spontaneum* density and biomass responded positively when both N and P were added together. This suggests that *S. spontaneum* is co-limited by both N and P in our system. Future studies should evaluate the physiological response of *S. spontaneum* to increased nutrients, competition, and mechanical *S. spontaneum* removal.

Introduction

Intense agriculture and grazing in tropical systems can lead to soil degradation and reduced productivity. When such lands are abandoned, natural succession is expected to lead to habitat recovery. However, low propagule availability, seed and seedling predation, seasonal drought, reduced nutrient availability, fire, and competition with weedy exotics are barriers that can slow the rate of succession (Nepstad et al. 1991, D'Antonio and Vitousek 1992, Aide and Cavelier 1994, Holl 1999, Moran et al. 2000). Active forest restoration can promote secondary succession through the re-introduction of desired forest species that can establish in harsh environments.

Tropical soils are diverse, but in general, tropical soils are less fertile than temperate soils and more likely to be phosphorus than nitrogen limited (Vitousek 1984, Sollins 1998, Townsend et al. 2007). Tropical nutrient availability is highly regulated by nutrient cycling through decomposition (Lu et al. 2002). Forest clearing and removal of above-ground biomass in agriculture reduces the amount of nutrients that cycle back into the system through decomposition (Newman 1997), leading to a rapid loss of P after as few as three cycles of shifting agriculture (Lawrence et al. 2007). As productivity decreases, increasing amounts of exposed soils can cause further losses of N, P, K, and other nutrients through erosion, leading to nutrient limitation and delayed succession (Vitousek 1984, Lu et al. 2002, Mercado et al. 2011, Baribault et al. 2012). Reduced nutrient availability can impede the establishment of native species (Lu et al. 2002, Chaer et al. 2011) and creates conditions that favor colonization by weedy exotic C_4 grasses (Nepstad et al. 1991, D'Antonio and Vitousek 1992, Williams and Baruch 2000, D'Antonio et al. 2001).

Numerous species of exotic C_4 grasses were introduced to the American tropics as pasture grasses, and many became pests (Parsons 1972, D'Antonio and Vitousek 1992, Aide and Cavelier 1994, Williams and Baruch 2000). In contrast to most native C_3 woody plants, C_4 grasses have high photosynthetic nitrogen use efficiency, allowing them to maintain high photosynthetic rates under the hot, sunny, low fertility conditions of abandoned agricultural lands; this gives the grasses a competitive advantage and delays succession to forest (Lambers et al. 2008, Edwards and Smith 2009). Exotic C_4 grasses can inhibit forest succession in degraded areas through increased nutrient competition and accelerated fire regimes (D'Antonio and Vitousek 1992, Aide and Cavelier 1994, Moran et al. 2000, Hooper et al. 2004, Hooper et al. 2005, D'Antonio et al. 2011).

Careful selection of tree species for planting in tropical forest restoration is crucial to maximize restoration success (Montagnini 2001, Hooper et al. 2002, Piotto et al. 2004, Wishnie et al. 2007, Aguirre et al. 2011, Hall et al. 2011b, van Breugel et al. 2011). In general, the most important attribute is the ability to quickly produce maximum canopy cover to shade out C_4 grasses. Legume trees in particular are often planted in restoration efforts because they establish well in degraded systems and rapidly close their canopy (Wishnie et al. 2007, Joslin et al. 2011).

Legumes have notably high demands for N and P, which suggest they may be good competitors for key nutrients (Israel 1987, Sprent et al. 1996, Vitousek et al. 2002, Houlton et al. 2008, Chaer et al. 2011). Legumes are able to fix their own nitrogen when soil nitrogen is limiting (Sprent et al. 1996, Vitousek et al. 2002, Chaer et al. 2011). But N fixation creates high P demands, and efficient mechanisms for P acquisition mean legumes may outcompete surrounding vegetation when P is limiting. For instance, fertilizer experiments show that the legume tree *Acacia tortilis* can reduce understory grass biomass through P competition rather than just through shading (Ludwig et al. 2001). High nutrient competition and dense shade produced by some legume species suggests legumes may be especially useful to inhibit exotic grasses and facilitate the establishment of native shade-tolerant species.

Saccharum spontaneum is an exotic C_4 grass that inhibits forest regeneration and succession in Panama. Deep shade inhibits *S. spontaneum* persistence (Hooper et al. 2002, Hooper et al. 2005, Wishnie et al. 2007, Joo Kim et al. 2008) and trials of various tree species show that those species with rapid canopy closure are most effective (Wishnie et al. 2007). Control of *S. spontaneum* during the establishment of trees for restoration is essential and reducing the duration of clearing can substantially reduce clearing costs. In Panama, I have received numerous anecdotal accounts of using *Cajanus cajan* (aka guandu, pigeon pea) to suppress *S. spontaneum* growth. *C. cajan* is a woody legume agricultural crop that grows up to 4 m tall, and can persist for 3-5 years (Duke 1981). The incorporation of agricultural species into restoration models has been suggested as a way to provide food security for small land holders and help offset management costs during restoration (Vieira et al. 2009) Fast growing agricultural species such as *C. cajan* could out-compete *S. spontaneum* and allow for supplemental planting of restoration trees . *C. cajan* has been used in conjunction with other species to suppress *S. spontaneum* growth during the formation of teak (*Tectona grandis*) plantations (Hammond 1998). In Brazil, *C. cajan* has also been used to increase nutrient availability on highly degraded soils (Chaer et al. 2011). However, no studies have been published to evaluate the influence of *C. cajan* on *S. spontaneum* regeneration.

The objective of this experiment was to test whether planting woody legumes could inhibit *S. spontaneum* performance, and whether such inhibition was mediated by N or P. We planted seeds of *C. cajan*, and then we manipulated nitrogen and phosphorus availability. We predicted that *S. spontaneum* growth would be inhibited by the presence of *C. cajan,* and that nutrient additions would relieve nutrient limitations imposed by *C. cajan* competition. In particular, the P fertilizer treatment should alleviate suppression of *S. spontaneum* by legumes if competition for P is important. The addition of N should alleviate the need to supplement N through nitrogen fixation and thus decrease the high demand for P associated with N fixation. Nutrient limitation should be alleviated in the +N+P plots, and *S. spontaneum* biomass and density should be highest in these plots. If the competitive effect of *C. cajan* on *S. spontaneum* was driven exclusively by shade and not nutrient competition, we would expect to see no interaction between the effect of *C. cajan* and the effect of nutrient additions. Finally, we expected that in the absence of *C. cajan*, *S. spontaneum* growth in tropical soils would be more P-limited than N-limited.

Methods:

Site Description

This project was established in abandoned agricultural lands now dominated by *S. spontaneum,* in Parque Nacional Soberanía in the Panama Canal Watershed, Republic of Panama. This site receives a mean rainfall of 2226 mm and 4.1 dry months annually from December to early April (defined as months with < 100 mm rainfall) (Wishnie et al. 2007). Soils at this site are characterized as ultisols; predominantly clay and silty clay loams (Park et al. 2010). Moist tropical forest was cleared from this site during the 1960's and the area was then used for grazing and small-scale agriculture, until it was abandoned around 1993.

Experimental Design

A factorial, split-plot design was used to test nutrient limitation of *S. spontaneum* re-growth and the effects of planting *C. cajan*, a fast-growing woody legume shrub. In June of 2011, above ground biomass of *S. spontaneum* was cleared with a machete from all blocks before applying nutrient fertilizers. This experiment consisted of six blocks, each containing two main plot (*C. cajan*/no *C. cajan*) treatments. In each main plot, four 5 × 5-m^2 sub-plots were established, each corresponding to a different nutrient treatment: no nutrient addition (control;

C), N addition (N), P addition (P), and N+P combination (N+P) (Fig. 1). A 2-m buffer was established between sub-plots, and a 3-m buffer was created between blocks. Buffer zones were maintained free of *S. spontaneum* above ground biomass to inhibit rhizomatous spread between the plots by *S. spontaneum*. In the *C. cajan* treatment plots, four seeds of *C. cajan* were planted at each of 16 points, 1m from the edge and 1m apart. After 3 weeks, and again after 5 weeks, seedlings were thinned and transplanted to points where seeds did not germinate to equalize *C. cajan* density across plots.

Dry fertilizer was added by hand after clearing *S. spontaneum* in July of 2011. Nitrogen was added as urea ($(NH_2)_2CO_2$), and phosphorus as triple super phosphate ($Ca(H_2PO_4)_2 \bullet H_2O$). Based on previous studies, nutrient application corresponded to 125 kg Ha^{-1} N and 50 kg Ha^{-1} P (Wright et al. 2011). Based on these values we calculated a bi-annual nutrient application of 135.63 g triple super phosphate, and 339.06 g urea per plot for each application. Soil cores were taken at the end of the study from each plot to compare final soil nutrient availability across treatments.

In September 2011 we measured *S. spontaneum* performance by comparing density and above ground biomass across treatments. We measured density in three randomly placed 1-m squares in each plot. *S. spontaneum* above ground biomass was removed from half of the 1-m area used for collecting *S.*

spontaneum density. *S. spontaneum* biomass was dried at 60°C for 3 days and weighed at the Smithsonian Tropical Research Institute in Panama City, Panama. At the end of September 2011, plots were cleared of *S. spontaneum*. In October 2011, we re-applied nutrients and allowed *S. spontaneum* to grow back until the onset of the dry season in December of 2011.

Data collection in December followed the methods used in September, however we also randomly collected and dried 10 individuals from each 1-m^2 density plot to determine biomass. Additionally, we collected soil and leaf nutrient data to compare nutrient treatments. We collected and homogenized 10 soil cores for each plot, and a subsample was used to extract soil nitrogen and phosphorus. Soil samples for N determination were placed in KCl solution immediately after field collection. We followed the KCl (Maynard DG 1993) and Mehlich (Mehlich 1984) standardized protocols to extract available nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{-2}) (Baldeck et al. 2013). We collected the 3rd mature leaf from the base of 15 randomly selected individuals in each plot. Leaves were dried for 3 days at 60°C, and samples were processed at the University of California Santa Cruz. Five leaves were selected from each plot and leaf N and P were extracted following the Kjeldahl acid digestion protocol (Novozamsky et al. 1983)using a Lachat BD 46 block digester (Lachat Instruments, Milwaukee, WI USA).

Data Analysis

We used a randomized complete block ANOVA with presence or absence of *C. cajan* as the whole plot effect and N, P, and the combination of N+P as the split plot effect. Blocks were included in the model as a random effect. Nutrient response variables included soil nitrate, ammonium, total N, phosphate, and N:P ratio. Plant growth response variables included *S. spontaneum* density and biomass measured in both September (July – September growth) and December (October – December growth). Plant nutrient variables were leaf N (%), leaf P (%), and leaf N:P ratios. Across all analyses we found no effect of *C. cajan* treatment, or its interactions with other factors ($p \geq 0.17$). Therefore, we re-analyzed all the data excluding the *C. cajan* treatment so that each plot was then considered a block (2 plots in each of 6 blocks, so final analysis on 12 randomized complete blocks).

Results

Plots with only nitrogen added had significantly higher levels of nitrate and total N, while ammonium was marginally significantly higher, and there was no effect on phosphate (Table 1; Fig #). In plots where only phosphorus was added, phosphate levels were marginally significantly higher and there was no effect on nitrogen (Table 1; Fig #). In plots where both N+P were added, ammonium and total N were significantly higher, while phosphate was marginally significantly higher and there was no increase in nitrate (Table 1; Fig #). There was no difference in soil N:P ratios across nutrient treatments (Table 1; Fig. 2).

S. spontaneum also responded to nutrient treatments by increasing internal nutrient concentrations. In plots where only N was added, we found a significantly higher *S. spontaneum* leaf N:P ratio although there was no significant effect on leaf N or P (Table 2; Fig). In plots where P was added, we found significantly higher leaf P and lower leaf N:P ratio, though no significant effect on leaf N (Table 2; Fig. 3). When both N and P were added *S. spontaneum* leaf P increased significantly, leaf N:P decreased significantly, and there was a marginally positive increase in leaf N (Table 2; Fig. 3) .

We found a significant positive effect of nitrogen and phosphorus addition on *S. spontaneum* biomass in both September and December in plots where both N and P were added (Table 3; Fig. 4). There was a marginally significant increase in *S. spontaneum* density in September in plots where both N and P were added, and a significantly positive increase in *S. spontaneum* density in December in plots where both N and P were added (Table 3; Fig. 5). We found no significant effect of N or P on *Saccharum* density (Table 3; Fig. 5) or biomass (Table 3; Fig. 4) when either were added alone.

Discussion

Effects of Cajanus cajan on S. spontaneum growth

We found no significant effect of *C. cajan* on *S. spontaneum* performance. This is likely due to late seed sowing of *C. cajan* and lack of *S. spontaneum* control during *C. cajan* establishment. In Panama, farmers that cultivate *C. cajan* sow their seeds during the onset of the rainy season in April, and cut back *S. spontaneum* until *C. cajan* is able to establish (personal communication). Due to logistical constraints, we were unable to establish our plots until July. In addition, *S. spontaneum* was only cleared once during the growing season, which was less frequent than traditional practices for establishing *C. cajan*.

Other studies have recommended mechanically clearing *S. spontaneum* multiple times annually during the establishment of restoration trees (Hooper et al. 2002, Craven et al. 2009). Although *C. cajan* grew as large as 169 cm tall during our study (data not presented), most of the plants were quite small (e.g. mean = 64 cm), which probably explains the lack of measurable effect of the treatment. Unfortunately, we are unable to use this experiment to evaluate the role of nutrient limitation in competition between *S. spontaneum* and legumes.

Physiological implications for increased S. spontaneum N and P in response to nutrient treatments

Final soil N was significantly higher in plots where N. Although soil P availability was only marginally significantly higher in plots where P was added, nutrient availability was higher throughout the study for a given nutrient treatment. Higher *S. spontaneum* leaf P in plots where P was added, and marginally significantly higher leaf N when both N and P were added indicates a positive *S. spontaneum* response to P addition, which leads to an increase in N uptake when N availability is increased. The positive response of *S. spontaneum* to nutrient addition suggests that *S. spontaneum* is co-limited by both nitrogen and phosphorus in this system. Increased foliar N when P is increased can lead to a variety of physiological responses, which will be discussed below.

We found a significant positive treatment effect of both added N and P on above ground biomass during both *S. spontaneum* harvests. Higher above-ground biomass may be linked to increased photosynthetic capacity under heightened nutrient availability. In one study, net CO_2 assimilation rate increased curvilinearly with increasing leaf N for eight members of the genus *Saccharum* spp. (Meinzer and Zhu 1998). In another study, leaf P and growth in C_4 grasses increased with increasing soil P supply (Ghannoum and Conroy 2007). Our results suggest that increased N in the presence of increased P may enhance *S. spontaneum* carbon capture through increased photosynthetic rates.

In one study that evaluated the photosynthesis-nitrogen relationship across 314 species photosynthetic rates increased with higher levels of both N and P (Reich et al. 2009). This would likely lead to either increased growth or carbon storage. In our system *S. spontaneum* biomass is increasing positively when both N and P are added. Although it is not likely that *S. spontaneum* is becoming nutrient saturated, future studies would need to evaluate the photosynthetic response, and above and below ground biomass response of *S. spontaneum* to a range of N, P, and N+P concentrations with limited above ground removal.

