Impact of invasive N-fixing tree species on soil dynamics

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Abstract

Invasive species cause impacts around the world affecting native communities through competition, hosting diseases and altering soil dynamics. Loss of native species and altered ecosystem services often result from invasion. Invasive species are often linked to their close association with humans, high dispersal rate, high reproductive capacity and wide range of adaptations. In Puerto Rico, historical deforestation for agricultural use and subsequent field abandonment left space for invasive species to establish and in most cases changed the forest species composition. Many of these invasive tree species (ITS) are N-fixing. This research looked at the ecosystem changes that occur when ITS are removed by bulldozing compared to areas with established ITS and planted native tree species. Bulldozing altered nutrient dynamics. Reference native (RN) plots had little variability and lower values (101.16 $\mu N/10cm^2/4weeks$) of total N flux compared to the N-fixing tree plots (339.93 $\mu N/10cm^2/4weeks$), which seemed to reflect the impact of invasive N-fixing trees in soil N flux. Total N flux, which was driven by nitrate, was higher in bulldozed plots compared to the reference native site and it increased during the dry season for all treatments. As herbaceous vegetation become established in bulldozed plots, available N decreased possibly due to plant uptake (or denitrification). High N:P flux ratios (60.0) suggested that additional nitrogen input from invasive trees could lead to phosphorus limitation, altering the soil nutrient cycle. Thus, the N-fixing tree species altered soil dynamics, and management by bulldozing can lead to ecosystem consequences. Management plans should consider these effects in restoration efforts so that N is not lost. Thus bulldozing should not be used as the only management practice when restoring an area. The restoration or management plan should include many factors like planting native species, watering new species and how to control grass.

Resumen

Las especies invasoras causan impactos alrededor del mundo, afectando negativamente las comunidades por competencia, como albergue de enfermedades y alterando la dinámica de suelo, entre otras. La pérdida de especies nativas y las alteraciones en los servicios de ecosistemas comúnmente es el resultado de invasiones y están muchas veces relacionadas a su asociación con los humanos, por su alta capacidad de dispersión, alta capacidad reproductiva y su amplia adaptación y establecimiento. En Puerto Rico, la deforestación para el uso agrícola de las tierras y luego el abandono de estas ha dejado un amplio espacio donde las especies invasivas han logrado establecerse y en muchos casos han cambiado la composición de las especies en los bosques. Muchas de estas especies invasoras son fijadoras de nitrógeno y el "bulldozing" es la práctica de manejo más común, pero también impacta el suelo. Esta investigación pretendió ver los cambios que ocurrían en el ecosistema al eliminar estas especies, comparándolo con áreas con arboles fijadores de nitrógeno establecidos y áreas con arboles nativos. Se encontró que la alta precipitación afecto directamente la biomasa y cobertura de especies herbáceas al igual que el flujo de N y P y su relación (N:P). La eliminación de las especies altero la dinámica de nutrientes en el suelo. Las parcelas de referencia nativas (RN) presentaron poca variabilidad y valores más bajos (101,16 μN/10cm2/4semanas) del flujo total de N en comparación con las parcelas de árboles fijadores de N (339,93 μN/10cm2/4semanas), que parecían reflejar el impacto de las especies invasoras fijadoras de N en el flujo total de N. El nitrógeno total disponible, que está liderado por nitrato, fue más alto en las parcelas donde estaban establecidas las fijadoras y aumentaba en la época seca. A medida que la vegetación herbácea volvía a establecerse, el N disminuyo probablemente utilizado por las plantas o desnitrificado. Altos valores en la proporción N:P (60.0) sugiere adiciones de N debido a los arboles fijadores y puede llevar a limitación del fosforo, alterando así el ciclo de nutrientes en el suelo. Este estudio demuestra que la presencia de arboles fijadores de nitrógeno en efecto afectan la dinámica del suelo, pero en ambientes específicos pueden ser también una herramienta de manejo y restauración. La utilización del "bulldozer" como método de control debe ser parte de un plan multifactorial de manejo y no como única herramienta.

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Introduction

Invasive species are often considered a problem to the ecosystem. The economic impact from their damaging effects and the efforts to control them is estimated to be $120 billion per year (Pimentel et al 2005). Due to characteristics of invasive species (opportunistic, high propagule pressure and establishment in novel environments) they have caused species declines and native habitat degradation (Ellison et al 2005, Ehrenfeld 2010). Examples of this can be seen in tropical forest around the world (Hughes et al. 2012, D'Antonio and Meyerson 2002). Both non-native grass and non-native tree establishment can alter forest function and composition. N-fixing trees invasions change soil more rapidly than non-fixing species, adding 2 to 3 times more N to the soil anually, leading to large changes in soil properties (Binkley and Giardina, 1998). For instance, a nitrogen fixing invasive tree in Samoa results in dense mono-specific stands that appear to replace native trees in the canopy (Hughes et al 2012). Non-native grasses do not allow natives to reestablish and grow (Thaxton et al. 2012). A fire-promoting invasive grass has contributed to the destruction and loss of tropical dry forest tree species in Hawaii (Cabin et al. 2002).

However, not all ecosystem impacts are necessarily negative. For instance, it has been shown that nitrogen-fixing trees can act as nurse trees to help reestablish native tree species in tropical dry forests (Santiago-García, 2010) and also for erosion control in degradated sites (Ali, 2010; Ssekabembe, 1992; Piggin, 2003).

While we have learned a lot about invasive species, like how they establish and spread the positive or negative ecological consequences of their removal are not always understood. I want to understand how invasive tree species affect soil nutrient supplies and how nutrients are altered when the invasive trees are removed.

The process of invasion includes: transport, establishment, spread, and impact. While the invasion process occurs, competition also occurs; adding problems of shifting vegetative composition where native species are replaced and soil dynamics are potentially altered. Finally, the impact stage represents the economic burden of both an ecological and economic problem for forest management. While management practices like bulldozing, planting and watering can be costly, so are the ecological consequences of losing species (Pimentel et al. 2005). Loss of foundation species can cause impacts on ecosystems processes such as C sequestration, nutrient fluxes, decomposition rates and energy flow (Ellison, et al. 2005).

Puerto Rico is no exception when it comes to landscapes dominated by invasive species. Changes in land use and purposeful introductions have created landscapes dominated by invasive nitrogen-fixing tree species (NFITS). The political status of Puerto Rico changed in the late 1940s, by then Puerto Rico was 94% deforested as a response to the conversion of forest to agricultural land (Lugo, 2004). Due to changes in the government and the economy, in the 1950's, people changed from agricultural to manufacturing jobs. As a result of this shift, agricultural lands were abandoned, by the 1980's, agriculture represented less than 5% GNP (Grau et al 2003).

The forests that regenerate on the abandoned fields are dominated by non-native forest species (Molina-Colón and Lugo 2006). While the species richness of Puerto Rican secondary forests recovered rapidly, the species composition was quite different in comparison with mature native forest sites (Aide et al. 2000, and Lugo 2004). It was suggested that this new assemblage of species represents a novel forest (Lugo and Helmer 2004, Lugo 2009), which are forests that have a combination and abundance of species that were not present previously (Hobbs et al 2006). These novel forests are a natural response to anthropogenic effects like deforestation and land use degradation that resulted from years of agriculture (Abelleira et al. 2010), and in certain areas of Puerto Rico are dominated by NFIT. Since these forests do not contain the same species as native forests, enrichment planting will be necessary to restore the original composition (Aide et al. 2000). While this is slightly different than invasive species invading systems dominated by native species, it still poses a challenge to farmers and land managers interested in restoring native species.

Invasive N-fixing trees have the potential to cause large changes due to their potential to alter nutrient dynamics. Yelenik et al. (2004) state that *Acacia saligna* (Labill.) Wendl., an N-fixing invasive tree (NFITS), altered physical soil characteristics by increasing moisture. As trees were removed, soil temperature increased due to decrease in shade. This resulted in changes in microbial communities and ultimately nutrient dynamics. NFIT's also increase N inputs to soil leading to acidification and high salinity (Adams, 2003; Garcia-Montiel and Binkley, 1998). This effect of soil acidification lowered soil fertility due to decrease in cation exchange capacity (Noble and Jones, 1997). As forest species composition changes, soil N and P concentrations can change. Studies in Hawaii demonstrated that soil under *Albizia sp*. had larger pools of available N, a reduction in P supply, and increased cation exchange capacity and exchangeable Al^{+3} (Binkley, 1997; Garcia-Montiel and Binkley, 1998). This could lead to environments that favor invasive species.

Despite these impacts, NFITS have been considered an option for restoration and conservation. It was suggested that *Leucaena leucocephala* could be used as nurse trees in reforestation efforts for native trees in Puerto Rico dry forests (Santiago-García, et al. 2008). Their experiments in Guánica Forest showed that native tree saplings grew better in the shade of *Leucaena leucocephala* trees. The conflict between the positive and negative ecosystem consequences of using NFIT in restoration lead to my question: Is bulldoze clearing the best option for managing N-fixing invasive trees?

Objectives

My project has three main objectives:

(1) Evaluate how do soil nutrient (C, N, P) dynamics change after clearing invasive tree species.

(2) Evaluate how does vegetation structure and composition change after bulldozing.

(3) Asses the cost and benefits of clearing invasive N-fixing tree species.

Literature Review

Soil Dynamics

Invasive species impact the structure and function of areas they colonize (Ehrenfeld 2010). In Puerto Rico where agricultural lands have been abandoned, invasive species can dominate the landscape for many years. Nitrogen fixing trees, such as *Leucaena leucocephala* (Lam.) de Witt and *Prosopis juliflora* (Sw.) DC.*,* also have the ability to alter the N cycling rates in soil by increasing the N input to the ecosystem. Hughes et al. (2012) reported an increase in soil available N associated with the presence of *Falcataria moluccana* (Miq.) Barneby & J.W. Grimes in Samoan forest, which were similar to results they saw in lowland wet forests of Hawaii (Hughes and Denslow 2005, Hughes and Uowolo 2006). In the wet lowland forest of Hawaii the *F. moluccana* forests had 121 times higher soil N availability compared to the 48 year old native site (Hughes and Denslow 2005). Also is stated that this N is then available for other species, facilitating the invasion of new species (Hughes and Denslow 2005). Fargione et al. (2003) proposed that invasive species use nutrients in different ways than natives (different times or depths) and that can result in an increase of the nutrient stocks. This can explain why invasives increased the overall net primary production of the ecosystem (Ehrenfeld 2003). It has been suggested that invasive species can promote and accelerate the rate of nitrification (Ehrenfeld 2003) and change and promote net N mineralization rates during the wet season while reducing them in the dry season (Mack and D'Antonio 2003). While these responses can be seen across many ecosystems, they can vary depending on the invading species or the invasion site characteristics (Ehrenfeld 2010, Yenelik et al. 2007). For instance, *Prosopis glandulosa* Torr. developing stands in Texas increased ecosystem C and N storage and cycling (Hughes et al. 2006). The authors suggest that stand age, soil type and functional plant traits will impact magnitude of the response.

Also leaves of invasive species are high in N and P and high litterfall rates will increase the soil nutrient pools (Lugo 2004, Liao et al. 2008, Erenfeld 2010). The leaf litter and roots also add organic matter (Lugo 2004). Root systems of invading species also change soil structure and chemistry. For example soil bulk density decreases significantly as forest succession advances (Lugo 2004, Weaver et al. 1987). The accumulation of nutrients and organic matter is driven by high concentrations of nutrients that can be found in leaves.

Cabin et al. (2002) stated that grasses with dense root systems may inhibit nutrient and water acquisition by native species, pointing that extensive aboveground biomass may decrease germination and establishment. Thaxton et al. (2012) support this by reporting soil moisture as the primary mechanism by which grasses limits native seedlings. Francis and Parrotta (2006) conclude that while suppressing dominant grasses, NFIT trees can facilitate regeneration of native dry forest species. They state that it will require decades before natural succession processes result in vegetation structure and species composition that resembles a native forest.

Brown and Ray (1993) suggest that N-fixing species can be helpful in the reestablishment of leached soils by improving soil nitrogen content. N input has been quantified from 0.2 to 18 kg N $ha^{-1}year^{-1}$ in Hawaii by the invasion of *Morella faya* (Aiton) Wilbur (Ehrenfeld 2010, Vitousek and Walker 1989). Some of the NFIT species that have a taproot system, like *Leucaena leucocephala*, have been used for erosion control around the world (Ali, 2010; Ssekabembe, 1992; Piggin, 2003). This helps prevent loss of topsoil from sites and allows organic matter to accumulate.

Bulldozing is one of the common management tools for NFIT removal in Puerto Rico, in both farming and restoration (Personal communication James Padilla, USFWS). This practice could lead to large detrimental ecosystem consequences, potentially establishing an environment where natives could not persist. Bulldozing disturbs the soil structure, which could lead to the leaching of nutrients in soil. The physical disruption of the soil has many consequences. One is that by removing the top layer of soil, organic carbon will directly be removed from the system. As the plants are removed light exposure to the soil will increase, which in turn increases soil temperature and nitrogen mineralization (D'Antonio and Meyerson 2002). Bulldozing also increases soil bulk density, compacts the soil, and alters its texture (Parsakhoo, et al. 2008). These processes could make restoration efforts challenging. In addition to the consequences on soil structure, removing trees by bulldozing can be detrimental for the entire ecosystem. Tropical forests are considered a sink for CO_2 (Grau, et al. 2003). If they are bulldozed then the stored CO_2 will be released to the atmosphere.

I wanted to know how nitrogen fixing tree species like *Leucaena* and *Prosopis* alter N cycling in an area which was recovering after abandoned from agriculture in Laguna Cartagena National Wildlife Refuge. Specifically, I Examined how soil N and P flux changed after elimination of established invasive N-fixing species by the most common management practice in Puerto Rico, bulldozing.

Vegetation Structure and Composition

As colonization of invasive species occurs, vegetation composition changes. Competition is one factor driving this change. High growth rates and fast establishment in less favorable conditions makes invasives good competitors for nutrients, sunlight and water. Levine et al. (2003) documented that competition affects growth and reproduction. Invasives reduce soil water availability to the natives and consequently reduces their growth and reproduction (D'Antonio and Mahall 1991). Invasion tends to result in establishment of monospecific stands, decreasing the number of natives (Vila and Weiner, 2004; Bakker and Wilson, 2001). Light competition in invaded areas has been reported as responsible for impacting species diversity, decreasing understory cover, growth and species richness in many studies around the world (Levine et al. 2003; Wyckoff and Webb, 1996).

Prior research points out that invasion leads to changes in vegetation composition. There are many examples of this around the globe (Levine et al. 2003, Hejda et al. 2009, Hughes et al. 2012, and Vitousek 1990). It has been suggested that N deposition after NFITS invasion leads to the exclusion of native species by more nitrophilic invasive species (Yelenik et al., 2004; Bobbink et al., 1998; Yoshida and Oka, 2004). Yelenik et al. (2004), found that the presence of *Acacia saligna* (a NFIT) stands increased growth rates of weedy grass, resulting from the alteration in N availability, making suggestions that after clearing *Acacia* stands more nitrophilic weedy species may invade. Yoshida and Oka (2004) found that the basal area of native tree species decreased with the invasion of *Leucaena leucocephala* (a NFIT) while the basal area of late-successional invasive species increased. Thus, NFIT seems to promote other invasive species in the understory due to changes in soil N availability.

Invasive species also increase the amount of standing biomass (Ehrenfeld 2003). Lugo (2004) stated that rapid development of tree density, basal area and tree height in alien forest demonstrated high levels of primary productivity. They report rate of basal area increase-two to seven times faster than succession on natives, but high rates of biomass accumulation will not always be found. Litter decomposition rates are on average 117% higher for invasive plants than co-ocurring native species, leading to higher element input on soil (Ehrenfeld 2010, Liao et al. 2008). Succession rates and processes are also influenced by the site history. Silver et al. (2000) found that biomass accumulates at slower rates during succession on cleared or abandoned agricultural lands as compared to abandoned pasture.

On the other hand there is the novel forest idea. Lugo (2004) states that Puerto Rico's native species tolerate and can benefit from the common alien species that are not shade-tolerant like many of the native species. Natives can grow under the canopy of invasive trees, and after around 40 years, when the invasive tree declines, the native trees will recolonize. Abelleira et al. (2009) state that non-native species like the African tulip tree (*Spathodea campanulata* P.Beauv.) restore forest structure and natives tree species in abandoned land in Puerto Rico. Lugo (2004) also states that with increasing age of the non-native species stands decreased in importance. He states that in the future Puerto Rico forest will continue to function as they do now but probably with different species composition and rates of processes.

Since invasive species and their management can alter vegetation communities, I want to understand if biomass differs between restored native tree areas and invaded areas. I also want to understand how long it takes for vegetative cover to be regained after bulldozing, how species richness changes, and if above ground biomass is altered as a result of bulldozing.