S. spontaneum density also responded positively to N and P additions. As previously mentioned, P appears to interact with N such that photosynthesis rate – N relationship can improve with increasing P. Although *S. spontaneum* density was only marginally significantly higher in the N+P addition plots in September, *S. spontaneum* density was significantly higher in N+P plots in December. This could be linked to increased tillering in N plots during both seasons. Lafarge (Lafarge 2006) found that tillering of tall fescue (*Festuca arundinacea*) increased with increasing N fertilizer. In another study conducted in a semiarid North American grassland, both C_3 and C_4 grasses increased tillering in response to the addition of livestock urine (Jaramillo and Detling 1992). Moyer and Sweeney (Moyer and Sweeney 2008) also found increased tillering in the North American C_4 grass *Tripsacum dactyloides* (L.) L in response to increased nitrogen. Our results suggest that *S. spontaneum* is responding similarly to grasses that occur in systems that are generally considered nitrogen limited and phosphorus abundant by increasing tillering in response to nitrogen additions.

Conclusion

Although we were unable to measure the potential for interspecific competition between C. cajan and *S. spontaneum,* S. *spontaneum* is nutrient co-limited in our system indicating nutrient limitation. Future studies should follow traditional *C. cajan* establishment practices to assess the competitive effects of *C. cajan* on *S. spontaneum* regeneration. To follow up on the influence of nutrients additions on *S. spontaneum* growth, future studies should evaluate the photosynthetic response of *S. spontaneum* to nutrient additions to determine whether increased nitrogen and phosphorus availability leads to increased photosynthetic rates resulting in increased above ground biomass accumulation in *S. spontaneum.*

Table 1. Two-way factorial ANOVA of final soil nutrient availability soil across treatments. Nutrients were added at the beginning of the study in July 2011 and again after the first harvest in October 2011. Nutrient additions are treated as fixed effects with blocks as random effects. Bold values represent significant treatment effects.

	Ammonium (mg/kg)			Nitrate (mg/kg)			Total N (mg/kg)			Phosphate (mg/kg)			N:P		
	df	t	P value	df	t	P value	df	t	P value	df	t	P value	df	t	P value
N no P	33	1.87	0.07	33	2.06	**0.05**	33	2.09	**0.04**	33	0.63	0.53	33	0.51	0.61
P no N	33	0.66	0.51	33	1.25	0.22	33	0.83	0.41	33	1.84	0.07	33	0.34	0.74
N+P	33	3.09	**0.004**	33	1.58	0.12	33	3.15	**0.004**	33	1.67	0.10	33	-0.22	0.83

Table 2. Two-way factorial ANOVAof *S. spontaneum* leaf nutrient concentrations across treatment. Leaves were collected during the December 2011 *S. spontaneum* harvest. Nutrient additions are treated as fixed effects with blocks as random effects. Bold values represent significant treatment effects.

	Leaf N			Leaf P			Leaf N:P		
	df	t	P value	df	t	P value	df	t	P value
N no P	33	1.30	0.20	33	-0.54	0.59	33	2.34	**0.03**
P no N	33	-1.47	0.15	33	5.50	**<0.001**	33	-7.45	**<0.001**
N+P	33	1.88	0.07	33	5.38	**<0.001**	33	-6.13	**<0.001**

Table 3. Two-way factorial analysis of *S. spontaneum* density and biomass in response to nutrient additions from July – September of 2011 and from October – December of 2011. Nutrient additions are treated as fixed effects with blocks as random effects. Bold values represent significant treatment effects.

	September Density			December Density			September Biomass			December biomass		
	df	t	P value	df	t	P value	df	t	P value	df	t	P value
N no P	33	0.48	0.63	33	1.26	0.21	33	1.12	0.27	33	-0.11	0.91
P no N	33	0.05	0.96	33	-0.07	0.94	33	1.04	0.30	33	-0.70	0.49
N+P	33	1.96	0.06	33	2.09	**0.04**	33	3.60	**0.001**	33	3.11	**0.004**

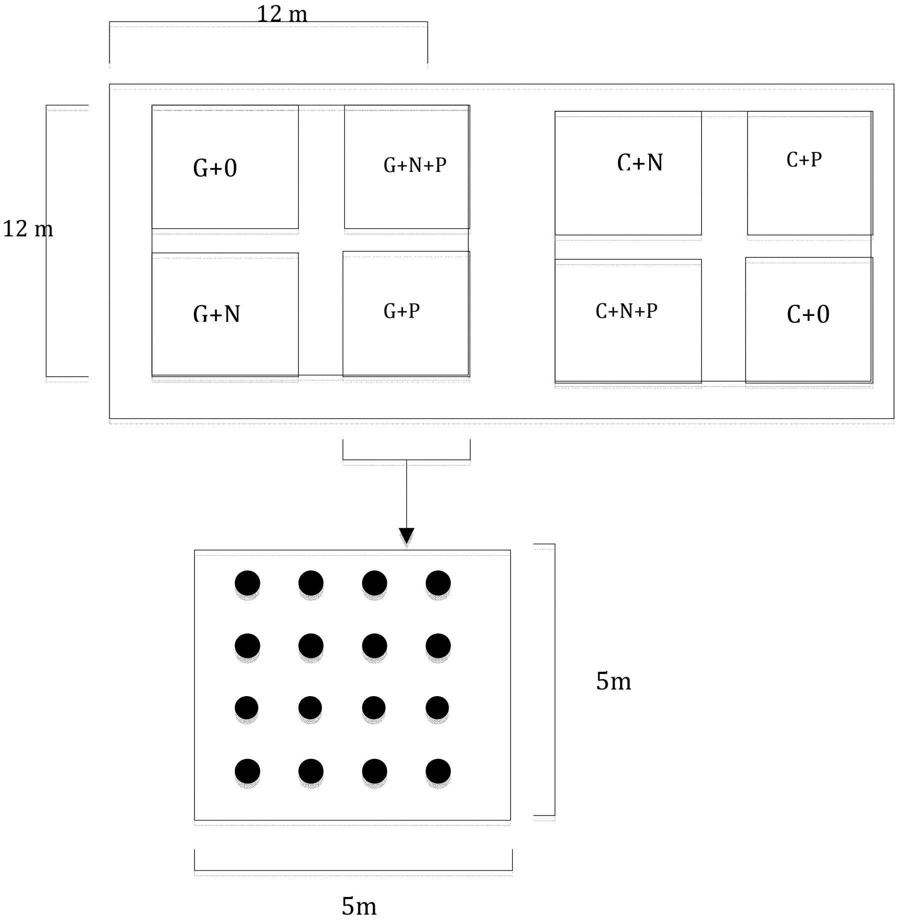

Fig. 1. A split-plot design showing an example of a block with main plots with (G) or without (C) *Cajanus cajan* and sub-plots with factorial N and P treatments (+0, +N, +P and +N+P). Black dots represent individual *Cajanus cajan* plants.

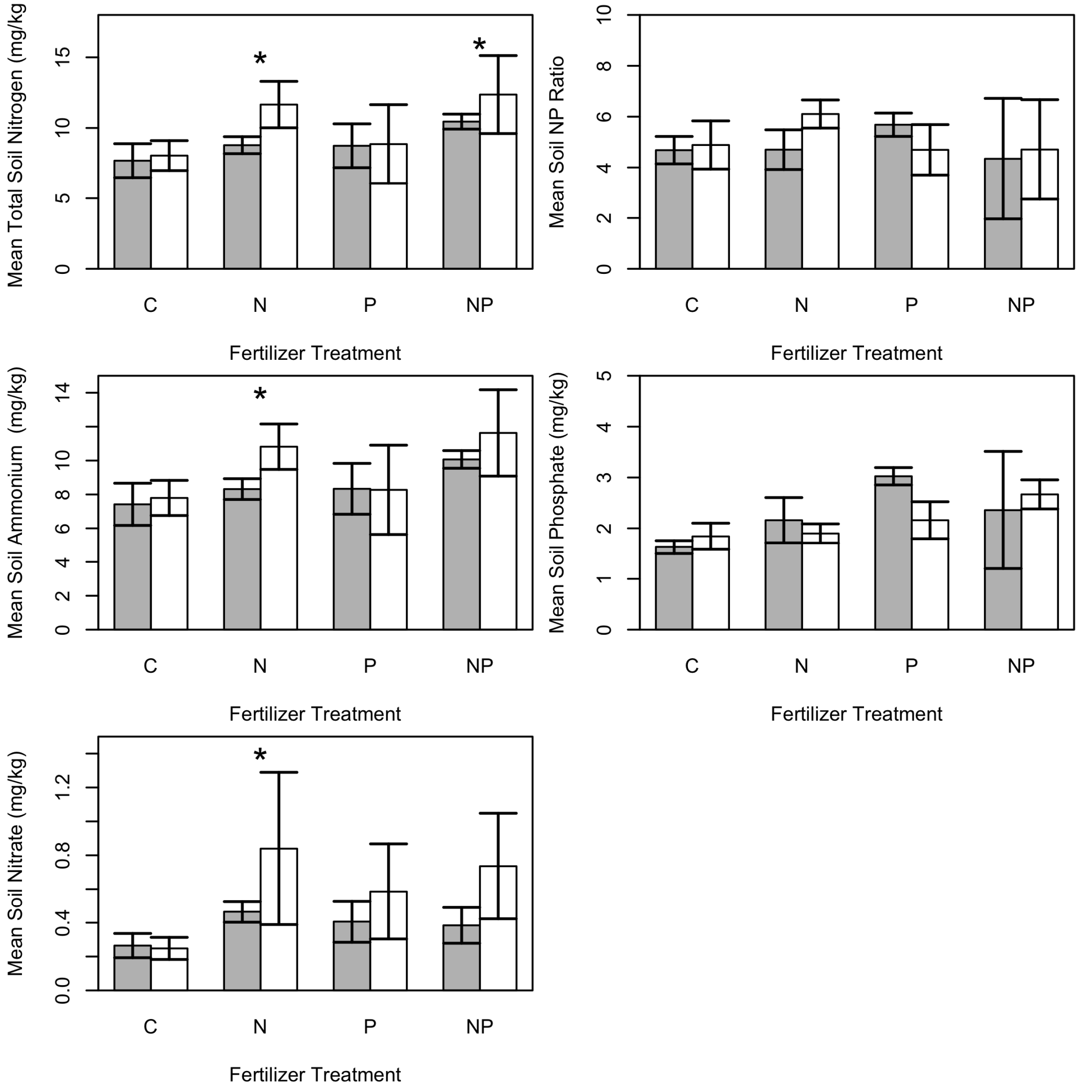

Figure 2. Mean soil nutrient concentrations between *C. cajan* (dark bars) and no *C. cajan* (light bars) sub plots across control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP) nutrient treatments (n=6/treatment). Mean soil nutrient concentrations were measured in December at the end of the study. Error bars represent the mean standard error for treatment sub plots. Asterisks represent treatments that are significantly different from the control.

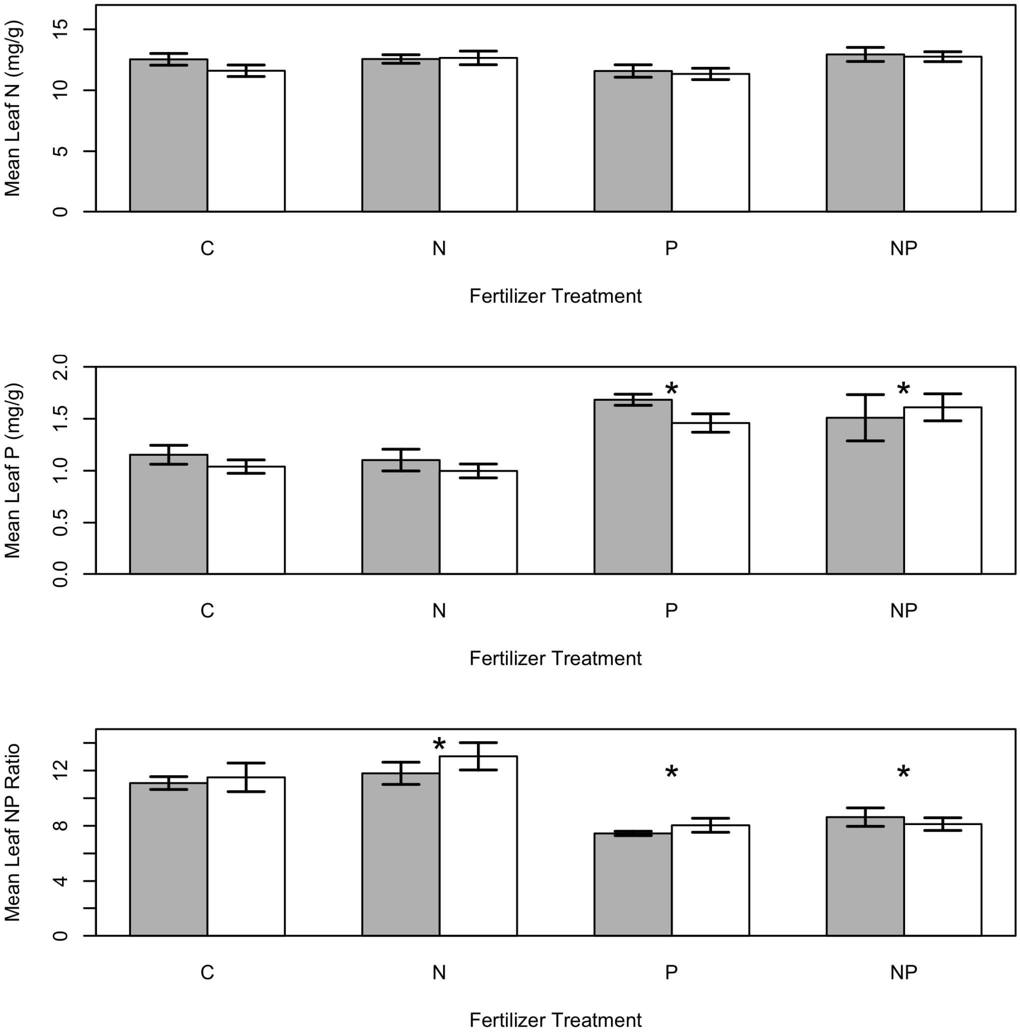

Figure 3. Mean leaf nutrient concentrations between *C. cajan* (dark bars) and no *C. cajan* (light bars) sub plots across control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP) nutrient treatments (n=30/treatment). Mean leaf nutrient concentrations were measured in December at the end of the study. Error bars represent the mean standard error for treatment sub plots. Asterisks represent treatments that are significantly different from the control