Materials and Methods

Site Description

This study was conducted at the Laguna Cartagena National Wildlife Refuge latitude 18.0139 and longitude -67.101 in the Lajas Valley of Puerto Rico (Fig.1.1). The driest months are January to March with an average temperature of 25.0°C and rainfall of 130 cm/yr (Weaver and Schwagerl, 2009). It is a lake with adjacent wetlands and uplands that include abandoned sugarcane fields and pasture. The area of experimentation was described by USFWS as a lowland dry shrubland and woodlands, but it can get flooded during the wet season. Nine 10 m x 10 m plots were established on the north side of Laguna Cartagena: three Control N-Fixing (CNF) plots with N-fixing trees (dominated by *Prosopis juliflora* with a few scattered *Leucaena leucocephala*, three Experimental N-Fixing (ENF) plots of N-Fixing trees cleared by bulldozing, and three Reference Native (RN) plots with native tree cover (Fig. 1.1).

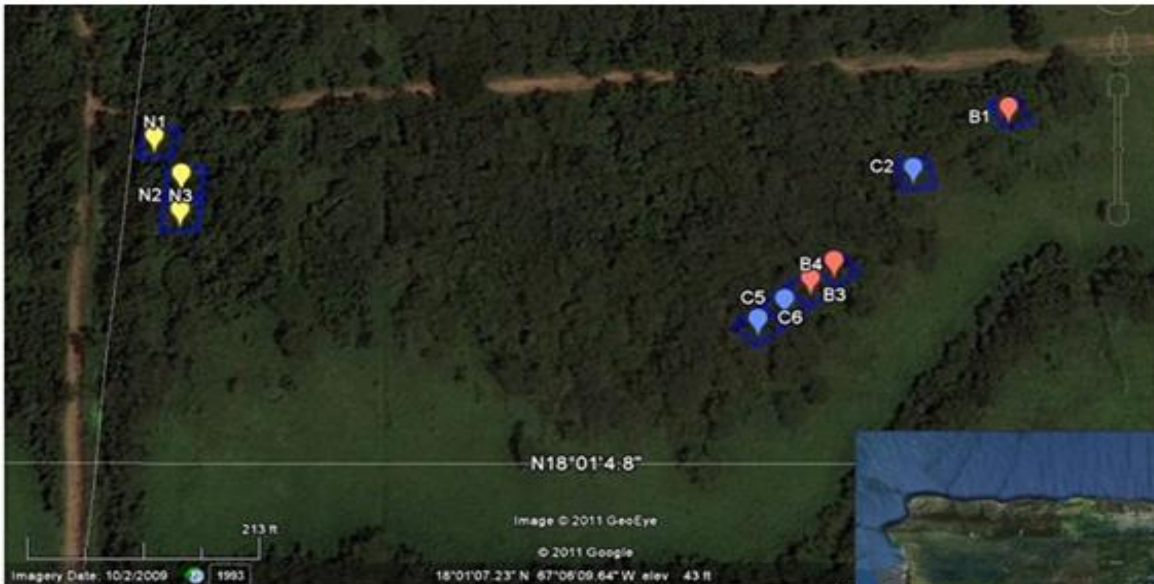

Fig 1.1 Map of research area on the north side of Laguna Cartagena in Lajas, PR. The squares are plots locations and the codes identify treatments as follows. Reference native (N1, N2, N3); control nitrogen fixing (C5, C5, C6) and experimental nitrogen fixing plots (B1, B3, B4).

The RN tree plots area were planted by the U.S. Fish and Wildlife Service (USFWS) about 20 years ago and include species like *Bucida buceras* L., *Zanthoxylum martinicense* (Lam.) DC. and *Ficus spp.* (Table 1.1) (Weaver and Schwagerl, 2008). All plots are on similar soils, which are Cartagena clay soils (Fine, mixed, superactive, isohyperthermic Sodic Haplusterts) that are somewhat poorly drained (USDA.gov). Plots were bulldozed with a D5K LGP CAT crawler dozer (~ 2000 lbs), passing through the field multiple times. Most of the top soil was removed in the process. Bulldozing was conducted in April 2010 and monitored for 12 months, until April 2011.

Table 1.1 List of species found in each treatment and classified as either native or introduced and by tree (T), grass (G) or forbs (F) described by the USDA plant database (http://plants.usda.gov). Red specie is the most common nitrogen-fixing tree.

Species	**Treatment**			**Classification**		**Form**
	ENF	CNF	RN	Native	Introduced	
Leucaena leucocephala (Lam.) de Witt	x	x	x		x	T
Ficus spp.			x		x	T
Zanthoxylum martinicense (Lam.) DC.			x	x		T
Prosopis juliflora (Sw.) DC.	x	x			x	T
Bucida buceras L.			x	x		T
Urochloa mutica (Forssk.) Stapf	x	x	x		x	G
Paspalum virgatum L.	x	x		x		G
Commelina diffusa Burm. f.	x	x		x		F
Jasminum fluminense Vell.	x				x	F
Merremia umbellate (L.) Hallier f.		x		x		F
Momordica charantia L.		x			x	F
Petiveria alliacea L.			x	x		F
Unknown		x	x			

Pre-Treatment soil analysis

To ensure similar soil characteristics across plots, soil was sampled from all plots pre-bulldozing. Five random soil samples from the top 10 cm were taken in each plot. Soil pH was measured in the lab using 25 grams of soil in 50 ml of water. The samples were analyzed by the USDA Forest Service-International Institute of Tropical Forestry-IITF Laboratory, for content of carbon, nitrogen, organic matter and other nutrients (Al, Ca, FE, K, Mg, Mn, Na and P). A second soil sampling was done in the wet season (October 2010) to see if there was an effect of bulldozing on the nutrient pools.

Post-Treatment soil analysis

Nutrients flux

N and P flux were measured using Plant Root Simulators or *PRS^{TM}-Probes.* Each sample consists of 3 pairs of cation and anion *PRS^{TM}-Probe (*Fig.1.2*). Probes* were placed in the ground during the first month after bulldozing. We randomly placed three samples in all nine plots. With the help of a chisel we opened the soil, inserted the *PRS^{TM}-Probes*, assuring that both sides of the membrane were in contact with the soil. *PRS^{TM}-Probes* were left in the field for four weeks. After four weeks, probes were taken out of soil, put in bags and placed in coolers until returned to lab. Once at the laboratory, the *PRS^{TM}-Probes* were cleaned with distilled water to remove all the soil and stored in the refrigerator until they were mailed to Western Ag Innovations Inc. Laboratory. This was repeated every month from May 2010 to April 2011 (total of 11 months, excluding January). Probes were placed in the originally established holes every month. Flux was measured based on the principle of Donnan exchange that describes the mechanism of ion absorption by plants roots (Western Ag Innovations Inc.) and was measured in micrograms/cm^2/4weeks, since the *PRS^{TM}-Probes* were left in field for 4 weeks.

Fig. 1.2 Example of PRS^{TM}-Probes samples and installation in field. (A) Three anion (orange) and three cation (purple) probes. (B) Probe installation and (C) probes installed at field.

N_2-fixation

Nitrogen fixation was measured in July 2010, 3 months after bulldozing using the acetylene reduction method (ARA) (Myrold, Ruess and Klug 1999). Nodulation on roots of N-fixing trees was examined to determine if the NFTS were fixing N.

Four 8 cm diameter soil cores were randomly extracted from each plot. Cores removed the top 10 cm of soil. The samples were collected during the months of July, August and October. Soil samples were placed in plastic bags and kept cool on the way to the laboratory. At the laboratory the 4 samples from the same plot were mixed and divided into four 1000 ml plastic bottles and hermetically sealed with rubber septa in the lid. Three of the bottles were injected with 10% of acetylene (C_2H_2) after removing 100 cc of air from the bottles. One bottle from each plot was not injected with acetylene as a control. All bottles were incubated for one hour. After incubation 1 cc samples were taken from each jar and then analyzed for ethylene concentration on an Agilent 6950 gas chromatograph with a flame ionization detector and a Poropak R column (100 to 200 mesh). We took samples at 1, 2, 9 and 25 hours. Rates were calculated based on the increase of ethylene over time.

Mineralization-Nitrification

Gross pattern N cycling was examined using in-situ soil cores for one month during the wet season. In November three PVC tubes were set in the field, capped and left to incubate for three weeks. At the same time, one soil core sample from the top 15 cm was taken and stored in the refrigerator for a month. These initial cores were used to determine initial levels of ammonia (NH_3^--N), nitrate (NO_3^--N) and nitrite ($NO_2^{\ -}$-N) (Appendix A) in order to calculate rates of net N mineralization and net nitrification as described by Robinson et al. (1999). I calculated N mineralized using the formula

$$[(Nitrate_f + Ammonium_f) – (Nitrate_0 + Ammonium_0)]/T_{days}.$$

Calculations for net nitrification were obtained using:

$$(Nitrate_f – Nitrate_0)/T_{Days.}$$

After three weeks initial and incubated soil cores were air dried for three days, ground and sieved with a 20 mesh size sieve. Extractions were obtained by shaking 5g of soil with 50 mL of 2M KCL solution for 30 minutes and then filtered through Whatman no.42 filter paper. The filtrate was kept cool for 12 hours then analyzed at the Central Analytic Laboratory-Agricultural Experimental Station, Rio Piedras, University of Puerto Rico.

Vegetation Structure and Composition

Trees

I measured tree height, diameter at 30 and 50 cm from ground and DBH for trees with DBH bigger than 2.5 cm in all plots prior to bulldozing and again after one year. From these data, tree biomass was estimated using allometric equations for dry forest species (Brown, Gillespie and Lugo 1989) and for *Prosopis* species (Padrón and Navarro 2004) (Appendix B).

Growth Rate was measured using the following equation: growth rate = (final basal area – initial basal area)/ time. We determined the growth rate of trees in Native and Control N-Fixing plots only since trees were removed from the bulldozed treatment. All instances of tree death were recorded.

Tree mortality (r_m) was measured to look at any differences between treatments, using the formula by Quinto-Mosquera et al. (2009):

$$r_m = [1 - (N_s/N_0)^{1/t}] \times 100$$

Where N_s is initial number of trees – dead trees, N_0 is initial number of trees, and t is time interval in years.

Herbaceous vegetation

Cover of herbaceous plants was determined in all plots while above ground biomass was measured for ENF and CNF treatments. Percent cover was estimated in three randomly selected 0.25 m^2 quadrates in each plot using the following cover classes: 0, 0-25, 25-50, 50-75 and 75-100. Cover measurements were made in June

August and December. To determine species cover vs. bare ground at the end of the experiment the point intercept method was used (Caratti 2006). Two transects of 10 m were established and the species present was recorded every 2 m (total of 12 points per plot). To estimate above ground biomass in ENF and CNF plots all above-ground vegetation was removed by clipping in the same quadrats used for percent cover estimation; however, biomass sampling only occurred in March, August and December 2010. The clipped plant material was oven dried at 50 ºC for 6 days and then weighed.

Below-ground biomass

Below-ground root length and biomass were estimated twice during the project, in March, pre-bulldozing, and on December, 8 months after bulldozing. Roots were collected from two 10 cm deep soil cores (15 cm diameter) in each plot. Roots were removed from the soil by suspension in water (100 ml of dishwater detergent in 5 gallons of water) and sieving out the soil using a 20 mesh size sieve to collect the roots. The roots were then preserved in a 60% ethanol solution. *Root length* (R) was determined using the line-intercept method described by Tennant (1975), where the number of times a root crossed the 2 cm grid lines was tallied. Root length was calculated using the following equation:

[(R) = .7857 x Number of intercepts (N) x Grid Unit]. A value of 1.574 was used as a length conversion factor for a 2 cm grid unit.

After determining root length, we measured root biomass by weighing air-dried roots after four hours.

Light Measurements

Light availability at the soil surface was measured every month at 3 random locations in each plot using a line sensor LI-COR- LI-250 Quantum/Radiometer/Photometer. Measurements were made at the same time (10:00am) in similar weather conditions (sunny days). (http://www.fondriest.com/pdf/li-cor_190_200_manual.pdf).

Data Analysis

ANOVA (InfoStat 2012, http://www.infostat.com.ar/) was used to look at the differences in nitrogen mineralization and nitrification, tree biomass and basal area growth rate. Chi square (InfoStat 2012e) was used to look at significant differences in herbaceous vegetation cover estimates. (Appendix C).

I examined differences in herbaceous vegetation biomass (Appendix D), root length and biomass and soil bulk nutrients (including C, N and P) (Appendix E) using a split-plot design ANOVA (SAS 9.1), where months were used as the split.

Changes in root length and biomass in the bulldozed plots were compared to each other pre- and post- treatment using split-plot design with ANOVA (SAS 9.1) to determine differences imposed by the action of bulldozing.

Differences in nutrient flux over time were determined using repeated measures ANOVA, with month as the repeated variable. Contrasts were used to look at differences between ENF tree areas vs CNF tree areas as well as CNF vs. RN. Slices were included in the model to see in which months treatments were different from each other as well as which treatment changed through time (Appendix F).

Results

Pre-Treatment soil nutrient analysis

Analysis of soil nutrient values show that there were no significant differences between treatments for the most important nutrients (% C, N, P and %OM), making it clear that soil nutrient pools were the same in all treatments (Table 1.3). However, there was a difference between seasons (dry vs. wet) for all nutrients except for percent organic matter, phosphorus and manganese.

Some other nutrients showed significant differences between treatments, aluminum (Al), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), and sodium (Na) (Table 1.3). Soil pH was similar in all treatment plots pre-bulldozing, with an average pH of 6.15 (Table 1.2).

Table 1.2 Mean soil pH for samples taken pre-treatment. (February 2010) (standard deviation)

Treatment	pH
Experimental-NF	6.25 (0.18) a
Control-NF	6.06 (0.65) a
Reference-N	5.89 (0.13) a

Means with the same letter have no significant differences $p<=0.05$.

Table 1.3 Mean nutrient concentrations from soil sample cores (0-10 cm depth) at Laguna Cartagena, Lajas Puerto Rico. Samples analyzed by USDA Forest Service International Institute of Tropical Forestry. Major nutrients had no significant treatment*month interactions. P-values < 0.05 (*), <0.01 (**), <0.001(***). Numbers in parenthesis are standard deviation.

Nutrient	%Carbon	%OM	N(mg/g)	P(mg/g)	K(mg/g)	Al(mg/g)	Fe(mg/g)	Ca(mg/g)	Mg(mg/g)	Mn(mg/g)	Na(mg/g)
Trt Main Effect											
ENF	2.8 a	14.4 a	2.3 a	0.74 a	1.1 a	20.9 a	36.4 a	4.3 a	3.6 a	0.91 a	0.31 a
CNF	3.0 a	14.5 a	2.4 a	0.77 a	1.1 a	19.8 b	35.5 a	4.2 a	3.5 a	0.86 a	0.30 ab
RN	3.0 a	14.4 a	2.2 a	0.73 a	1.1 a	20.2 ab	33.5 b	5.9 b	4.2 b	0.90 a	0.23 b
Season Main Effect											
February (Dry)	3.29 (0.58) A	14.2 (1.1) A	2.7 (0.50) A	0.75 (0.07) A	0.85 (0.13) A	13.8 (0.64) A	30.7 (1.9) A	5.0 (0.98) A	3.3 (0.31 A	0.88 (0.12) A	0.21 (0.60) A
August (Wet)	2.59 (0.72) B	14.6 (1.2) A	1.9 (0.68) B	0.74 (0.10) A	1.4 (0.22) B	26.8 (2.0) B	39.6 (2.7) B	4.6 (.10) B	4.3 (0.54) B	0.89 (0.13) A	0.34 (0.12) B
Trt	NS	NS	NS	NS	NS	*	***	***	***	NS	*
Month	***	NS	***	NS	***	***	***	*	***	NS	***
Trt*Month	NS	NS	NS	NS	NS	**	NS	*	NS	NS	NS

Means with the same letter have no significant differences p<=0.05.

Post-Treatment soil nutrient analysis

Nutrient Ratios

N:P ratios were the same in all plots while C:N ratios showed that RN areas had the highest C:N ratio while CNF that has the lowest (Table 1.4). ENF mean value of 13.21 was not significantly different from the other two treatments (Table 1.4).

Table 1.4 Mean N:P and C:N ratios calculated from soil sample data.

Treatment	N:P Ratio		C:N Ratio	
ENF	3.01	a	13.21	ab
CNF	3.18	a	12.50	a
RN	3.03	a	13.52	b

Means with the same letter have no significant differences p<=0.05.

Nutrient flux

Nitrogen

Nutrient fluxes varied between treatments and over time (Fig. 1.3). N increased in both CNF and ENF plots until June when they began to decrease (Fig. 1.3). By July, ENF had the lowest total N-flux (TN) with 19.9 µg/cm^2/4 wks, which was maintained until December. The only time TN flux did not differ between treatments was in November and December (Appendix F). All treatments increased through time from February to April with CNF having the highest flux and ENF having intermediate N flux rate. Control-NF has the highest flux of Total N year round with an average value of 378 μg/cm^2/4 wks. The N-flux seems to be driven by nitrate flux rather than ammonium (Fig 1.3b and 1.3c). While ammonium concentrations change through time in each treatment there is no clear pattern other than the concentrations decreased.