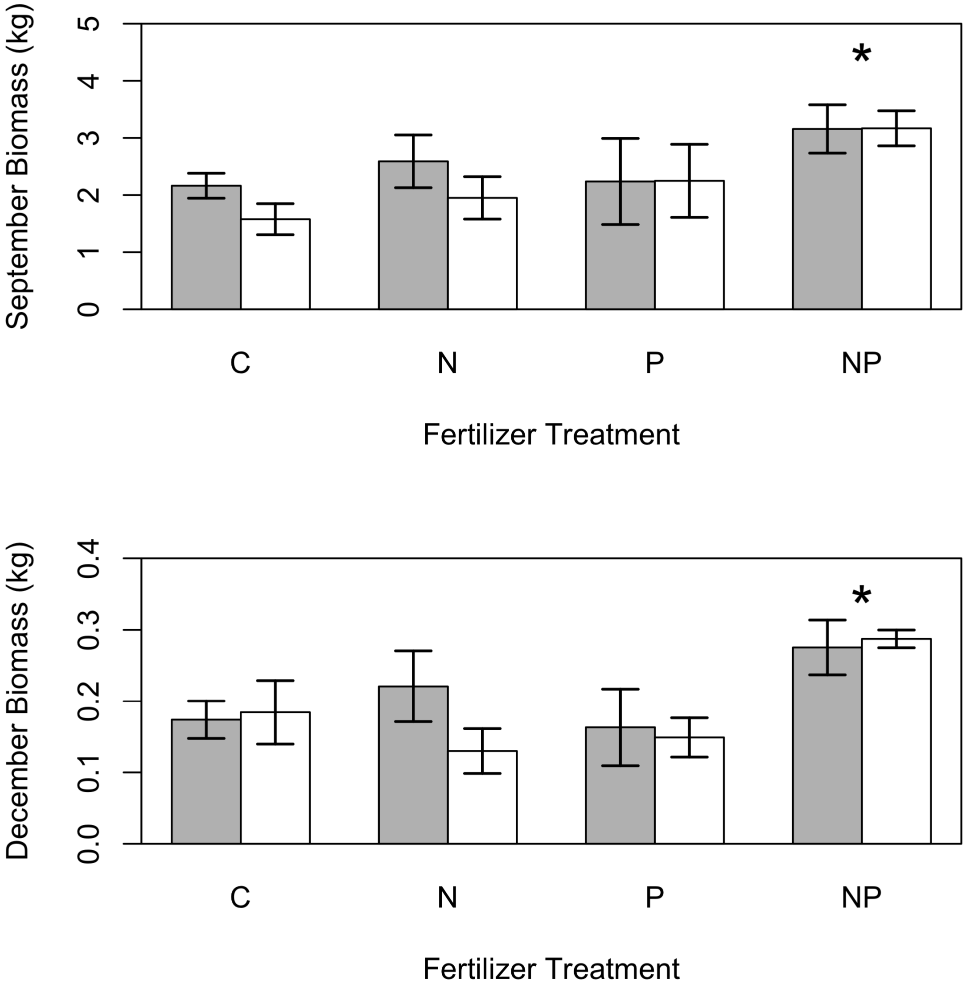

Figure 4. Mean *Saccharum spontaneum* biomass between *C. cajan* (dark bars) and no *C. cajan* (light bars) sub plots across control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (N+P) nutrient treatments in September and December. Error bars represent the mean standard error for treatment sub plots. Asterisks represent treatments that are significantly different from the control.

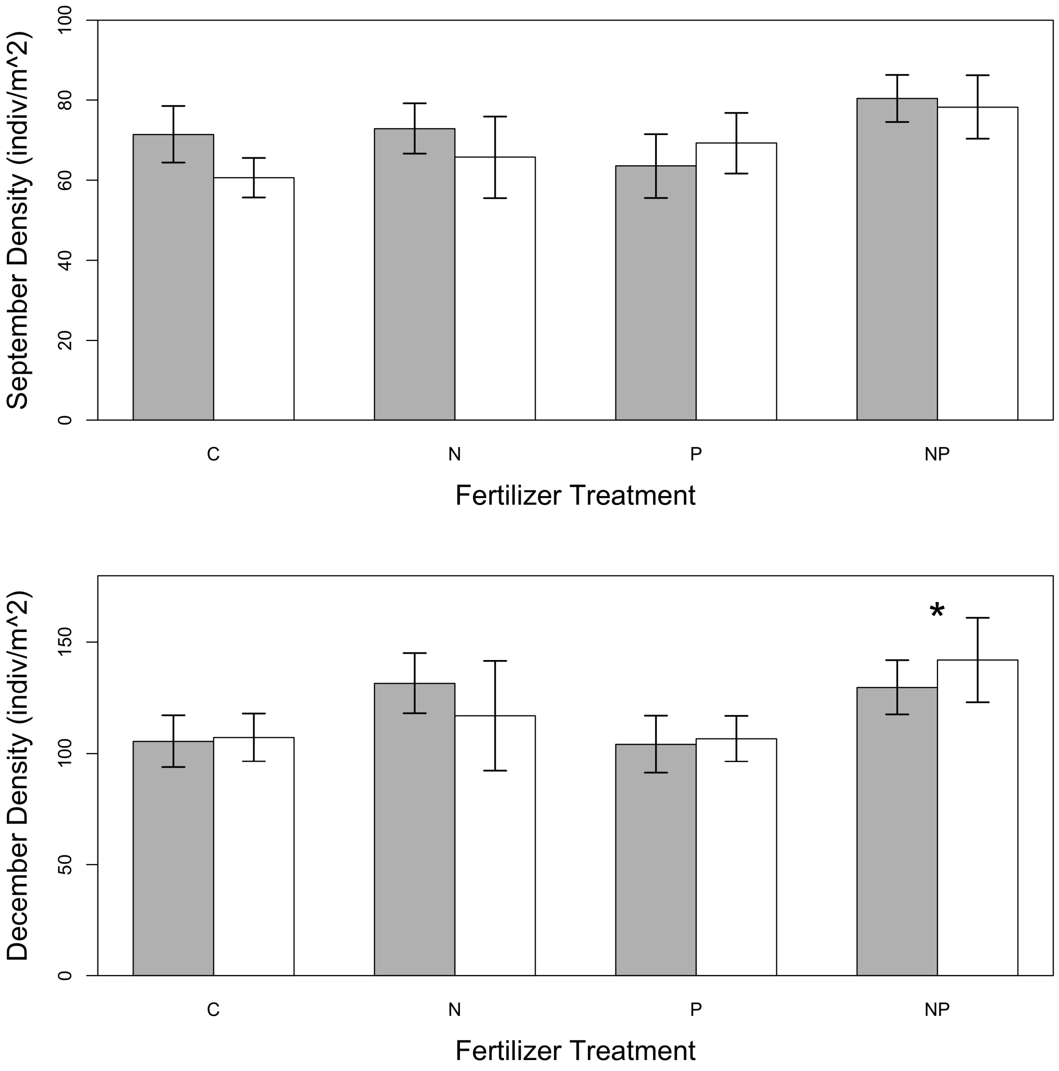

Figure 5. Mean *Saccharum spontaneum* density between *C. cajan* (dark bars) and no *C. cajan* (light bars) sub plots across control (C), nitrogen (N), phosphorus (P), and nitrogen and phosphorus (NP) nutrient treatments in September and December. Error bars represent the mean standard error for treatment sub plots. Asterisks represent treatments that are significantly different from the control.

Works Cited

Aguirre, N., X. Palomeque, M. Weber, B. Stimm, and S. Guenter. 2011. Reforestation and natural succession as tools for restoration on abandoned pastures in the Andes of South Ecuador. Pages 513-524 *in* S. Gunter, M. Weber, B. Stimm, and R. Mosandl, editors. Silviculture in the Tropics. Springer, 233 Spring Street, New York, Ny 10013, United States.

Aide, T. M. and J. Cavelier. 1994. Barriers to lowland tropical forest restoration in the Sierra Nevada de Santa Marta, Colombia. Restoration Ecology **2**:219-229.

Baldeck, C. A., K. E. Harms, J. B. Yavitt, R. John, B. L. Turner, R. Valencia, H. Navarrete, S. J. Davies, G. B. Chuyong, D. Kenfack, D. W. Thomas, S. Madawala, N. Gunatilleke, S. Bunyavejchewin, S. Kiratiprayoon, A. Yaacob, M. N. N. Supardi, and J. W. Dalling. 2013. Soil resources and topography shape local tree community structure in tropical forests. Proceedings of the Royal Society B-Biological Sciences **280**.

Baribault, T. W., R. K. Kobe, and A. O. Finley. 2012. Tropical tree growth is correlated with soil phosphorus, potassium, and calcium, though not for legumes. Ecological Monographs **82**:189-203.

Chaer, G. M., A. S. Resende, E. F. C. Campello, S. M. de Faria, and R. M. Boddey. 2011. Nitrogen-fixing legume tree species for the reclamation of severely degraded lands in Brazil. Tree Physiology **31**:139-149.

Craven, D., J. Hall, and J. M. Verjans. 2009. Impacts of herbicide application and mechanical cleanings on growth and mortality of two timber species in *Saccharum spontaneum* grasslands of the Panama Canal Watershed. Restoration Ecology **17**:751-761.

D'Antonio, C. M., R. F. Hughes, and J. T. Tunison. 2011. Long-term impacts of invasive grasses and subsequent fire in seasonally dry Hawaiian woodlands. Ecological Applications **21**:1617-1628.

D'Antonio, C. M., R. F. Hughes, and P. M. Vitousek. 2001. Factors influencing dynamics of two invasive C-4 grasses in seasonally dry Hawaiian woodlands. Ecology **82**:89-104.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Duke, J. A. 1981. Handbook of legumes of world economic importance. Plenum Press New York.

Edwards, E. J. and S. A. Smith. 2009. Phylogenetic analyses reveal the shady history of C4 grasses. Proceedings of the National Academy of Sciences **107**:2532.

Ghannoum, O. and J. P. Conroy. 2007. Phosphorus deficiency inhibits growth in parallel with photosynthesis in a C-3 (*Panicum laxum*) but not two C-4 (*P. coloratum and Cenchrus ciliaris*) grasses. Functional Plant Biology **34**:72-81.

Hall, J. S., B. E. Love, E. J. Garen, J. L. Slusser, K. Saltonstall, S. Mathias, M. van Breugel, D. Ibarra, E. W. Bork, D. Spaner, M. H. Wishnie, and M. S. Ashton. 2011. Tree plantations on farms: Evaluating growth and potential for success. Forest Ecology and Management **261**:1675-1683.

Hammond, B. W. 1998. *Saccharum spontaneum* (Gramineae) in Panama. Journal of Sustainable Forestry **8**:23-38.

Holl, K. D. 1999. Factors limiting tropical rain forest regeneration in abandoned pasture: seed rain, seed germination, microclimate, and soil. Biotropica **31**:229-242.

Hooper, E., R. Condit, and P. Legendre. 2002. Responses of 20 native tree species to reforestation strategies for abandoned farmland in Panama. Ecological Applications **12**:1626-1641.

Hooper, E., P. Legendre, and R. Condit. 2005. Barriers to forest regeneration of deforested and abandoned land in Panama. Journal of Applied Ecology **42**:1165-1174.

Hooper, E. R., P. Legendre, and R. Condit. 2004. Factors affecting community composition of forest regeneration in deforested, abandoned land in Panama. Ecology **85**:3313-3326.

Houlton, B. Z., Y. P. Wang, P. M. Vitousek, and C. B. Field. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature **454**:327-U334.

Israel, D. W. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation   Plant Physiology **84**:835-840.

Jaramillo, V. J. and J. K. Detling. 1992. Small-scale heterogeneity in a semiarid North-American grassland. 1. Tillering, N-uptake, and retranslocation in simulated urine patches Journal of Applied Ecology **29**:1-8.

Joo Kim, T., F. Montagnini, and D. Dent. 2008. Rehabilitating abandoned pastures in Panama: Control of the invasive exotic grass, *Saccharum spontaneum* L., using artificial shade treatments. Journal of Sustainable Forestry **26**:192-203.

Joslin, A. H., D. Markewitz, L. A. Morris, F. D. Oliveira, R. O. Figueiredo, and O. R. Kato. 2011. Five native tree species and manioc under slash-and-mulch agroforestry in the eastern Amazon of Brazil: plant growth and soil responses. Agroforestry Systems **81**:1-14.

Lafarge, M. 2006. Reproductive tillers in cut tall fescue swards: differences according to sward age and fertilizer nitrogen application, and relationships with the local dynamics of the sward. Grass and Forage Science **61**:182-191.

Lambers, H., F. S. Chapin, and T. L. Pons. 2008. Plant physiological ecology. Springer Verlag.

Lawrence, D., P. D'Odorico, L. Diekmann, M. DeLonge, R. Das, and J. Eaton. 2007. Ecological feedbacks following deforestation create the potential for a catastrophic ecosystem shift in tropical dry forest. Proceedings of the National Academy of Sciences of the United States of America **104**:20696-20701.

Lu, D., E. Moran, and P. Mausel. 2002. Linking amazonian secondary succession forest growth to soil properties. Land Degradation & Development **13**:331-343.

Ludwig, F., H. de Kroon, H. H. T. Prins, and F. Berendse. 2001. Effects of nutrients and shade on tree-grass interactions in an East African savanna. Journal of Vegetation Science **12**:579-588.

Maynard DG, K. Y. 1993. Nitrate and exchangeable amonium nitrogen Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Fla.

Mehlich, A. 1984. Mehlich-3 soil test extractant- A modification of Mehlich-2 extractant Communications in Soil Science and Plant Analysis **15**:1409-1416.

Meinzer, F. C. and J. Zhu. 1998. Nitrogen stress reduces the efficiency of the C-4 CO2 concentrating system, and therefore quantum yield, in *Saccharum* (sugarcane) species. Journal of Experimental Botany **49**:1227-1234.

Mercado, L. M., S. Patino, T. F. Domingues, N. M. Fyllas, G. P. Weedon, S. Sitch, C. A. Quesada, O. L. Phillips, L. Aragao, Y. Malhi, A. J. Dolman, N. Restrepo-Coupe, S. R. Saleska, T. R. Baker, S. Almeida, N. Higuchi, and J. Lloyd. 2011. Variations in Amazon forest productivity correlated with foliar nutrients and modelled rates of photosynthetic carbon supply. Philosophical Transactions of the Royal Society B-Biological Sciences **366**:3316-3329.

Montagnini, F. 2001. Strategies for the recovery of degraded ecosystems: Experiences from Latin America. Interciencia **26**:498-+.

Moran, E. F., E. S. Brondizio, J. M. Tucker, M. C. da Silva-Forsberg, S. McCracken, and I. Falesi. 2000. Effects of soil fertility and land-use on forest succession in Amazonia. Forest Ecology and Management **139**:93-108.

Moyer, J. L. and D. W. Sweeney. 2008. Long-term responses in the yield of Eastern Gamagrass *Tripsacum dactyloides* (L.) L. to nitrogen fertilizer under two harvest regimes in the United States. Grass and Forage Science **63**:390-397.

Nepstad, D. C., C. Uhl, and E. A. S. Serrao. 1991. Recuperation of a degraded Amazonian landscape - forest recovery and agricultural restoration Ambio **20**:248-255.

Newman, E. I. 1997. Phosphorus balance of contrasting farming systems, past and present. Can food production be sustainable? Journal of Applied Ecology **34**:1334-1347.

Novozamsky, I., V. J. G. Houba, R. Vaneck, and W. Vanvark. 1983. A novel digestion technique for multi-element plant analysis Communications in Soil Science and Plant Analysis **14**:239-248.

Park, A., M. van Breugel, M. S. Ashton, M. Wishnie, E. Mariscal, J. Deago, D. Ibarra, N. Cedeno, and J. S. Hall. 2010. Local and regional environmental variation influences the growth of tropical trees in selection trials in the Republic of Panama. Forest Ecology and Management **260**:12-21.

Parsons, J. J. 1972. Spread of African pasture grasses to the American tropics. Journal of Range Management **25**:12-17.

Piotto, D., E. Viquez, F. Montagnini, and M. Kanninen. 2004. Pure and mixed forest plantations with native species of the dry tropics of Costa Rica: a comparison of growth and productivity. Forest Ecology and Management **190**:359-372.

Reich, P. B., J. Oleksyn, and I. J. Wright. 2009. Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia **160**:207-212.