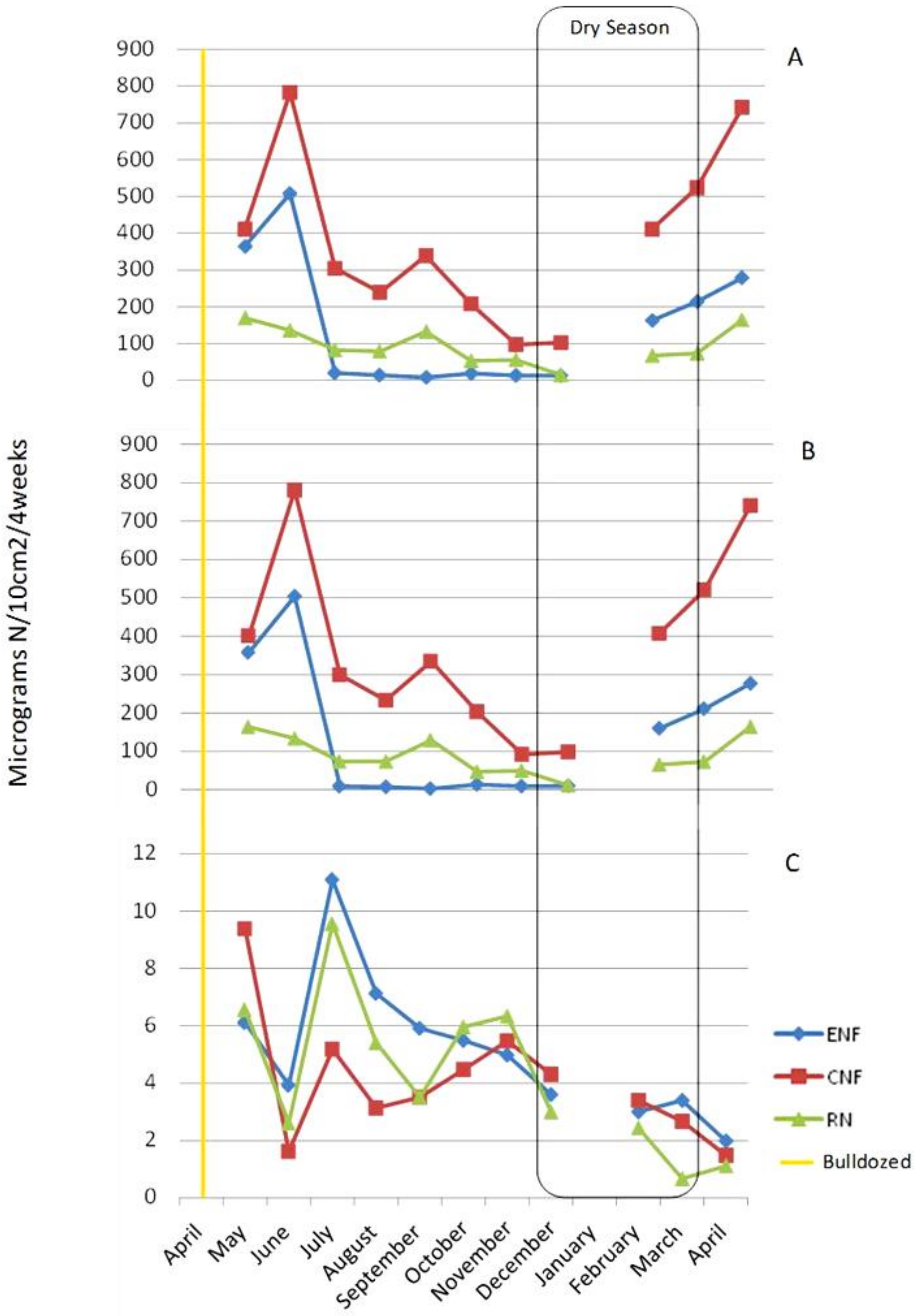

Fig. 1.3 Mean monthly flux of total N (A), nitrate (B), and ammonium (C). Data from PRS™-Probes. Means calculated from 3 samples per plot for a total of 9 samples for each data point. The box indicates the months that correspond to the dry season. ENF is the diamond, CNF is the square, RN is the triangle and the line in April denotes the month when bulldozing occurred.

Phosphorus

Reference native plots had the highest flux of phosphorus, with a maximum rate of 23.8 µg/cm^2/4wks. Mean P flux values were different between treatments; however, phosphorus flux initially increased in all treatments through time but was low in the dry season in February and March (Fig1.4). Removal of NF trees had significant impact on P flux (Fig. 1.4) (F=14.69, df=1, p=0.002, Appendix G). This appears to be important immediately following bulldozing, when P flux in ENF is almost 2 times lower than CNF in the first two month post bulldozing.

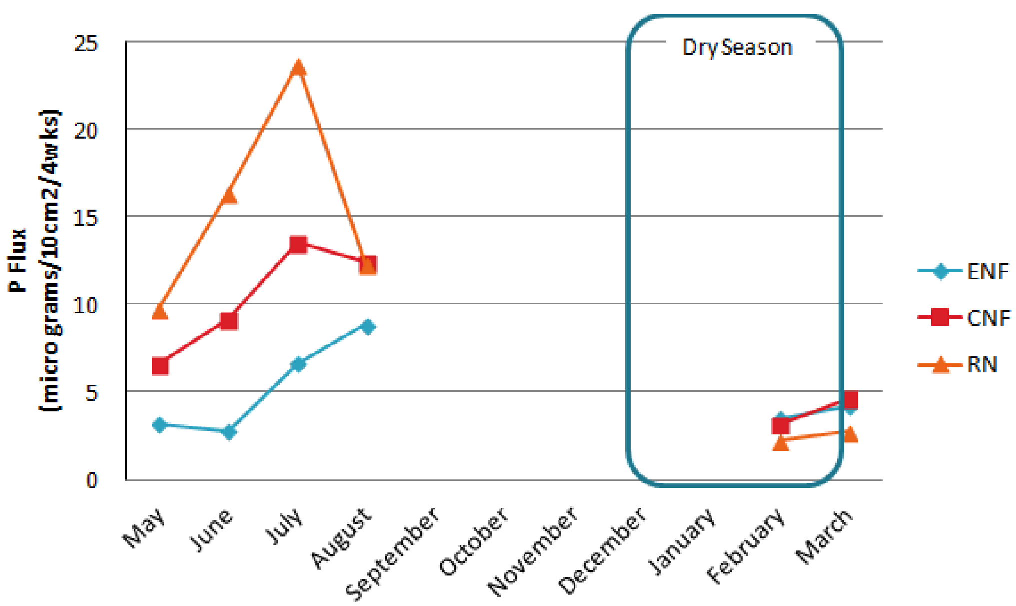

Fig. 1.4 Mean monthly phosphorus flux from PRS^{TM}- Probes.

N:P Ratios

N:P flux ratios were calculated for May, June, July, August of 2010 and February and March 2011. Native plots always had low N:P. The N:P ratio in RN was not significantly different between seasons and averaged 17.14. The ENF plots had the highest N:P flux ratio seen, which occurred in May and June and then dropped to levels seen in the RN plots before increasing again in the dry season (Fig.1.5). At the end of the experiment, CNF had the highest N:P ratios. All months, except for July and August, show significant differences between CNF with ENF and RN.