Sollins, P. 1998. Factors influencing species composition in tropical lowland rain forest: Does soil matter? Ecology **79**:23-30.

Sprent, J. I., I. E. Geoghegan, P. W. Whitty, and E. K. James. 1996. Natural abundance of N-15 and C-13 in nodulated legumes and other plants in the cerrado and neighbouring regions of Brazil. Oecologia **105**:440-446.

Townsend, A. R., C. C. Cleveland, G. P. Asner, and M. M. C. Bustamante. 2007. Controls over foliar N : P ratios in tropical rain forests. Ecology **88**:107-118.

van Breugel, M., J. S. Hall, D. J. Craven, T. G. Gregoire, A. Park, D. H. Dent, M. H. Wishnie, E. Mariscal, J. Deago, D. Ibarra, N. Cedeno, and M. S. Ashton. 2011. Early growth and survival of 49 tropical tree species across sites differing in soil fertility and rainfall in Panama. Forest Ecology and Management **261**:1580-1589.

Vieira, D. L. M., K. D. Holl, and F. M. Peneireiro. 2009. Agro-successional restoration as a strategy to facilitate tropical forest recovery. Restoration Ecology **17**:451-459.

Vitousek, P. M. 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests Ecology **65**:285-298.

Vitousek, P. M., K. Cassman, C. Cleveland, T. Crews, C. B. Field, N. B. Grimm, R. W. Howarth, R. Marino, L. Martinelli, E. B. Rastetter, and J. I. Sprent. 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry **57**:1-45.

Williams, D. G. and Z. B. Baruch. 2000. African grass invasion in the Americas: ecosystem consequences and the role of ecophysiologys. Biological Invasions **2**

:123-140.

Wishnie, M. H., D. H. Dent, E. Mariscal, J. Deago, N. Cedeno, D. Ibarra, R. Condit, and P. M. S. Ashton. 2007. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. Forest Ecology and Management **243**:39-49.

Wright, S. J., J. B. Yavitt, N. Wurzburger, B. L. Turner, E. V. J. Tanner, E. J. Sayer, L. S. Santiago, M. Kaspari, L. O. Hedin, K. E. Harms, M. N. Garcia, and M. D. Corre. 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. Ecology **92**:1616-1625.

CHAPTER 3

Allelopathy: A tool for weed management in forest restoration

Abstract

Forest restoration uses active management to re-establish natural forest habitat after disturbance. However, competition from early successional species, often aggressively invasive exotic plant species, can inhibit tree establishment and forest regeneration. Ideally, restoration ecologists can plant native tree species that not only establish and grow rapidly, but also suppress exotic competitors. Allelopathy may be a key mechanism by which some native trees could reduce the abundance and impact of exotic species. Allelopathy is a recognized tool for weed management in agriculture and agroforestry, but few studies have considered how allelopathic interactions may aid restoration. Here we introduce the "Homeland Security" hypothesis, which posits that some naïve exotic species may be particularly sensitive to allelochemicals produced by native species, providing a tool to reduce the growth and impacts of invasive exotic species on reforestation. This article explores how exploiting allelopathy in native species could improve restoration success and the re-establishment of natural successional dynamics.

We review the evidence for allelopathy in agroforestry systems, and consider its relevance for reforestation. We then illustrate the potential for this approach with a case study of tropical forest restoration in Panama. C_4 grasses heavily invade deforested areas in the Panama Canal watershed, especially *Saccharum spontaneum* L. We measured the effect of leaf litter from 17 potential restoration tree species on the growth of invasive C_4 grasses. We found that leaf litter from legume trees had a greater inhibitory effect on performance of *S. spontaneum* than did litter from non-legume trees. However, allelopathic effects varied greatly among species within tree functional groups. Further evaluation of intra- and inter-specific interactions will help to improve our selection of restoration species.

Key words: *Saccharum spontaneum*, legumes, novel weapons hypothesis, homeland security hypothesis, allelopathy, forest restoration

Introduction

Anthropogenic disturbance of natural ecosystems is one of the biggest threats to biodiversity (Vitousek et al. 1994, Vitousek et al. 1997, Zhang and Fu 2009), and impacts are often exacerbated when disturbance facilitates invasion by exotic species (Mack et al. 2000). For example, the intentional conversion of tropical forest to pastures of exotic grasses to raise cattle led to the subsequent colonization and spread of exotic C_4 grasses throughout disturbed areas in the new world tropics (Parsons 1972, D'Antonio and Vitousek 1992, Condit et al. 2001, Carpenter et al. 2004). Establishment of exotic C_4 grasses tends to be limited to disturbed habitats (Hooper et al. 2004, MacDonald 2004, Leung et al. 2009). Lack of establishment in forests may be limited by abiotic factors and biotic interactions with native species (Hou et al. 2011, Hou et al. 2012). Identifying traits of native species that impede invasion by exotic species may be useful when attempting to protect disturbed areas from invasion or restore them to forest.

Forest restoration attempts to return a disturbed area to a historical forest state. This includes the re-establishment of successional dynamics, nutrient and hydrological cycles, native species diversity, habitat connectivity, ecosystem services (Palmer et al. 1997, Young 2000, Clewell and Aronson 2006, Satake and Rudel 2007, Little and Lara 2010, Aerts and Honnay 2011), and increased carbon sequestration (Huang et al. 2012) . Global initiatives such as REDD (www.unredd.org) that offer financial incentives for countries to restore and maintain forest cover have prompted research to improve the efficiency and efficacy of forest restoration techniques (Donald and Evans 2006, Putz and Redford 2010, Thangata and Hildebrand 2012).

Much of restoration ecology focuses on overcoming critical barriers to succession such as seed dispersal (Wunderle 1997, Holl 1999, Murray et al. 2008), abiotic factors (VanDuren et al. 1997, Loik and Holl 1999, 2001, McNamara et al. 2006) and identification of species that establish and grow rapidly in degraded environments (Nepstad et al. 1996, Hooper et al. 2002, Chazdon 2003, Jones et al. 2004, Hooper et al. 2005, Wishnie et al. 2007, Park et al. 2010, Hall et al. 2011a, Hall et al. 2011b, van Breugel et al. 2011). Weedy exotic plants and other aggressive early successional species are important barriers to reforestation. Such species can delay or prevent succession from continuing to a desired restoration end point (Hobbs and Huenneke 1992, Aide and Cavelier 1994, Aide et al. 1995, Nepstad et al. 1996, Aide et al. 2000, Guariguata and Ostertag 2001, Chazdon 2003). For example, exotic C_4 grasses can prevent recolonization by native species in disturbed tropical forest landscapes (Hooper et al. 2004, Jones et al. 2004). Removal of forests and increased light conditions that favor growth rates of C_4 grasses can lead to dense stands of these exotic invaders (Sage and Kubien 2003, Joo Kim et al. 2008, Foxcroft et al. 2010). Such invaders can further alter the natural successional trajectory if they generate feedbacks such as intensified fire regimes (D'Antonio and Vitousek 1992, Aide and Cavelier 1994). Reducing the abundance of invasive grasses is then critical to decrease fuel loads and allow the establishment of trees in the system. Since mechanical or chemical weed control can be prohibitively expensive, successful restoration may sometimes depend on the ability of planted species themselves to suppress exotic weeds (Hooper et al. 2002, Jones et al. 2004, Wishnie et al. 2007).

Certain functional traits may be key to the success of restoration species. Trees that rapidly produce deep shade may outcompete shade-intolerant exotic species (Jones et al. 2004, Joo Kim et al. 2008). Some planted trees can also affect growth of understory plants through their effects on nutrient availability (Pearson and Vitousek 2001, Dakora and Phillips 2002, Ashton et al. 2008) and soil pH (Haynes 1983). Less explored, however, is whether some tree species produce allelochemicals that inhibit germination or growth of understory plants. Native species that produce allelopathic compounds may be useful to control exotic weeds as part of forest restoration efforts.

In this paper we explore the potential use of allelopathy as a tool in forest restoration and exotic species management. We begin by reviewing literature from agroforestry, summarizing the approaches taken and evidence provided for allelopathic effects of trees on herbaceous weeds and crops. We then focus on allelopathy in the context of invasive species, examining situations where allelopathic species and response species do not share an evolutionary history. Finally, we present data from a greenhouse study that tests the allelopathic potential of leaves from trees used for forest restoration to suppress the growth of the exotic invasive grass *Saccharum spontaneum* L.

Allelopathy and Agroforestry

Agroforestry is an approach to sustainable land management that integrates trees with agricultural crops and/or livestock (Rizvi et al. 1999). Inhibition of understory growth in the absence of competition for light, water, or nutrients suggests that allelopathy may play a role in suppressing growth of some plants in the agroforest understory (Lodhi and Rice 1971, Chou and Kuo 1986, Bhatt et al. 1997, Bhatt et al. 2010). For example, black walnut (*Juglans nigra* L.) is commonly grown for its nuts and wood in North America. Sensitivity of understory crops to allelochemicals produced by *Juglans* spp. has required careful evaluation of which species can be effectively incorporated into *Juglans* agroforestry models (Willis 2000). Similar interactions in other intercropping systems have stimulated interest in determining optimal crop/tree combinations. In addition to the interspecific allelopathic effects of agroforestry trees on understory crops, some studies have documented inhibitory allelopathic effects of trees on weed germination and growth (Chou and Kuo

1986, Babu and Kandasamy 1997, Williams and Hoagland 2007, Matok et al. 2009, Kaur et al. 2011, Wu et al. 2011).

Allelopathy has been suspected in a wide range of tree species (Table 1). In addition to *Juglans* (walnut), the allelopathic effects of numerous agroforestry genera, including *Eucalyptus* spp*.* (Espinosa-Garcia 1996), and *Leucaena* spp*.* (John and Narwal 2003), have been particularly well studied (Table 1). Robust conclusions about the importance of allelopathic interactions in field systems require extensive and interlinked experimental bioassays, biochemical characterization, and field experiments (Inderjit and Callaway 2003). Unfortunately, there are few data available to evaluate allelopathic activity for most suspected species (Table 1).

The Petri dish bioassay of leaf extracts (leachates) on seed germination and seedling growth is perhaps the most common approach to studying allelopathy. Many studies (Table 1) that used leaf extract bioassays found a negative effect of tree leaf material on crop seedlings (Lisanework and Michelsen 1993, Bhatt et al. 2010, Cui et al. 2011). However, the most consistent result is a tremendous variation among response species in their degree of susceptibility to extracts (Table 1).

There are fewer studies that measure natural levels of allelochemicals in the soil or that quantify the allelopathic effects of trees on herbaceous plants in the field (Table 1). Singh and Kohli (1992) found that crops grown closer to *Eucalyptus* shelterbelts (windbreak plantations) had reduced yields, and that soil extracts from near *Eucalyptus* suppressed germination. More field studies are needed to quantify the strength of allelopathic effects of trees on crops and other herbaceous species.

The combined results of these studies have stimulated interest in the use of allelopathic trees as part of crop production and pest management systems. Depending on the crop-tree (Chaturvedi and Jha 1992, Lisanework and Michelsen 1993, Alrababah et al. 2009, Li et al. 2010b) or weed-tree (Babu and Kandasamy 1997, El-Rokiek and Eid 2009, Kaur et al. 2011) combination, the effect of tree allelochemicals may vary from inhibitory to stimulatory. For example, when investigating the effects of leaf extracts on two perennial weeds, fresh leaf leachates of *Eucalyptus globulus* Labill. inhibited the growth of bermuda grass (*Cynodon dactylon* L. Pers.) but promoted the growth of purple nutsedge (*Cyperus rotundus* L.) (Babu and Kandasamy 1997). Similarly, germination of cereal crops was less affected than legume crops by fresh and dry leaf extracts and soil extracts from *Quercus coccifera* L. and *Pinus halpensis* Mill. (Alrababah et al. 2009). This demonstrates the need for careful evaluation of tree-crop/weed combinations when designing agroforestry systems.

Understanding the mechanisms of differential allelopathic effects can open opportunities for creative forest restoration and agroforestry management, by excluding recruitment of undesired species while promoting others. *Terminalia* spp. (Bhatt et al. 1997, Bhatt et al. 2010, Baratelli et al. 2012), *Gliricidia sepium* (Jacq.) Kunth. (Kamara et al. 2000a, Kamara et al. 2000b, John et al. 2007, Williams and Hoagland 2007)*,* and *Tectona grandis* (Linn. f.) (John et al. 2007, Sahoo et al. 2007, Bhatt et al. 2010) have been suggested to be allelopathic towards crops and weeds, and are also used commonly for restoration (Wishnie et al. 2007, van Breugel et al. 2011). These studies point to possible strategic uses of allelopathic trees in restoration or forestry. Here we explore the idea of incorporating these concepts into forest restoration models to improve restoration in areas where invasive weeds inhibit restoration success.

Resisting invasion and Restoration: the "Homeland Security" Hypothesis

Some aggressive invaders are thought to be successful in part because of allelochemicals they produce. A number of invasive species are thought to have allelopathic properties (Wink 2002, Haubensak and Parker 2004, Inderjit et al. 2006, Jarchow and Cook 2009, Lankau 2010, Valera-Burgos et al. 2012); however, it is not clear how often allelopathy contributes to invasion success.

Allelochemicals produced by introduced species may facilitate invasion when native species cannot tolerate what to them are novel chemical compounds (Bais et al. 2003, Callaway and Ridenour 2004, Pollock et al. 2008). The Novel Weapons Hypothesis (Callaway and Ridenour 2004), suggests that an introduced species may have strong allelopathic effects on neighboring plant species in a novel environment, even though it does not show similar effects on species in its native range where plants share an evolutionary history. For instance, competitive dominance of *Centaurea diffusa* Lam. in invaded range of North America has been attributed to the release of allelopathic compounds into the environment (Callaway and Aschehoug 2000, Ridenour and Callaway 2001). Studies using the congener *Centaurea maculosa* Lam. (now *C. stoebe*) showed similar negative allelopathic effects on plant species in its invaded range of North America (He et al. 2009, Thorpe et al. 2009). Increasing evidence suggests that, for some species, allelopathy may play a major role in invasion success.

The concept of “novel weapons” could also operate in reverse to increase the resistance of native communities to invasion. We call this the “Homeland Security” hypothesis. Introduced plants may be naïve and particularly susceptible to allelochemicals produced by local native plant species, which could impede invasion. Thus allelopathy may contribute to invasion resistance of native communities. For example, in Florida’s sand pine scrub habitats, allelochemicals released by *Polygonella myriophylla* (Small. Horton) and other native scrub vegetation inhibit invasion by invasive grasses such as bahiagrass *Paspalum notatum* (Small) Horton (Weidenhamer and Romeo 2005).

Variation in allelopathy of native plant species may also contribute to variation in invasibility across plant communities. *Mikania micrantha* H.B.K. is a highly invasive perennial vine that has spread widely throughout China, invading forests and agricultural areas (Hou et al. 2012). Variation in *M. micrantha* invasion success raised the question of whether allelopathy from certain local trees might suppress growth of the exotic. Hou et al. (2012) tested the allelopathic response of *M. micrantha* to six dominant tree species: *Pinus massoniana* Lamb. , *Schima superba* (Gardner & Champ)*, Castanopsis chinensis, Castanopsis fissa, Cryptocarya chinensis* (Hance) Hemsel*,* and *Cryptocarya concinna* (Hance) Hemsl from 3 different forest types. All six tree species inhibited root growth after germination in laboratory bioassays using leaf litter extracts. In a pot experiment, individual seedlings of five of the tree species were planted in pots with 50 seeds of *M. micrantha* and grown for 5 months. Growth of *M. micrantha* was inhibited by four of the species, while one (*Cryptocarya concinna*) enhanced stem length and biomass (Hou et al. 2012). This study suggests that invasion resistance of intact communities may be partially due to allelochemical growth inhibition of exotic species. However, we need more studies that examine the interactions between potentially allelopathic natives and exotic species.

Evaluating which native species may provide an allelopathic arsenal against invaders currently requires a species-by-species, empirical approach. Assessing the usefulness of functional groups for predicting allelopathy is especially important in tropical forest restoration, where hyperdiverse native communities offer hundreds of tree species as possible choices. For example, fast-growing pioneer species in areas with high resource availability tend not to invest heavily in secondary chemistry (Fine et al. 2006), and may be less likely to have allelopathic effects. Certain families or genera are associated with high concentrations or high diversity of secondary compounds that could produce allelopathic effects (Hadacek 2002, Singh et al. 2003, Wink 2003). For instance, the high levels of glucosinolates found in many Brassicaceae and alkaloids in many Fabaceae may increase the probability that plants in these groups could have allelopathic effects.

Incorporating allelopathy into forest restoration strategies: A case study from Panama

In many parts of the tropics, weedy C_4 grasses colonize degraded deforested areas (Nepstad et al. 1996, Batianoff and Franks 1998, Hooper et al. 2004, Jones et al. 2004, Wishnie et al. 2007). In Panama, *Saccharum spontaneum* is a large exotic grass species that invades abandoned agricultural fields and other disturbed sites. Like other C_4 grasses, *S. spontaneum* is adapted to drought, establishes and grows rapidly in high light conditions, and promotes intense and frequent fires that kill young trees. It is very difficult to eradicate and is considered one of the most serious weeds in many tropical areas (Holm et al. 1979, Hooper et al. 2004).

In Panama, PRORENA (The Native Species Reforestation Program) has examined numerous strategies for inhibiting *S. spontaneum* and restoring diverse, native tropical forests. In an abandoned agricultural area dominated by *S. spontaneum,* PRORENA established replicated monospecific reforestation test plots to evaluate survival, growth, and canopy cover of over 75 different native and commercially important tree species (Hooper et al. 2002, Jones et al. 2004, Wishnie et al. 2007, Park et al. 2010, Hall et al. 2011b, van Breugel et al. 2011). In previous work, these species were characterized for a number of functional traits, but not for allelopathic effects.

We observed great variation in the density and growth of *S. spontaneum* under different tree species in the PRORENA plots. Not surprisingly, density of the invader was lower in deep shade, but it was even lower under legume than under non-legume tree species (J. Cummings, unpublished data). These observations led to the hypothesis that allelopathic effects of legumes might contribute to suppression of *S. spontaneum.*

Because litter decomposition can be an important source of allelochemicals (Reigosa et al. 1999), we focused on the effects of leaf litter from legume and non-legume trees on growth of *S. spontaneum.* Leaf litter would be expected to deliver nutrients to the plants as well, meaning that allelopathic effects can be modulated by other factors in litter addition studies. However, from a practical perspective, litter addition is a sensible first step to evaluate whether a candidate species for restoration might help control weeds (Babu and Kandasamy 1997). In a shade house, we placed 4g of fresh mature leaves from 3 legumes, (*Gliricidia sepium, Diphysa americana* (Mill.) M. Sousa*,* and *Inga punctata* Willd.), 3 non-legume (*Tectona grandis* L. f.*, Pachira quinata* (Jacq.) W.S. Alverson*,* and *Terminalia amazonia* (J.F.Gmel.)), all tree species from the PRORENA plots, on the surface of nursery bags containing potting soil and test plants of *S. spontaneum* (n=15 per tree species). Asexually produced *S. spontaneum* tillers were removed from the maternal plant and planted in the bags containing soil just prior to placing leaves on the soil surface. We included a control group that was identical to our treatments except no leaves were added to the bags. This project was establish at the end of May 2011 and harvested after 7 weeks. Bags were surface-watered during this time to allow for the breakdown of leaf material and release of compounds. We used a nested ANOVA (species within functional groups) to compare performance measures (leaf node height, above-and below-ground biomass, and mortality) at harvest, and general linear models with binomial variables for mortality. Results of the control treatments are presented graphically, but are not included in statistical analyses because the nested structure (Species within Legume/NonLegume), could not easily incorporate a comparison to the control (which was not replicated in the same way as functional group). However, the controls serve as a reference in the figures to evaluate the net of positive and negative effects of leaves from legume and non-legume trees. All data were analyzed using the statistical program R version 2.14.0.

Survival of *S. spontaneum* was significantly reduced by applying leaves of legume overstory species compared to non-legume leaves (Fig. 1A). Change in height was also marginally lower when leaves were from legume trees (Fig. 1B). Aboveground (F=0.64; df=2,4; p=0.62) and belowground (F=0.73; df=2,4; p=0.49) biomass was not significantly different. *Tectona grandis* did not show a strong negative effect on *S. spontaneum* (Fig. 1), which was surprising since leaf extracts of *T. grandis* have been shown to inhibit the germination, growth, and biomass yield of *Arachis hypogeae* L. and *Brassica campestris* L. (Bhatt et al. 2010).

We found that decomposing leaves from legumes appeared to reduce the persistence of *S. spontaneum,* but there was also much variation among legume tree species. We conducted a larger experiment with 17 candidate tree species, including 6 species of legume (*Gliricidia sepium, Inga punctata, Enterolobium cyclocarpum* (Jacq). Griseb*, Diphysa americana* (Mill.) M. Sousa*, Albizia guachapeli* (Kunth)*,* and *Albizia adenocephalia*) and 11 species of non-legume trees (*Terminalia amazonica* (J.F. Gmel) Exell*, Tectona grandis, Tabebuia rosea*

DC*, Tabebuia guayacan* (Seem.) Hemsl. *Pachira quinata* (Jacq.)*, Ochroma pyramidae* (Cav. ex Lamk)*, Luehea semannii* Planch. & Triana*, Guazuma ulmifolia* Lam*, Columbrina glandulosa, Cedrela odorata* L.*,* and *Calycophylum candidissimun* (Vahl.) DC) that were being evaluated as forest restoration species in the PRORENA plots. This project was implemented for 6 weeks from Oct-Nov 2011 with the same design to that above (n=15 individuals per species). In this experiment, plants treated with litter from legume trees showed marginally lower aboveground biomass (Fig. 2A) and belowground biomass (Fig. 2B), than those with non-legume litter; there was no effect of legumes (Z=0.38; df=17; p=0.70) or non-legumes on mortality (Z=1.03, df=17; 0.92). Interestingly, application of non-legume litter resulted in significantly higher biomass than controls with no litter, suggesting a possible nutritional benefit from litter.

The two studies together suggest that different tree species and perhaps functional groups of trees will have different effects on *S. spontaneum*, beyond effects of direct competition and shading. These patterns could be consistent with positive fertilization effects as well as negative allelopathic effects from canopy tree litter.

Future Directions

The primary measure of forest restoration success is the survival and growth of focal tree species (Jones et al. 2004, Wishnie et al. 2007, Hall et al. 2011b, van

Breugel et al. 2011). However, trees planted for restoration may also be able to suppress exotic weed species (Jones et al. 2004); this is particularly important when invasive exotic species promote fire that threatens long term forest recovery. Under these conditions, the allelopathic potential of native species should be assessed along with growth rates and other traits for evaluating restoration candidates.

Variation in the impact of restoration species on the performance of an invasive grass beyond that expected from shade alone suggests that allelopathy may be a useful tool for forest restoration. While other studies suggest the trees produce allelochemicals, we found fairly weak evidence for allelopathic effects on the grasses in this study; in some cases addition of tree litter even enhanced growth and survival of the grass. This suggests caution and particular attention to species-specific effects when recommending particular species for restoration.

Susceptibility to allelopathic chemicals can vary among recipient species (Rizvi et al. 1999, Caamal-Maldonado et al. 2001, Bhatt et al. 2010, Zhang and Fu 2010). The choice of restoration species may depend on which weeds are problems at a given site. We currently know little about how much variation to expect in the response of functionally similar species –among different tropical C_4 grasses, for example – to allelopathic litter. Thus, incorporating allelopathy into restoration strategies will require testing across response species to determine whether effects are general or species specific.

Stressful conditions can also amplify both the production and the effects of allelochemicals (Reigosa et al. 2006, Blanco 2007, Hooper et al. 2009, Graneli and Salomon 2010, Tesio and Ferrero 2010, Varkitzi et al. 2010). For example, N and P deficiency can enhance the production of allelochemicals in some species (Graneli and Salomon 2010, Varkitzi et al. 2010) as well as sensitivity to allelochemicals (Einhellig 1996). Water stress (Tang et al. 1995, Tongma et al. 2001) and interspecific competition (Lankau and Strauss 2007) can similarly stimulate the production of allelochemicals. In one study, stress effects were found to be most intense when plants were exposed to a combination of allelochemicals, salt, and drought (Reigosa et al. 2006). In some cases, microbes enhance allelopathic effects by converting allelopathic compounds into more toxic forms (Gagliardo and Chilton 1992, Bains et al. 2009), or by redistributing allelochemicals through fungal networks (Barto et al. 2011). The stress of growing under low light, such as found in the PRORENA plots in Panama, can sometimes act synergistically with allelopathic chemicals (Hussain et al. 2011, Lorenzo et al. 2011). The combination of stressful conditions and allelopathy could thus work in conjunction to inhibit growth more than competition alone.

Plants species that share an evolutionary history with allelopathic species may have evolved resistance to the allelochemicals that allow them to co-exist (Vivanco et al. 2004, Pollock et al. 2008, Thorpe et al. 2009). For example, the allelochemical (-) catechin, produced by the exotic invader *Centaurea maculosa*, reduced growth when applied to pots containing grasses from its invaded range of North America, but did not affect the growth of grasses from its native range (Bais et al. 2003). Other studies have found similar patterns where allelopathic species have neutral interactions with species from their native range, whereas they inhibit species with which they lack an evolutionary history (Callaway and Aschehoug 2000, Thorpe et al. 2009, Ni et al. 2010, Kim and Lee 2011, Callaway et al. 2012). Some allelochemicals can even promote the growth of species that share a coevolutionary history (Kim and Lee 2011). The Homeland Security hypothesis suggests that allelopathic effects may provide useful antagonistic tools early in restoration to provide an advantage to native species over exotics, but then gradually fade in importance as the restored sites are colonized by additional native species. Careful selection and monitoring of early restoration species not only for their growth characteristics but also for their ability to inhibit weedy exotics and favor native biodiversity may improve restoration success.

We recommend that to evaluate the utility of allelopathy, forest restoration studies should:

1. Monitor response of invader species to plantings of native tree species
2. Examine interactions between candidate reforestation species and locally problematic weeds through extract, litter, and field experiments.

3. Monitor natural recruitment of native species under allelopathic restoration trees.
4. Investigate interactions between allelopathic effects and other stressors, including nutrients, drought, and light.

To test the "Homeland Security" Hypothesis, studies using native species should quantify the relative importance of allelopathy effects on other natives vs. allelopathy effects on introduced species. Ideally, these studies will go beyond leaf extract bioassays and will include evidence from field studies as well.

Based on the ecological role of allelopathy, and in keeping with the Homeland Security Hypothesis, we propose that identifying and incorporating allelopathic native species into restoration models may help to improve restoration by disproportionately suppressing invasive exotics. Research on forest restoration has greatly improved our ability to reforest degraded areas (Hooper et al. 2002, Wishnie et al. 2007, Hall et al. 2011a, Hall et al. 2011b). Allelopathy may be an additional useful tool to help reduce the persistence of exotic weeds and expedite the restoration of our world's forests.

Table 1. Representative studies investigating allelopathy of trees on understory plants. For each study, we report 1) the putatively allelopathic tree species that was the focal species for the investigation, 2) the species that were measured for their response to allelopathy, the type of approach taken in the study, a briefly stated main result of the study, the citation, and the location of the study. Unless otherwise stated, effects were measured in petri plate bioassays.

Tree taxon	Response species	Type of response spp.	Approach	Main result	Citation	Location
Juglans regia	*Atractylodes macrocephala*, *Isatis tinctoria*, *Polygala tenuifolia*, *Salvia mitirrhiza*	Medicinal plants	Effect of leaf extracts on seed germination	Variation among response species	(Li et al. 2010b)	China
Juglans regia	Turnip (*Brassica rapa*)	Crops	Effect of leaf extracts on germination and growth	Older trees produced stronger effects	(Cui et al. 2011)	China
Juglans regia	Dandelions (*Taraxacum officinale*)	Weed	Effects of extracts of fallen leaves, husks, and roots on seed germination and growth	Reduced germination	(Matok et al. 2009)	Poland
Juglans regia, *Pinus* spp.	Bread wheat (*Triticum aestivum*)	Crop	Field experiment: leaf and extract effects on growth	Reduced grain yield	(Akkaya et al. 2006)	Turkey

Pinus halepensis, Quercus coccifera	Wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), fava bean (*Vicia faba*)	Crops	Effects of leaf extracts on germination	Legume crop germination was more sensitive to extracts than cereal crops	(Alrababah et al. 2009)	Jordan
Cupressus lusitanica, Eucalyptus globulus, Eucalyptus camaldulensis, Eucalyptus saligna	Chickpea (*Cicer arietinum*) maize (*Zea mays*), pea (*Pisum sativum*), teff (*Eragrostis tef*)	Crop	Effects of leaf extracts on germination, seedling growth	Reduced germination and growth. Effects of *C. lusitanica* < *E. globulus* < *E. saligna* < *E. camaldulensi*	(Lisanework and Michelsen 1993)	Ethiopia
Eucalyptus tereticornis	Chickpea (*Cicer arietinum*), lentil (*Lens esculentum*), wheat (*Triticum aestivum*), cauliflower (*Brassica oleracea*), toria (*Brassica campestris*), Beerseem Clover (*Trifolium alexandrinum)*	Crop	Distance from shelterbelts (windbreak plantations in agricultural landscapes) as a proxy for chemical influence on the soil	Bioassays showed negative effects of soil extracts close to the shelterbelt, crop yield increased with distance	(Singh and Kohli 1992)	India

Eucalyptus urophylla	*Delonix regia, Eleocarpus sylvestris, Schima superba, Michelia macclurel*	Native spp.	Factorial field experiment manipulating presence of roots and litter	Germination was depressed in the presence of *Eucalyptus* roots or litter. Seedling growth was reduced with roots but not litter	(Zhang and Fu 2009)	China
Eucalyptus camaldulensis	Spinach (*Spinacia oleracea*), onion (*Allium cepa*), garden cress (*Lepidium sativum*), barnyard grass (*Rumex acetosella*), maize (*Zea mays*), tomato (*Solanum lycopersicon*), wild oat (*Avena fatua*)	Crops and weeds	Effects of leaf and volatile oil extracts on the Hill reaction in chloroplasts (spinach), mitosis (onion), meristematic root tips and radical growth, and peroxidase activity (all other spp.)	Decreased cell division in the root apical meristem (*A. cepa*), inhibition of the Hill reaction (spinach), reduction in radical growth across all other spp. except *Z. mays*	(Moradshahi et al. 2003)	Iran