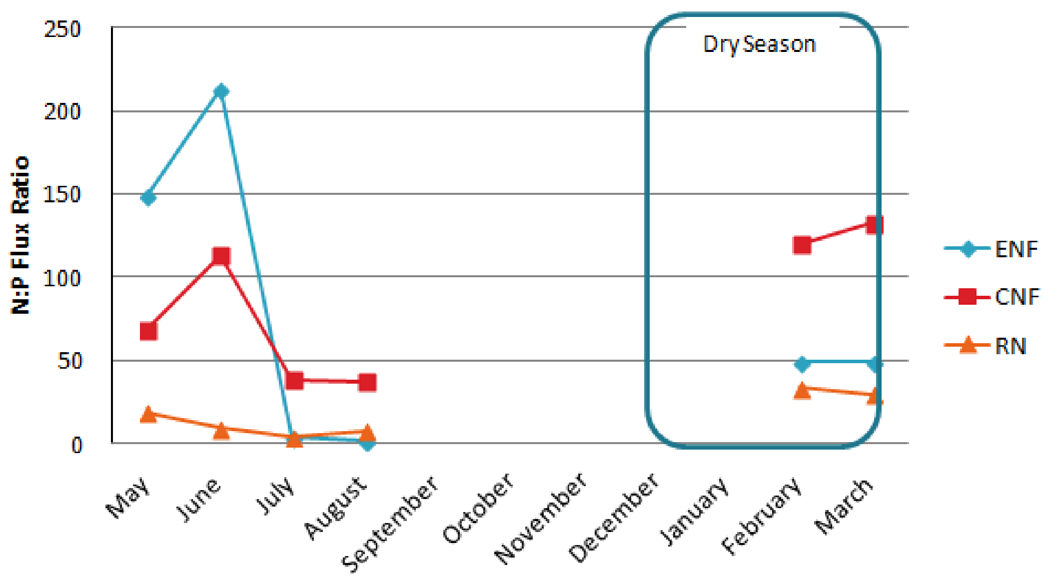

Fig. 1.5 Mean N:P flux ratio from PRS^{TM}- Probes data by treatment and time.

N mineralization and nitrification

N mineralization and nitrification was measured to determine the effectiveness of the PRS^{TM}- Probes to measure N flux. The highest mineralization and nitrification rates for the month of November were seen in CNF (Table 1.6). There was a lot of variability in both nitrification and mineralization in all plots, but specially ENF and RN. Regardless, the trends seen here are similar to those found with the PRS^{TM}- Probes. The TN, nitrate and ammonium flux data for the month of November, where CNF was high and ENF was very low (Fig. 1.3 and Table 1.5).

Table 1.5 Mean mineralization and nitrification rates. Values in parenthesis are standard deviations.

Treatment	Mineralization Rate (mg N*kg-1*d-1)	Nitrification Rate (mg NO3*kg-1*d-1)
ENF	0.75 (3.46) a	0.37 (1.68) a
CNF	6.19 (6.67) b	3.00 (3.24) b
RN	1.40 (4.89) ab	0.68 (2.37) ab

Means with the same letter have no significant differences p<=0.05.

N-fixation

N-fixation was not detected by the ARA method in any of the treatments. This was supported by the lack of nodules found on tree roots including the CNF.

Vegetation Structure and Composition

Trees

Total tree biomass was the same between treatments prior to bulldozing (Table 1.7), with an average biomass of 8.7 kg/m^2. However, in terms of the biomass of individual trees, Control N-Fixing trees had 7.44 times more biomass per tree than the other two treatments using Padrón and Navarro (2004) specific equation for *Prosopis* trees (Table 1.6) (Appendix C).

Table 1.6 Above ground biomass pre-bulldoze using equations describe by Brown (1989) and Padrón and Navarro (2004).

	Brown (1989)				**Padrón and Navarro (2004)**			
Treatment	AGB (kg/tree)		AGB (kg/m2)		AGB (kg/tree)		AGB (kg/m2)	
ENF	58.1 (167.4)	a	4.37 (2.18)	a	72.2 (178.4)	a	5.87 (2.52)	a
CNF	318.6 (406.33)	b	9.56 (6.97)	a	476.4 (368.38)	b	14.41 (7.88)	a
RN	55.7 (85.27)	a	6.31 (1.93)	a	55.7 (85.27)	a	6.31 (4.59)	a

Means with the same letter have no significant differences p<=0.05.

No trees sprouted in the ENF after bulldozing. Trees did not grow in the CNF or RN areas, as indicated by basal area growth rates that were not significantly different from zero (Appendix C). However, there was some tree mortality in RN. The RN treatment had 10% annual rate mortality while no trees died in CNF (Table 1.8).

Table 1.7 Total Tree, Mortality and Regrowth by treatment

Total number of trees 2010	**73**
RN	34
ENF	30
CNF	9
Total number of trees 2011	**45**
RN	32
ENF	0
CNF	9
Total number of dead trees	**2**
RN	2
ENF	0
CNF	0
Annual Mortality Rate	
RN	10%
ENF	100%
CNF	0

Herbaceous vegetation

I measured above ground herbaceous biomass only in CNF and ENF treatments during the first year following bulldozing. Average above ground biomass for all areas was 1244.5 g/m^2. Biomass changed through time in the CNF areas, ranging from 911.6 g/m^2 to 1882.1 g/m^2 *(*Fig.1.6). Biomass in the CNF plots decreased from March to December. As I expected, ENF plots decreased to 0 right after bulldozing and increased in biomass through time, until December. In 8 months it increased from zero to 1081.7 g/m^2, which was not significantly different than the biomass found in CNF.

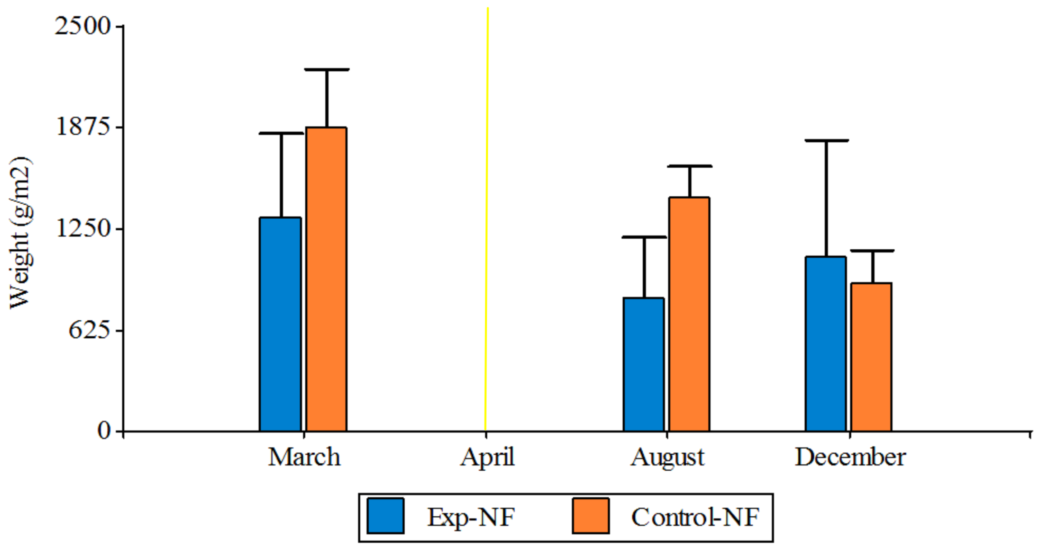

Fig. 1.6 Mean above ground biomass of herbaceous vegetation pre-bulldozing (March), 4 months after (August) and 8 months after bulldozing (December). The lines represent standard deviation. Yellow line in April represents the month that ENF was bulldozed.

Cover Estimation and Species Richness

As expected, herbaceous plant cover increased rapidly after bulldozing. Just three months after bulldozing the ENF plots were 100% covered. However, this was not maintained. By December, five months after bulldozing, the percent of ground covered decreased in all plots. Percent cover in ENF dropped to 31.9%, a 70% decrease from August while CNF decreased to 70.8% (Fig.1.7). RN plots initially had no significant herbaceous vegetation in the plots. However, understory vegetation increased during the study, with maximum cover of 40% in August, which was maintained through the wet season. Two years after bulldozing (Spring 2012) both CNF and ENF had 100% herbaceous cover (based on the point intercept method) while RN had almost 100% bare ground cover.

Herbaceous species richness was lowest in RN with only 2 species sampled, while there were no differences between ENF and CNF (4 and 5 species respectively). A maximum of 6 species per plot was found in June 2010, by February 2012 a maximum of 4 species per plot were found in all plots (Table 1.1). The very invasive *Urochloa mutica* (Forssk.) Stapf was found in all treatments. *Paspalum virgatum L.* had the highest frequency followed by *Urochloa mutica* in ENF and CNF treatments.

Below ground biomass

There was no significant difference between treatments or month for either of the measurements, root biomass or length. Average root length was 62.4 m/soil m^3 while root biomass was 115.1 g/soil m^3.