Eucalyptus citriodora	*Avena fatua*, *Hippeastrum hubridum*	Weeds	Effect of leaf extracts on growth and germination of oat weed. Greenhouse study of leaf extracts on oat weed and amaryllis growth and flowering	Inhibition of oat weed in lab and greenhouse. Stimulation of *Amaryllis* growth	(El-Rokiek and Eid 2009)	Egypt
Eucalyptus dundasii	*Lolium rigidum*, *Hordeum glaucum*	Weeds	Extract & Oil fraction effects on germination and growth	Oils depressed germination and sometimes growth	(Wu et al. 2011)	Australia
Eucalyptus globulus	*Cyperus rotundus*, *Cynodon dactylon*	Weeds	Greenhouse bioassay of fresh and dry leaf extract and fresh leaf cuttings on germination and growth	Fresh extracts inhibited bermuda grass but promoted purple nutsedge. Dry leaf extract had differential effects	(Babu and Kandasamy 1997)	India
Eucalyptus spp., *Acacia auriculiformis*	Rice (*Oryza sativa* var. Moroberekan)	Crop	Effects of leaf litter extracts on young rice seedlings	Increase in the root weight/root length ratio	(Bernhard-Reversat 1999)	Congo

Eucalyptus tereticornis	*Amaranthus viridis*	Weed	Chemical composition and effect of volatile oils on seed germination and seedling growth	Reduced seedling growth, photosynthesis and energy metabolism	(Kaur et al. 2011)	India
Eucalyptus urophylla	Native trees: *Cinnamomum burmanni, Cryptocarya concinna, Machilus chinensis, Photinia benthamiana, Pygeum topengii, Diospyros morrisiana, Pterospermum lanceaefolium;* Exotic trees: *Acacia confusa, Albizia lebbeck*, *Albizia falcataria*	Native and exotic trees	Effects of leaf extracts and volatiles on seed germination and seedling growth	Variation among response species and response variables	(Fang et al. 2009)	China

Chukrasia tabularis, Tectona grandis, Terminalia myriocarpa, Trema orientalis	Rice (*Oryza sativa*), maize (*Zea mays*), *Vigna radiata, Vigna umbellate, Arachis hypogea, Brassica campestris*	Crops	Effects of leaf extracts on germination and on growth in pots	Response species varied: Oilseed crops were most susceptible, followed by cereal crops and finally legumes	(Bhatt et al. 2010)	India
Pinus pinea	*Cistus salvifolius, Cistus libanotis, Halimium halimifolium*	Native shrubs	Needle extracts crossed with a physical barrier (toothpicks) treatment	Extracts reduced germination; responses varied across shrub species	(Valera-Burgos et al. 2012)	Spain
Pinus massoniana, Schima superba, Castanopsis chinensis, Castanopsis fissa, Cryptocarya chinensis, Cryptocarya concinna	*Mikania micrantha*	Weed	Leaf litter extract bioassays, 5-mo. pot experiment using 5 of the 6 spp.	Extracts from all spp. reduced early growth. Tree seedlings suppressed the invader in four cases but not in one for the pot experiment	(Hou et al. 2012)	China

Gliricidia sepium, Diphysa americana, Inga punctata, Tectona grandis, Pachira quinata, Terminalia amazonia	*Saccharum spontaneum*	Weed	Leaf litter addition in pot experiment	Effect of litter on survival was negative with legume trees but not with non-legumes	Cummings et al. (this paper)	Panama
17 tree species (see Figure 2)	*Saccharum spontaneum*	Weed	Leaf litter addition in pot experiment	Overall effect of litter on growth positive; with legume trees, less positive than non-legumes	Cummings et al. (this paper)	Panama
Acacia delbata	Lettuce (*Lactuca sativa*), maize (*Zea mays*), *Dactylis glomerata*, *Arabidopsis thaliana*, *Acacia delbata*	Crops, herbs, and tree spp.	Throughfall and litter extract effects on seed germination and seedling growth	Differential effects on test spp. with mostly stimulatory effects	(Lorenzo et al. 2010)	Spain

Acacia melanoxylon, Quercus robur, Pinus radiata, Eucalyptus globulus	Lettuce (*Lactuca sativa*)	Crop	Effects of extracts of decomposing leaf material on germination	Inhibitory effects varied by species and with time	(Gonzales et al. 1995)	Spain
Acacia melanoxylon	Cocksfoot (*Dactylis glomerata*) ryegrass (*Lolium perenne*), common sorrel (*Rumex acetosa*), lettuce (*Lactuca sativa*)	Native spp. and lettuce	Effects of phylode and flower extracts on germination and physiological function in pearlite	Reduction in growth and physiological function in all spp.	(Hussain et al. 2011)	Spain
American chestnut (*Castanea dentate*)	Red maple (*Acer rubrum*), sugar maple (*A. saccharum*), eastern white pine (*Pinus strobus*), eastern hemlock (*Tsuga canadensis*), yellow-poplar (*Liriodendron tulipifera*), rosebay rhododendron (*Rhododendron maximum*), lettuce (*Lactuca sativa*)	Native trees and lettuce	Extract effects on seed germination and seedling growth	Reduction in germination and radical growth	(Vandermast et al. 2002)	USA

Celtis laevigata	*Andropogon gerardi, A. scoparius, Panicum virgatum, Sorghastrum nutans*	Native spp.	Field study comparing stands of *C. laevigata* and *Prunus*; Greenhouse experiment on effects of dried leaf litter, extracts, and *s*oil on germination and growth	Inhibitory effects of decaying leaves, extracts, and soils	(Lodhi and Rice 1971)	USA
Leucaena leucocephala	Rice (*Oryza sativa*), *Acacia niolotica, Casuarina equisetifolia*, *Pongamia pinnata, Lagerstroemia speciosa*, *Sesbania grandiflora, Leucaena leucocephala*	Crop (rice) and tree spp.	Effects of leaf extracts on germination and growth. Evaluation of mimosine content in leaves of *Leucaena*	Variation in mimosine content between varieties. Differential germination among test species	(Chaturvedi and Jha 1992)	India
Eucalyptus camaldulensis,	*Amaranthus hybridus, Portulaca oleracea*	Weeds	Oil extraction and effects on seedling growth and germination	Complete inhibition of germination and seedling growth	(Verdeguer et al. 2009)	Spain

Leucaena leucocephala	Lettuce (*Lactuca sativa*), rice (*Oryza sativa*), rye grass (*Lolium multiflorum*), *Acacia confusa, Alnus formosana, Casuarina glauca, Pinus taiwanensis, Liquidambar formosana, Miscanthus floridulus, Mimosa pudica, Ageratum conzoides*	Crops, weeds, and trees	Comparison of understory biomass between *Leucaena* and grasslands. Seedling response to dry leaf extracts and to soil from beneath *Leucaena*.	Lower biomass under *Leucaena*. Extracts reduced germination and growth of all test spp except *P. taiwanensis* and *M. floridulus*	(Chou and Kuo 1986)	Taiwan

Leucaena leucocephala, Gliricidia sepium, Senna siamea	*Talinum triangulare* Sedges (*Cyperus* & *Mariscus* spp.), *Panicum maximum, Digitaria horizontalis Triumfetta* spp., *Chromolaena odorata, Centrosema pubescens, Celosia leptostachya, Calopogonium mucunoides, Commelina* spp., *Physalis angulata, Desmodium scorpirus, Oldenlandia corymbosa*, *Tridax procumbens*, *Psophocarpus palustris*, *Euphorbia hirta*, other grass weeds, other broadleaf weeds	Weeds	Field experiment: mulch suppression of local weeds	Mulch from *G. sepium* and *S. siamea* suppressed weed growth, but no effect of *L. leucocephala*	(Kamara et al. 2000a)	Nigeria

Sonneratia apetala, *Spartina alterniflora*	Alfalfa (*Medicago sativa*), ryegrass (*Lolium perenne*), sudangrass (*Sorghum sudanense*)	Weeds and crops	Effect of leaf volatiles and soil on germination and growth	Volatiles reduced germination and growth. Soil from *S. apetala* suppressed *S. alterniflora* growth	(Li et al. 2010a)	China

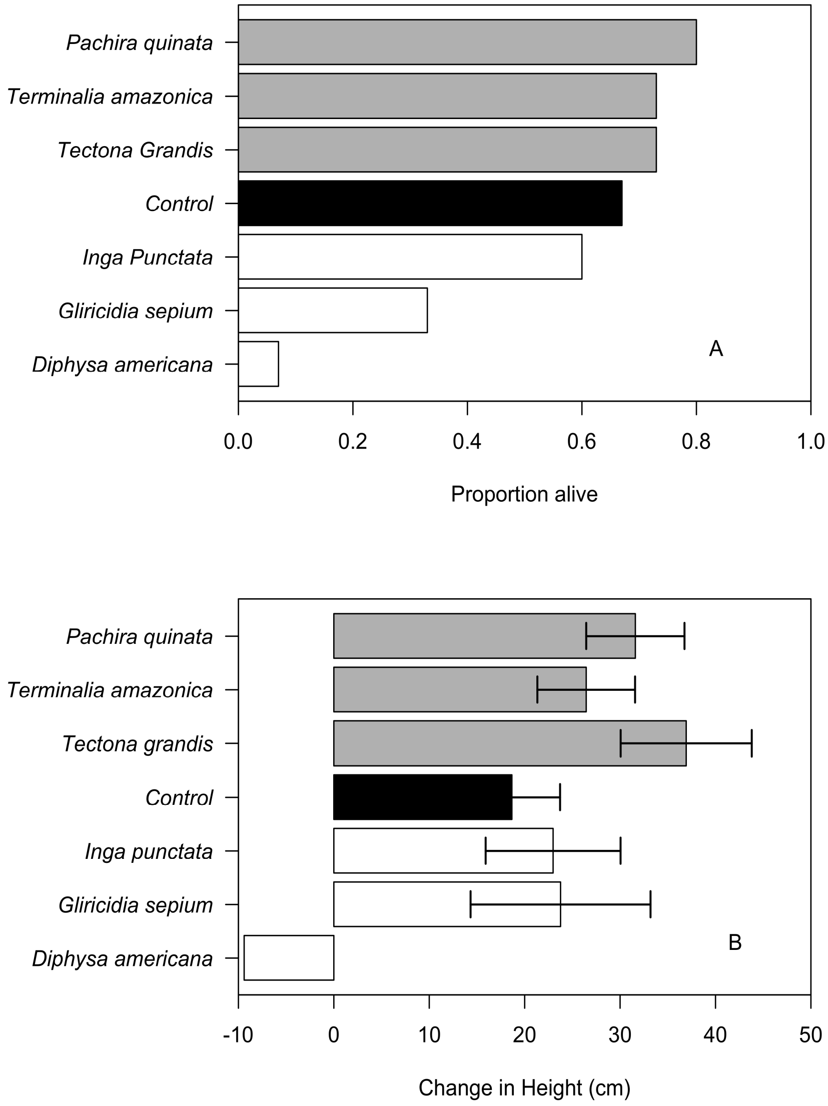

Figure 1. (A) Survival of *Saccharum spontaneum* for 7 weeks was significantly less after applying litter from legume (white) than non-legume (gray) tree species (df=5; Z= 3.88; p<0.001). (B) Mean change in height (± S.E.) for *S. spontaneum* grown with litter from legume and non-legume overstory trees. Legumes and non-legumes were marginally different (df=1,4; F=2.95; p=0.09). Black bar is the no-litter control.

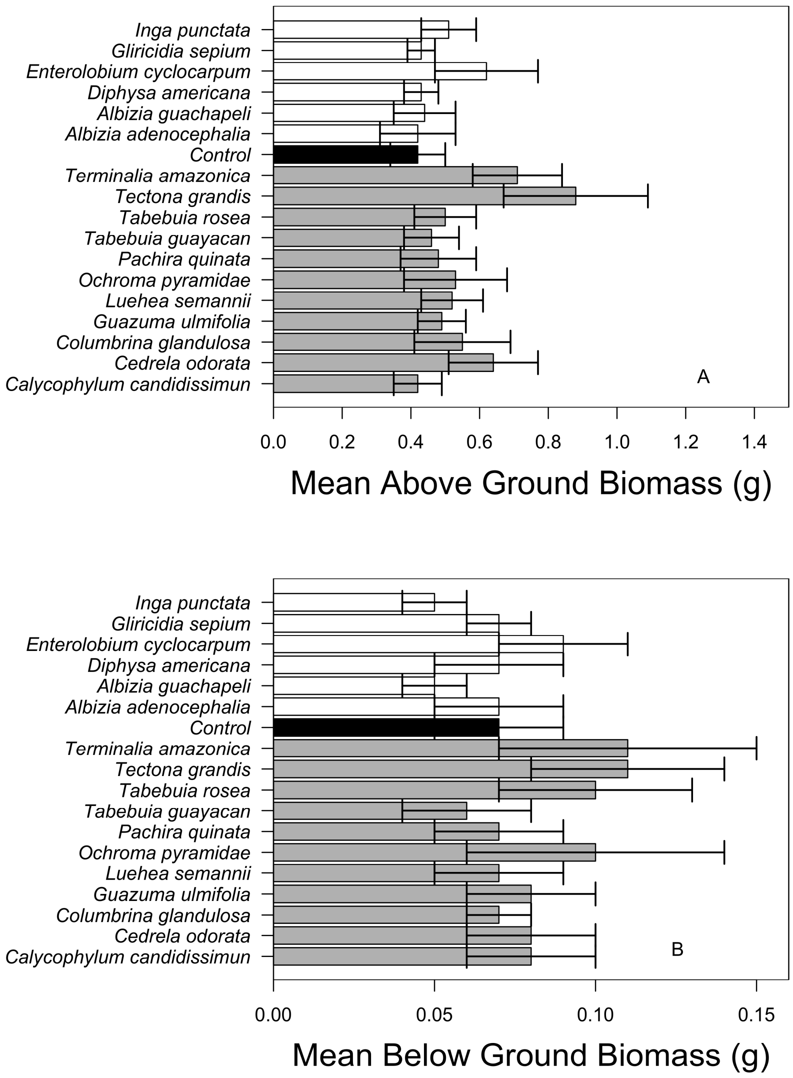

Figure 2. (A) Mean (±S.E.) above ground biomass (df=1,15; F=3.45; p=0.07) and (B) mean (±S.E.) below ground biomass (df=1,15; F=3.45; p=0.07) of *S. spontaneum* grown in the shade house with litter from legume (white) and non-legume (gray) tree species. *S. spontaneum* biomass was marginally lower in legume treatments than non-legume treatments. No-leaf controls (black bars) are show for visual comparison.

Works Cited

Aerts, R. and O. Honnay. 2011. Forest restoration, biodiversity and ecosystem functioning. BMC Ecology **11**:29.

Aide, T. M. and J. Cavelier. 1994. Barriers to lowland tropical forest restoration in the Sierra Nevada de Santa Marta, Colombia. Restoration Ecology **2**:219-229.

Aide, T. M., J. K. Zimmerman, L. Herrera, M. Rosario, and M. Serrano. 1995. Forest recovery in abandoned tropical pastures in Puerto Rico. Forest Ecology and Management **77**:77-86.

Aide, T. M., J. K. Zimmerman, J. B. Pascarella, L. Rivera, and H. Marcano-Vega. 2000. Forest regeneration in a chronosequence of tropical abandoned pastures: Implications for restoration ecology. Restoration Ecology **8**:328-338.

Alrababah, M. A., M. J. Tadros, N. H. Samarah, and H. Ghosheh. 2009. Allelopathic effects of *Pinus halepensis* and *Quercus coccifera* on the germination of Mediterranean crop seeds. New Forests **38**:261-272.

Ashton, P. M. S., S. J. Samarasinghe, I. Gunatilleke, and C. V. S. Gunatilleke. 2008. Role of legumes in release of successionally arrested grasslands in the central hills of Sri Lanka. Restoration Ecology **5**:36-43.

Babu, R. C. and O. S. Kandasamy. 1997. Allelopathic effect of *Eucalyptus globulus* Labill. on *Cyperus rotundus* L. and *Cynodon dactylon* L. Pers. Journal of Agronomy and Crop Science-Zeitschrift Fur Acker Und Pflanzenbau **179**:123-126.

Bains, G., A. S. Kumar, T. Rudrappa, E. Alff, T. E. Hanson, and H. P. Bais. 2009. Native plant and microbial contributions to a negative plant-plant interaction. Plant Physiology **151**:2145-2151.

Bais, H. P., R. Vepachedu, S. Gilroy, R. M. Callaway, and J. M. Vivanco. 2003. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. Science **301**:1377-1380.

Baratelli, T. D., A. C. C. Gomes, L. A. Wessjohann, R. M. Kuster, and N. K. Simas. 2012. Phytochemical and allelopathic studies of Terminalia catappa L. (Combretaceae). Biochemical Systematics and Ecology **41**:119-125.

Barto, E. K., M. Hilker, F. Mueller, B. K. Mohney, J. D. Weidenhamer, and M. C. Rillig. 2011. The fungal fast fane: Common mycorrhizal networks extend bioactive zones of allelochemicals in soils. Plos One **6**.

Batianoff, G. N. and A. J. Franks. 1998. Weed invasion of the tropical Mackay coast, Queensland, Australia. Plant Protection Quarterly **13**:123-130.

Bhatt, B. P., M. Kumar, and N. P. Todaria. 1997. Studies on the allelopathic effects of *Terminalia* species of Garhwal Himalaya. Journal of Sustainable Agriculture **11**:71-84.

Bhatt, B. P., J. K. Singh, L. Barooah, and Imtimongla. 2010. Phytotoxic influence of agroforestry tree species on food crops in Eastern Himalaya, India. Allelopathy Journal **25**:485-495.

Blanco, J. A. 2007. The representation of allelopathy in ecosystem-level forest models. Ecological Modelling **209**:65-77.

Caamal-Maldonado, J. A., J. J. Jimenez-Osornio, A. Torres-Barragan, and A. L. Anaya. 2001. The use of allelopathic legume cover and mulch species for weed control in cropping systems. Agronomy Journal **93**:27-36.

Callaway, R. M. and E. T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science **290**:521-523.

Callaway, R. M. and W. M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment **2**:436-443.

Callaway, R. M., U. Schaffner, G. C. Thelen, A. Khamraev, T. Juginisov, and J. L. Maron. 2012. Impact of *Acroptilon repens* on co-occurring native plants is greater in the invader's non-native range. Biological Invasions **14**:1143-1155.

Carpenter, F. L., J. D. Nichols, and E. Sandi. 2004. Early growth of native and exotic trees planted on degraded tropical pasture. Forest Ecology and Management **196**:367-378.

Chaturvedi, O. P. and A. N. Jha. 1992. Studies on allelopathic potential of an important agroforestry species. Forest Ecology and Management **53**:91-98.

Chazdon, R. L. 2003. Tropical forest recovery: legacies of human impact and natural disturbances. Perspectives in Plant Ecology Evolution and Systematics **6**:51-71.

Chou, C. H. and Y. L. Kuo. 1986. Allelopathic research of subtropical vegetation in Taiwan. 3. Allelopathic exclusion of understory by *Leucaena leucocephala* (Lam) Dewit. Journal of Chemical Ecology **12**:1431-1448.

Clewell, A. F. and J. Aronson. 2006. Motivations for the restoration of ecosystems. Conservation Biology **20**:420-428.

Condit, R., W. D. Robinson, R. Ibanez, S. Aguilar, A. Sanjur, R. Martinez, R. F. Stallard, T. Garcia, G. R. Angehr, L. Petit, S. J. Wright, T. R. Robinson, and S. Heckadon. 2001. The status of the Panama Canal watershed and its biodiversity at the beginning of the 21st century. Bioscience **51**:389-398.

Cui, C., J. Cai, Z. M. Jiang, and S. X. Zhang. 2011. Effects of walnut (*Juglans regia* L.) root exudates on germination, seedling growth and enzymatic activities of turnip (*Brassica rapa L.*). Allelopathy Journal **28**:237-249.

D'Antonio, C. M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63-87.

Dakora, F. D. and D. A. Phillips. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil **245**:35-47.

Donald, P. F. and A. D. Evans. 2006. Habitat connectivity and matrix restoration: the wider implications of agri-environment schemes. Journal of Applied Ecology **43**:209-218.

Einhellig, F. A. 1996. Interactions involving allelopathy in cropping systems. Agronomy Journal **88**:886-893.

El-Rokiek, K. G. and R. A. Eid. 2009. Allelopathic effects of *Eucalyptus citriodora* on *Amaryllis* and associated grassy weeds Planta Daninha **27**:887-899.

Espinosa-Garcia, F. J. 1996. Review on alleopathy of Eucalyptus L'Herit. Boletin de la Sociedad Botanica de Mexico **0**:55-74.

Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Saaksjarvi, L. C. Schultz, and P. D. Coley. 2006. The growth-defense tradeoff and habitat specialization by plants in Amazonian forests. Ecology **87**:S150-S162.

Foxcroft, L. C., D. M. Richardson, M. Rejmanek, and P. Pysek. 2010. Alien plant invasions in tropical and sub-tropical savannas: patterns, processes and prospects. Biological Invasions **12**:3913-3933.

Gagliardo, R. W. and W. S. Chilton. 1992. Soil transformation of 2(3H)-benzoxazolone of rye into phytotoxic 2-amino-3H-phenoxazin-3-one Journal of Chemical Ecology **18**:1683-1691.

Graneli, E. and P. S. Salomon. 2010. Factors influencing allelopathy and toxicity in *Prymnesium parvum*. Journal of the American Water Resources Association **46**:108-120.

Guariguata, M. R. and R. Ostertag. 2001. Neotropical secondary forest succession: changes in structural and functional characteristics. Forest Ecology and Management **148**:185-206.

Hadacek, F. 2002. Secondary metabolites as plant traits: Current assessment and future perspectives. Critical Reviews in Plant Sciences **21**:273-322.

Hall, J. S., M. S. Ashton, E. J. Garen, and S. Jose. 2011a. The ecology and ecosystem services of native trees: Implications for reforestation and land restoration in Mesoamerica. Forest Ecology and Management **261**:1553-1557.

Hall, J. S., B. E. Love, E. J. Garen, J. L. Slusser, K. Saltonstall, S. Mathias, M. van Breugel, D. Ibarra, E. W. Bork, D. Spaner, M. H. Wishnie, and M. S. Ashton. 2011b. Tree plantations on farms: Evaluating growth and potential for success. Forest Ecology and Management **261**:1675-1683.

Haubensak, K. A. and I. M. Parker. 2004. Soil changes accompanying invasion of the exotic shrub *Cytisus scoparius* in glacial outwash prairies of western Washington USA. Plant Ecology **175**:71-79.

Haynes, R. J. 1983. Soil acidification induced by Leguminous crops. Grass and Forage Science **38**:1-11.

He, W.-M., Y. Feng, W. M. Ridenour, G. C. Thelen, J. L. Pollock, A. Diaconu, and R. M. Callaway. 2009. Novel weapons and invasion: biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (+/-)-catechin. Oecologia **159**:803-815.

Hobbs, R. J. and L. F. Huenneke. 1992. Disturbance, diversity, and invasion - Implications for conservation. Conservation Biology **6**:324-337.

Holl, K. D. 1999. Factors limiting tropical rain forest regeneration in abandoned pasture: seed rain, seed germination, microclimate, and soil. Biotropica **31**:229-242.

Holm, L., J. V. Pancho, J. P. Herberger, and D. L. Plucknett. 1979. A geographical atlas of world weeds. New York.

Hooper, A. M., A. Hassanali, K. Chamberlain, Z. Khan, and J. A. Pickett. 2009. New genetic opportunities from legume intercrops for controlling Striga spp. parasitic weeds. Pest Management Science **65**:546-552.

Hooper, E., R. Condit, and P. Legendre. 2002. Responses of 20 native tree species to reforestation strategies for abandoned farmland in Panama. Ecological Applications **12**:1626-1641.

Hooper, E., P. Legendre, and R. Condit. 2005. Barriers to forest regeneration of deforested and abandoned land in Panama. Journal of Applied Ecology **42**:1165-1174.

Hooper, E. R., P. Legendre, and R. Condit. 2004. Factors affecting community composition of forest regeneration in deforested, abandoned land in Panama. Ecology **85**:3313-3326.

Hou, Y.-P., S.-L. Peng, B.-M. Chen, and G.-Y. Ni. 2011. Inhibition of an invasive plant (*Mikania micrantha* H.B.K.) by soils of three different forests in lower subtropical China. Biological Invasions **13**:381-391

.

Hou, Y. P., S. L. Peng, G. Y. Ni, and L. Y. Chen. 2012. Inhibition of invasive species *Mikania micrantha* HBK by native dominant trees in China. Allelopathy Journal **29**:307-314.

Huang, L., J. Liu, Q. Shao, and X. Xu. 2012. Carbon sequestration by forestation across China: Past, present, and future. Renewable & Sustainable Energy Reviews **16**:1291-1299.

Hussain, M. I., L. Gonzalez, C. Souto, and M. J. Reigosa. 2011. Ecophysiological responses of three native herbs to phytotoxic potential of invasive *Acacia melanoxylon* R. Br. Agroforestry Systems **83**:149-166.

Inderjit and R. M. Callaway. 2003. Experimental designs for the study of allelopathy. Plant and Soil **256**:1-11.

Inderjit, R. M. Callaway, and J. M. Vivanco. 2006. Can plant biochemistry contribute to understanding of invasion ecology? Trends in Plant Science **11**:574-580.

Jarchow, M. E. and B. J. Cook. 2009. Allelopathy as a mechanism for the invasion of *Typha angustifolia*. Plant Ecology **204**:113-124.

John, J. and S. S. Narwal. 2003. Allelopathic plants. 9. *Leucaena leucocephala* (Lam.) de Wit. Allelopathy Journal **12**:13-36.

John, J., K. M. Sreekumar, and P. Rekha. 2007. Allelopathic effects of leaf leachates of multipurpose trees on vegetables. Allelopathy Journal **19**:507-516.

Jones, E. R., M. H. Wishnie, J. Deago, A. Sautu, and A. Cerezo. 2004. Facilitating natural regeneration in *Saccharum spontaneum* (L.) grasslands within the Panama Canal Watershed: effects of tree species and tree structure on vegetation recruitment patterns. Forest Ecology and Management **191**:171-183.

Joo Kim, T., F. Montagnini, and D. Dent. 2008. Rehabilitating abandoned pastures in Panama: Control of the invasive exotic grass, *Saccharum spontaneum* L., using artificial shade treatments. Journal of Sustainable Forestry **26**:192-203.

Kamara, A. Y., I. O. Akobundu, D. Chikoye, and S. C. Jutzi. 2000a. Selective control of weeds in an arable crop by mulches from some multipurpose trees in Southwestern Nigeria. Agroforestry Systems **50**:17-26.

Kamara, A. Y., I. O. Akobundu, N. Sanginga, and S. C. Jutzi. 2000b. Effect of mulch from selected multipurpose trees (MPTs) on growth, nitrogen nutrition and yield of maize (Zea mays L.). Journal of Agronomy and Crop Science-Zeitschrift Fur Acker Und Pflanzenbau **184**:73-80.

Kaur, S., H. P. Singh, D. R. Batish, and R. K. Kohli. 2011. Chemical characterization and allelopathic potential of volatile oil of *Eucalyptus tereticornis* against *Amaranthus viridis*. Journal of Plant Interactions **6**:297-302.

Kim, Y. O. and E. J. Lee. 2011. Comparison of phenolic compounds and the effects of invasive and native species in East Asia: support for the novel weapons hypothesis. Ecological Research **26**:87-94.

Lankau, R. 2010. Soil microbial communities alter allelopathic competition between *Alliaria petiolata* and a native species. Biological Invasions **12**:2059-2068.

Lankau, R. A. and S. Y. Strauss. 2007. Mutual feedbacks maintain both genetic and species diversity in a plant community. Science **317**:1561-1563.

Leung, G. P. C., B. C. H. Hau, and R. T. Corlett. 2009. Exotic plant invasion in the highly degraded upland landscape of Hong Kong, China. Biodiversity and Conservation **18**:191-202.

Li, Q. A., J. Cai, Z. M. Jiang, and S. X. Zhang. 2010. Allelopathic effects of walnut leaves leachate on seed germination, seedling growth of medicinal plants. Allelopathy Journal **26**:235-242.

Lisanework, N. and A. Michelsen. 1993. Allelopathy in agroforestry systems- the effects of leaf extracts of *Curpress lusitanica* and 3 *Eucalyptus* spp. on 4 Ethiopian crops. Agroforestry Systems **21**:63-74.

Little, C. and A. Lara. 2010. Ecological restoration for water yield increase as an ecosystem service in forested watersheds of south-central Chile. Bosque **31**:175-178.

Lodhi, M. A. K. and E. L. Rice. 1971. Allelopathic effects of *Celtis laevigata*. Bulletin of the Torrey Botanical Club **98**:83-&.

Loik, M. E. and K. D. Holl. 1999. Photosynthetic responses to light for rainforest seedlings planted in abandoned pasture, Costa Rica. Restoration Ecology **7**:382-391.

Loik, M. E. and K. D. Holl. 2001. Photosynthetic responses of tree seedlings in grass and under shrubs in early-successional tropical old fields, Costa Rica. Oecologia **127**:40-50.

Lorenzo, P., A. Palomera-Perez, M. J. Reigosa, and L. Gonzalez. 2011. Allelopathic interference of invasive *Acacia dealbata* Link on the physiological parameters of native understory species. Plant Ecology **212**:403-412.

MacDonald, G. E. 2004. Cogongrass (*Imperata cylindrica*) - Biology, ecology, and management. Critical Reviews in Plant Sciences **23**:367-380.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications **10**.

Matok, H., B. Leszczynski, G. Chrzanowski, and C. Sempruch. 2009. Effects of walnut phenolics on germination of dandelion seeds. Allelopathy Journal **24**:177-182.

McNamara, S., D. V. Tinh, P. D. Erskine, D. Lamb, D. Yates, and S. Brown. 2006. Rehabilitating degraded forest land in central Vietnam with mixed native species plantings. Forest Ecology and Management **233**.

Murray, K. G., K. Winnett-Murray, J. Roberts, K. HorJus, W. A. Haber, W. Zuchowski, M. Kuhlmann, and T. M. Long-Robinson. 2008. The roles of disperser behavior and physical habitat structure in regeneration of post-agricultural fields. Pages 192-215 *in* R. W. Myster, editor. Post - Agricultural Succession in the Neotropics. Springer, 233 Spring Street, New York, Ny 10013, United States.

Nepstad, D. C., C. Uhl, C. A. Pereira, and J. M. C. da Silva. 1996. A comparative study of tree establishment in abandoned pasture and mature forest of eastern Amazonia. Oikos **76**:25-39.