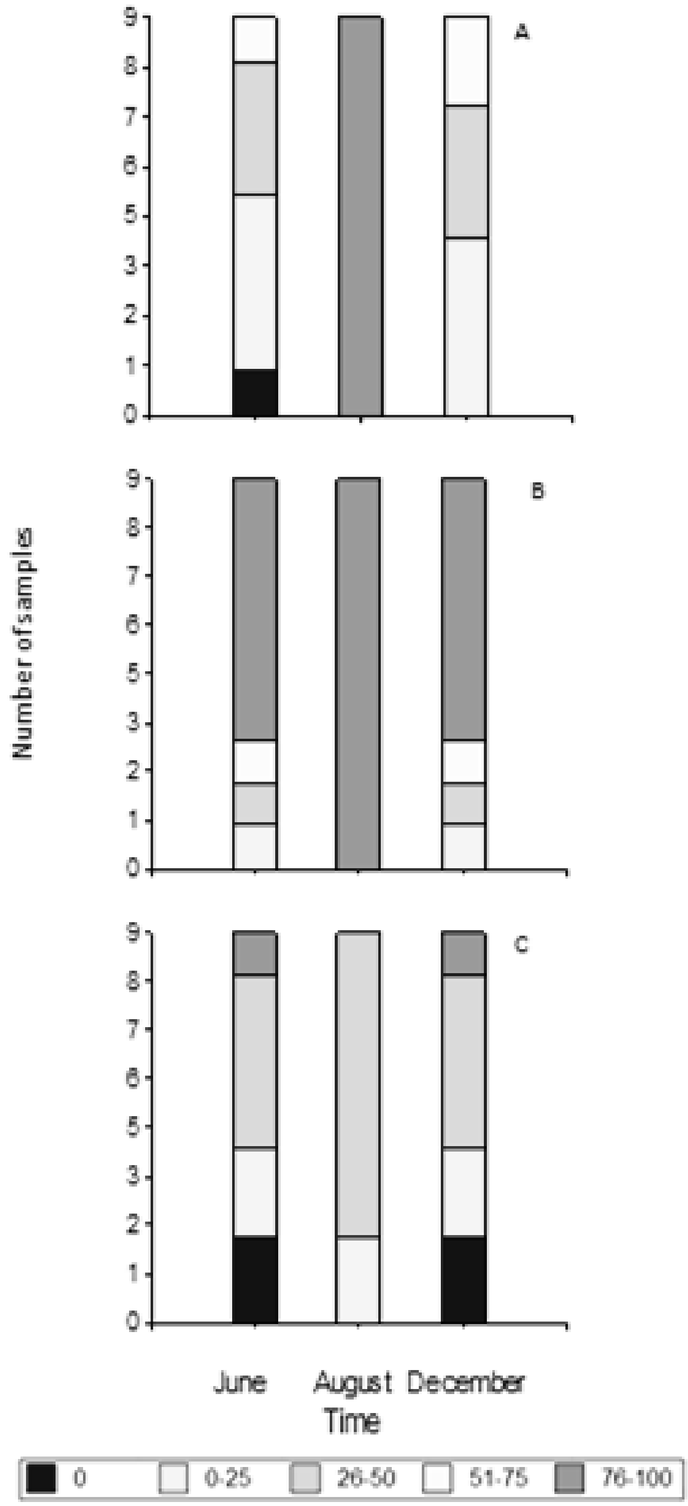

Fig. 1.7 Above ground herbaceous vegetation cover estimation for 2, 4 and 8 months (June, August and December 2010) following bulldozing. Treatments are represented by (A) ENF, (B) CNF and (C) RN.

Discussion

Nutrient availability is different in each forest type and is altered by bulldozing NFIT. Nutrient availability also changes through the year. Since the invasive species removed were nitrogen fixing trees, nitrogen was the focus of the study. The patterns in nitrogen availability were affected mainly by forest cover type and precipitation. Removal of NF trees decreased the N availability in soils not only by leaching of exposed soil but also by plant nutrient uptake while species recolonize As expected, CNF areas had the highest nitrogen availability, with the highest availability in the early spring. The N in ENF initially resembled the patterns seen in CNF, although at lower concentrations, suggesting that there might be some legacy of N-fixing trees (NF). However, this N quickly disappeared, probably due to the uptake by regrowth of vegetation, denitrification, leaching or a combination. Thus the legacy of the NF trees was short lived. It was not unexpected that the presence of NF trees resulted in higher N availability. Presumably, this would be a result of nitrogen fixation; however nitrogen fixation was not detected in any forested area. It is possible that nitrogen fixation is not necessary due to the high levels of available nitrogen in the soils. Another explanation for not detecting N-fixation could be high N in litterfall and high litterfall rates, the recycling of available nutrients between the litterfall and microbial communities.

The pattern seen in total nitrogen was due to changes in nitrate and not ammonium concentrations. Ammonia is the result of ammonification which is the conversion of organic nitrogen to ammonia. This process can be aerobic or anaerobic. The ammonia is used by nitrifying bacteria and converted to nitrate. Nitrification is an aerobic process. In this study when ammonium is at low concentrations and nitrate was high the nitrifying bacteria were likely using ammonia. Ammonium levels could also be lower due to the attraction of ammonium ions to soil particles. Nitrate, which is often abundant in soil water (Camberato 2001) is soluble and thus more mobile and easily leached.

Nitrogen availability increased with the initial rain events in the season, but decreased through the rainy season. The nitrate that was there was either denitrified, taken up by plants, or leached downstream due to its high solubility. The excessive rains that lead to saturated soils and flooding likely decreased the microbial activity. This decrease could result from the soils becoming anaerobic during the wet season due to saturation with water. As mentioned above nitrifiers are aerobic organisms that convert ammonia to nitrate. As nitrate decreased, ammonia availability decreased, suggesting that nitrifiers were not as active. Litterfall nutrient composition and litterfall rates were not measured in this experiment; however, these inputs have been reported as being important in N availability for other N-fixing systems (with *Acacia saligna* (Yelenik, Stock and Richardson 2004). Even though the N flux decreases with removal of NF trees (and some of the top soil) by bulldozing it still has higher levels than areas with native trees. The amount of N available does not change through time in areas with native trees. This is probably due to the absence of N-fixing trees that has driven lower values of nitrification and mineralization.

These results are congruent with those reported by Hughes and Denslow (2005) in wet lowland forest of Hawaii. Invaded sites in these forests had 121 times higher soil N availability compared to 48 year old native site. The invaded site was dominated by *Falcataria moluccana,* a N-fixing tree. Similar results were also found with another N-fixing tree, *Acacia saligna* in South Africa (Yelenik et al. 2004). There, available soil N showed significantly higher concentrations in *Acacia spp.* stands (~ 2,000 mgN/m^2/y^{-1}) than in the native dominated areas (~550 mgN/m^2/y^{-1}). They suggest that alteration in Acacia stands are probably a consequence of the seasonal moisture stimulating mineralization, just as hypothesized above. Thus N availability is influenced by precipitation at least in areas with N-fixing trees. As precipitation increased, nitrate flux in soils decreased (Fig.1.8). In 2010, there was a heavy rainy season at Laguna Cartagena. Precipitation reports show a mean annual rainfall of 116.8 centimeters for years 1948-2012 (South East Regional Climate Center for Lajas Sub-station). Annual precipitation for our experiment year (May 2010 to April 2011) was 167.4 centimeters (Fig.1.8). High precipitation and flooding conditions reduce nitrification by creating anaerobic conditions, which are undesirable for nitrifier aerobic bacteria species such as *Nitrosomas* and *Nitrobacter*. ENF and CNF N flux patterns support this by decreasing during the rainy season and increasing after it, in December (Fig.1.8).

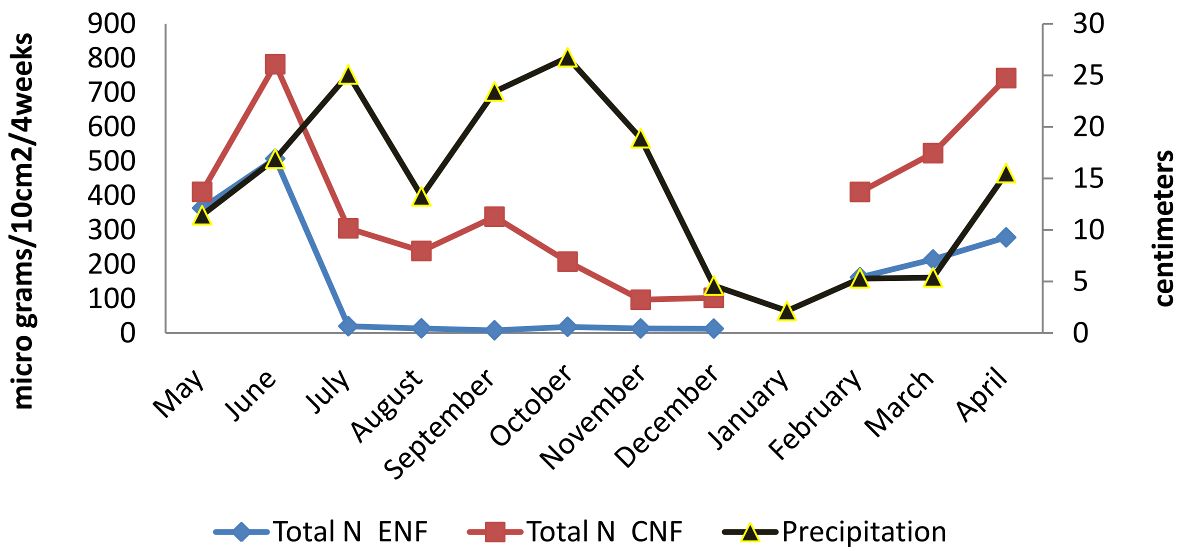

Fig 1.8 Mean monthly total N for the experimental nitrogen fixing (ENF) and control nitrogen fixing (CNF) tree plots. Total monthly rainfall (cm) for (2010-2011)