Ni, G.-Y., U. Schaffner, S.-L. Peng, and R. M. Callaway. 2010. *Acroptilon repens*, an Asian invader, has stronger competitive effects on species from America than species from its native range. Biological Invasions **12**:3653-3663.

Palmer, M. A., R. F. Ambrose, and N. L. Poff. 1997. Ecological theory and community restoration ecology. Restoration Ecology **5**:291-300.

Park, A., M. van Breugel, M. S. Ashton, M. Wishnie, E. Mariscal, J. Deago, D. Ibarra, N. Cedeno, and J. S. Hall. 2010. Local and regional environmental variation influences the growth of tropical trees in selection trials in the Republic of Panama. Forest Ecology and Management **260**:12-21.

Parsons, J. J. 1972. Spread of African pasture grasses to the American tropics. Journal of Range Management **25**:12-17.

Pearson, H. L. and P. M. Vitousek. 2001. Stand dynamics, nitrogen accumulation, and symbiotic nitrogen fixation in regenerating stands of Acacia koa. Ecological Applications **11**:1381-1394.

Pollock, J. L., T. R. Seastedt, R. M. Callaway, J. Kaur, and Inderjit. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biological Invasions **10**:875-890.

Putz, F. E. and K. H. Redford. 2010. The importance of defining 'Forest': Tropical forest degradation, deforestation, long-term phase shifts, and further transitions. Biotropica **42**:10-20.

Reigosa, M., N. Pedrol, and L. Gonzalez. 2006. Allelopathy: A physiological process with ecological implications. Allelopathy: A physiological process with ecological implications.

Reigosa, M. J., A. Sanchez-Moreiras, and L. Gonzalez. 1999. Ecophysiological approach in allelopathy. Critical Reviews in Plant Sciences **18**:577-608.

Ridenour, W. M. and R. M. Callaway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia **126**:444-450.

Rizvi, S. J. H., M. Tahir, V. Rizvi, R. K. Kohli, and A. Ansari. 1999. Allelopathic interactions in agroforestry systems. Critical Reviews in Plant Sciences **18**:773-796.

Sage, R. F. and D. S. Kubien. 2003. Quo vadis C-4? An ecophysiological perspective on global change and the future of C4 plants. Photosynthesis Research **77**:209-225.

Sahoo, U. K., K. Upadhyaya, and C. B. Meitei. 2007. Allelopathic effects of *Leucaena leucocephala* and T*ectona grandis* on gerrmnation and growth of maize. Allelopathy Journal **20**:135-143.

Satake, A. and T. K. Rudel. 2007. Modeling the forest transition: Forest scarcity and ecosystem service hypotheses. Ecological Applications **17**:2024-2036.

Singh, H. P., D. R. Batish, and R. K. Kohli. 2003. Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. Critical Reviews in Plant Sciences **22**:239-311.

Tang, C. S., W. F. Cai, K. Kohl, and R. K. Nishimoto. 1995. Plant stress and allelopathy. Allelopathy: Organisms, Processes, and Applications **582**:142-157.

Tesio, F. and A. Ferrero. 2010. Allelopathy, a chance for sustainable weed management. International Journal of Sustainable Development and World Ecology **17**:377-389.

Thangata, P. H. and P. E. Hildebrand. 2012. Carbon stock and sequestration potential of agroforestry systems in smallholder agroecosystems of sub-Saharan Africa: Mechanisms for 'reducing emissions from deforestation and forest degradation' (REDD+). Agriculture Ecosystems & Environment **158**:172-183.

Thorpe, A. S., G. C. Thelen, A. Diaconu, and R. M. Callaway. 2009. Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. Journal of Ecology **97**:641-645.

Tongma, S., K. Kobayashi, and K. Usui. 2001. Allelopathic activity of Mexican sunflower (*Tithonia diversifolia* (Hemsl.) A. Gray) in soil under natural field conditions and different moisture conditions. Weed Biology and Management **1**:115-119.

Valera-Burgos, J., M. C. Diaz-Barradas, and M. Zunzunegui. 2012. Effects of *Pinus pinea* litter on seed germination and seedling performance of three Mediterranean shrub species. Plant Growth Regulation **66**:285-292.

van Breugel, M., J. S. Hall, D. J. Craven, T. G. Gregoire, A. Park, D. H. Dent, M. H. Wishnie, E. Mariscal, J. Deago, D. Ibarra, N. Cedeno, and M. S. Ashton. 2011. Early growth and survival of 49 tropical tree species across sites differing in soil fertility and rainfall in Panama. Forest Ecology and Management **261**:1580-1589.

VanDuren, I. C., D. Boeye, and A. P. Grootjans. 1997. Nutrient imitations in an extant and drained poor fen: implications for restoration. Plant Ecology **133**:91-100.

Varkitzi, I., K. Pagou, E. Graneli, I. Hatzianestis, C. Pyrgaki, A. Pavlidou, B. Montesanto, and A. Economou-Amilli. 2010. Unbalanced N:P ratios and nutrient stress controlling growth and toxin production of the harmful dinoflagellate *Prorocentrum lima* (Ehrenberg) Dodge. Harmful Algae **9**:304-311.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science **277**.

Vitousek, P. M., D. R. Turner, W. J. Parton, and R. L. Sanford. 1994. Litter decomposition on the Mauna-Loa environmental matrix, Hawaii- patterns, mechanisms, and models Ecology **75**:418-429.

Vivanco, J. M., H. P. Bais, F. R. Stermitz, G. C. Thelen, and R. M. Callaway. 2004. Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. Ecology Letters **7**:285-292.

Weidenhamer, J. D. and J. T. Romeo. 2005. Allelopathy as a mechanism for resisting invasion: the case of Polygonella myriophylla.

Williams, R. D. and R. E. Hoagland. 2007. Phytotoxicity of mimosine and albizziine on seed germination and seedling growth of crops and weeds. Allelopathy Journal **19**:423-430.

Willis, R. J. 2000. *Juglans* spp, juglone and allelopathy. Allelopathy Journal **7**:1-55.

Wink, M. 2002. Production of quinolizidine alkaloids in in vitro cultures of legumes. Biotechnology in Agriculture and Forestry. Medicinal and aromatic plants XII **51**:118-136.

Wink, M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry **64**:3-19.

Wishnie, M. H., D. H. Dent, E. Mariscal, J. Deago, N. Cedeno, D. Ibarra, R. Condit, and P. M. S. Ashton. 2007. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. Forest Ecology and Management **243**:39-49.

Wu, H. W., J. B. Zhang, R. Stanton, M. An, D. L. Liu, and D. Lemerle. 2011. Allelopathic effects of *Eucalyptus dundasii* on germination and growth of ryegrass and barley grass. Allelopathy Journal **28**:87-94.

Wunderle, J. M. 1997. The role of animal seed dispersal in accelerating native forest regeneration on degraded tropical lands. Forest Ecology and Management **99**:223-235.

Young, T. P. 2000. Restoration ecology and conservation biology. Biological Conservation **92**:73-83.

Zhang, C. and S. Fu. 2010. Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops. Allelopathy Journal **26**:91-99.

Zhang, C. L. and S. L. Fu. 2009. Allelopathic effects of eucalyptus and the establishment of mixed stands of eucalyptus and native species. Forest Ecology and Management **258**:1391-1396.

CONCLUSION:

Reforestation in the tropics has been impeded by the invasion of C_4 grasses. Interactions between reforestation species and grasses may determine restoration success. The overall objective of these studies was to determine whether certain restoration tree species were able to inhibit *S. spontaneum* regeneration more than others. Results from chapters one and two suggest that *S. spontaneum* regeneration was most limited by light. In addition, legume overstory species further reduced *S. spontaneum* abundance below the expected reductions associated with shade. This result led to an evaluation of factors that could lead to increased suppression of *S. spontaneum* under legume trees.

In plantations in Panama, as in other field studies (Joo Kim et al. 2008) light availability limits *S. spontaneum* persistence. Among the diversity of trees tested as candidates for restoration planting, legume species increased canopy cover more rapidly than non-legumes, which decreased *S. spontaneum* density under legume trees (Chapter 1). This result was supported by the out-planting study in chapter one where *S. spontaneum* survival was reduced under the deeper shade of legume trees even in the absence of below ground interactions. Previous studies also found that legumes often produced denser canopies more rapidly than non-legume species (Jones et al. 2004, Wishnie et al. 2007). Our results suggest that legume trees continue to become darker overtime long after establishment. In addition, outplanted *S. spontaneum* tended to produce less below ground biomass, which can hinder nutrient capture in low light understory environments.

Competition with overstory trees can increase nutrient limitation and further reduce understory C_4 grass survival (Ludwig et al. 2001). I found a trend towards higher soil N and lower P in plots with legume trees when compared to non-legume plots. This trend paralleled significantly higher *S. spontaneum* leaf N:P ratio and a trend towards lower *S. spontaneum* leaf P in legume plots. These results taken together suggest that legumes may be competing for P with *S. spontaneum*.

Other studies also suggest that nutrient competition may play a role in *S. spontaneum* suppression. When *S. spontaneum* was grown under similar light conditions in the greenhouse, and in soils removed from the understory of mono-specific legume and non-legume tree plots, *S. spontaneum* growth and persistence was unaffected (Chapter 1). A lack of soil effect suggests that overstory trees are likely competing for nutrients in plots where *S. spontaneum* growth is suppressed.

Under high light conditions, and in the absence of competition, *S. spontaneum* growth was limited by both N and P availability (Chapter 2). Across both harvest dates *S. spontaneum* biomass increased significantly in plots where both N and P were added together. Similarly, *S. spontaneum* density increased significantly in plots where both N and P were added in December, although this response was only marginally significantly positive in the September harvest. These results are consistent with another study, which suggests that increasing P for a given N can increase the photosynthetic rate – N relationship (Reich et al. 2009).

In conjunction with nutrient competition, allelopathic chemicals in tree leaf litter may also be involved in the antagonistic interactions that suppress *S. spontaneum* growth under legume trees. Results from greenhouse studies in chapter 3-revealed weak evidence for allelopathic effects of leaf decomposition on *S. spontaneum* growth and persistence. In one study, survival was significantly reduced and height was marginally reduced for *S. spontaneum* for individuals that received leaf treatments from legume trees. In a second study, both above- and below-ground biomass were marginally reduced for individuals that received leaf treatments from a wider range of legume species. Although the results of the two experiments were not consistent, both point to physiological effects that can result in reduced *S. spontaneum* survival. Reduced height, above and below ground biomass can reduce competition for light and nutrients, and long-term carbohydrate storage, leading towards *S. spontaneum* mortality. Different responses in the two studies may be due to variations in allelochemical allocation in the trees at different parts of the growing season. This will need to be evaluated in future studies.

In summary, light appears to be the primary factor that reduces *S. spontaneum* performance below trees planted for restoration (Chapter 1). In the absence of below ground competition, *S. spontaneum* appears to be nitrogen and phosphorus co-limited in our system (Chapter 2). Although future studies should continue to evaluate the suppressive effects of legumes on *S. spontaneum*, our results suggest that legume trees suppress *S. spontaneum* growth more than non-legume trees. The production of deep shade appears to be linked to decreased below ground growth, which reduces the ability of *S. spontaneum* to forage and compete for nutrients (Chapter 1). Our results also suggest that legume trees may further inhibit *S. spontaneum* growth through competition for nutrients (Chapter 1) and possibly allelopathy (Chapter 3). Future studies should consider the following:

1. Photosynthetic response of *S. spontaneum* to different nutrient additions along a light gradient. This type of study would give insight towards understanding how nutrient availability influences photosynthesis rate. Similar studies should also investigate the influence of competitors on *S. spontaneum* nutrient uptake and photosynthesis rates along a light gradient.

2. Temporal variation in resource allocation for *S. spontaneum* under different nutrient treatments. If nutrient allocation is shifting from above ground investment during the early growing season towards below ground growth late in the growing season this would allow for a determination of whether there are seasonal shifts in resource allocation. In addition, growth under different nutrient treatments would allow for a determination of how nutrient addition alleviate nutrient limitation and the impact on *S. spontaneum* resource allocation and regeneration.

3. Extraction, isolation, and identification of allelochemicals from overstory trees and the response of *S. spontaneum* to their application. This would allow for a direct assessment of whether allelochemicals are produced by trees, and the extent to which those chemicals influence *S. spontaneum* growth.

4. Seasonal variation in allelochemical production in leaves across legume and non-legume overstory trees. If allelochemical production varies with season, this could give insight into when to control *S. spontaneum* so that allelochemicals can reinforce the suppression of *S. spontaneum* regeneration.

5. Compare nutrient resorption and uptake of legume and non-legume trees. Our data suggests that legume trees are taking up more phosphorus than non-legume trees. Also, if trees in this system are experiencing phosphorus limitation, we expect high P resorption in legume trees and low P concentrations in senesced tissues. This would give insight behind the competitive potential of restoration trees. Additionally, results from these studies could give insight into the nutrient cycling rate of N and P in plots undergoing restoration and how this changes as trees establish.

6. Compare techniques for establishing legume trees for reforestation of areas dominated by *S. spontaneum.* Planting tree stakes, as opposed to seedlings, has been shown to be an effective technique to expedite restoration tree establishment and canopy cover (Zahawi and Holl 2009). Incorporating these techniques into restoration models in *S. spontaneum* dominated systems could hasten the restoration process. However, variation in stake establishment across tree species suggests that species trials of tree stakes from trees recommended for restoration are necessary.

In conclusion, shade appears to be the primary factor that limits understory *S. spontaneum* growth. Legume trees are producing deeper shade faster that non-legume trees, and have the potential to further suppress *S. spontaneum* through nutrient competition and allelopathy. These results emphasize the importance of incorporating legume trees into restoration models where *S. spontaneum* suppression is desired. Future studies will need to further evaluate competitive interactions between *S. spontaneum* and legume trees. Based on the results presented in this thesis, restoration practitioners should consider establishing mixed species stands dominated by legume tree species when reforesting areas dominated by *S. spontaneum* to expedite the reforestation process. Supplemental planting of non-legume species could follow to increase understory diversity after *S. spontaneum* inhibition.

Works Cited

Jones, E. R., M. H. Wishnie, J. Deago, A. Sautu, and A. Cerezo. 2004. Facilitating natural regeneration in *Saccharum spontaneum* (L.) grasslands within the Panama Canal Watershed: effects of tree species and tree structure on vegetation recruitment patterns. Forest Ecology and Management **191**:171-183.

Joo Kim, T., F. Montagnini, and D. Dent. 2008. Rehabilitating abandoned pastures in Panama: Control of the invasive exotic grass, *Saccharum spontaneum* L., using artificial shade treatments. Journal of Sustainable Forestry **26**:192-203.

Ludwig, F., H. de Kroon, H. H. T. Prins, and F. Berendse. 2001. Effects of nutrients and shade on tree-grass interactions in an East African savanna. Journal of Vegetation Science **12**:579-588.

Reich, P. B., J. Oleksyn, and I. J. Wright. 2009. Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia **160**:207-212.

Wishnie, M. H., D. H. Dent, E. Mariscal, J. Deago, N. Cedeno, D. Ibarra, R. Condit, and P. M. S. Ashton. 2007. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the Republic of Panama. Forest Ecology and Management **243**:39-49.

Zahawi, R. A. and K. D. Holl. 2009. Comparing the Performance of Tree Stakes and Seedlings to Restore Abandoned Tropical Pastures. Restoration Ecology **17**:854-864.