The soil incubations done in November support the idea that both mineralization and nitrification were occurring and were higher in CNF compared with ENF (Table 1.6). This led to higher values of TN in CNF. A low C:N ratio (less than 15:1) in pre-treatment soil samples suggests that carbon could be limiting microbial activity; however it is lowest in the CNF plots where TN availability is highest. High nitrogen content in litterfall leads microorganisms to release N from decomposing plant material (Camberato 2001). This higher N would result in a lower C:N ratio in the soil, which is what was seen in CNF (Table 1.4). Higher C:N ratio and lower TN availability in the wet season may be explained by the increased activity of the anaerobic microbial community, specifically denitrifiers, and decomposition of organic matter (Hoorman and Islam 2010).

As with nitrogen, P availability is likely driven by rainfall. Lower values on the ENF and CNF treatment compared to RN seem to be driven by plant uptake. RN trees are stressed by the flooding and the understory vegetation is almost non-existent. While in CNF and ENF, herbaceous vegetation, some which can withstand flooding, were likely using P for growth. High N:P ratios (Table 1.4) seemed to show how excess N in the system decreased the need for N fixation but created potential phosphorus limitation.

N:P flux ratios show that available phosphorus was limiting in most months, except during the wet season with values higher than 16. ENF plots had the highest N:P in the first two months post bulldozing even though it did not have the highest available TN. This suggests that removal of NFIT can have large impacts on both P an N nutrient cycles.

Vegetation rapidly recovered after bulldozing and was recolonized mainly by grass. Three months after bulldozing, ENF plots were 100% covered by grass and herbaceous vegetation. This is the same time over which N availability decreased. Like nutrients; the amount of cover by herbaceous vegetation was also affected by precipitation. After high constant precipitation months (September and October) estimated cover decreased, especially in ENF treatment (Fig. 1.9). It was observed that every time soil was saturated by water, significant areas of grass died in ENF and CNF plots.

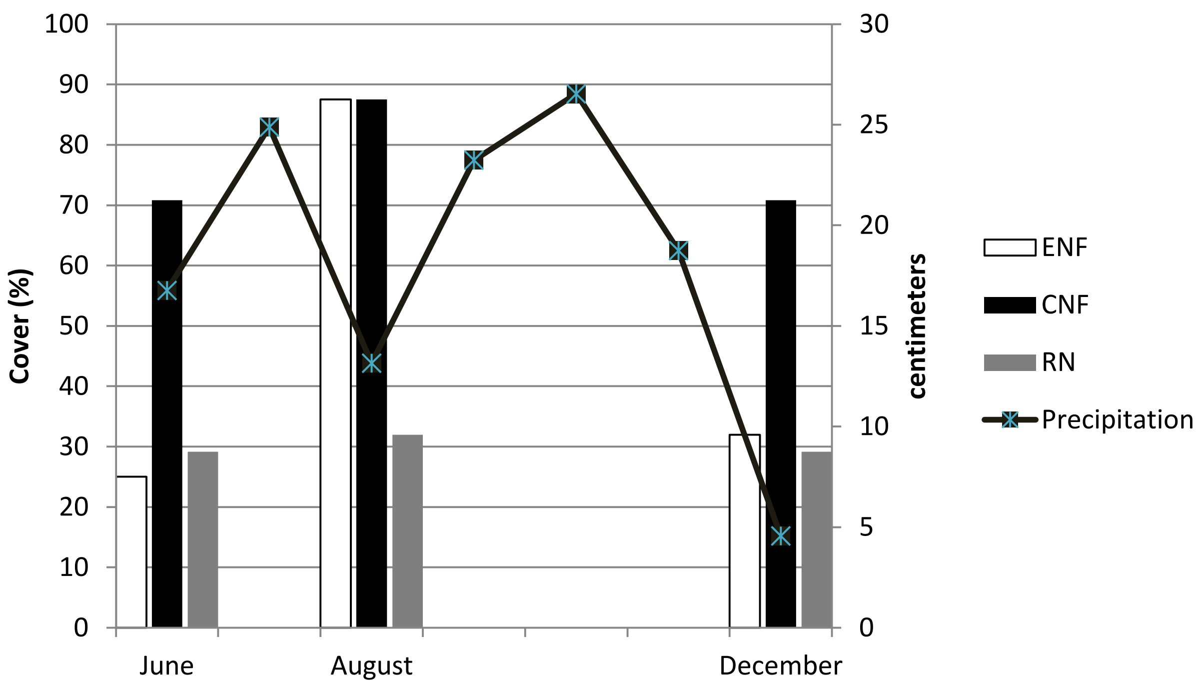

Fig. 1.9 Mean monthly precipitation (mm) overlay on the mean percent cover for treatment percentage of cover of above ground biomass and precipitation.

Differences in herbaceous biomass between treatments can be explained by tree canopy. Even though ENF had more light available for grasses to establish, soil exposure after bulldozed and its effects on physical characteristics of soil allowed for more soil erosion, water saturation and flooding. This could have decreased the amount of vegetation growth compared to CNF, which has tree canopy.

While biomass changed over time, species composition remained constant. The maximum number of species per plot changed from 6 to 4 in ENF and from 5 to 4 in CNF. RN had significantly lower number of species in the understory, with only 1-2 species at any given time. RN was mostly covered by bare ground. Thus invaded areas tend to have higher diversity than reference native plot. However, many of the recolonizing species are invasive as well. Unlike my findings, previous work has shown that lower richness of seedlings and saplings were found in invaded areas compared to uninvaded forest (Martin, Canham and Marks 1999). In the Czech Republic invasion of *Heracleum mantegazzianum*, an herb, reduced species diversity compared to uninvaded areas (Pysek 1994). One reason for this higher diversity in invaded areas could be due to the nature of RN plots. The native trees in RN plots were planted close together and allowed very little light into the understory, an average of 91 $\mu mol/m^2/s$. Trees in CNF plots were fewer in number, established on their own and were not close to each other. Thus, there was almost 5 times more light in the understory (446.7 $\mu mol/m^2/s$). Loss of species over time can be caused by the persistence of grass that competes with the other species. The shade of tall grass, that can increase 4 times in height in one month, does not allow other lower herbaceous species to grow well. Native seeds, which cannot reach the mineral soil, also have to compete for light. *Paspalum virgatum* was the most common species seen followed by *Urochloa mutica.* In nearby more open plots, *Urochloa mutica,* an invasive grass, dominated (Almodovar unpublished data, 2012). The difference in grass dominance is explained by the presence of water. The Food and Agriculture Organization described *U. mutica* as an introduced species that is adapted to high rainfall, but also tolerates drought (FAO-online, May 15, 2013). The more open area tends to accumulate more water than this experimental area.

Tree biomass per m^2 was the same regardless of whether it was native or invasive. This was not unexpected, since studies have shown that invaded and native intact forest had no differences in total biomass (D'Antonio et al. 2011). However, trees in the CNF plots had more individual tree biomass than the other treatments showed by both equations. The CNF plots were dominated by a few (n = 9) large (DBH 4.8 cm - 64.1 cm) Prosopis trees while the other plots contained more numerous smaller-diameter stems – primarily either *L. leucocephala* (ENF) or *Bucida buceras* (RN). The disparity between the two equations could be due to the difference between measurements used for the calculations. The Brown equation used DBH and Padrón and Navarro (2004) used diameter at base (30 cm). Both equations were from dry forests areas. Nevertheless Padrón and Navarro (2004) reported values of tree biomass ranging from 72.0 to 1893.0 kg for *Prosopis.* I have values ranging from 75.7 to 1137.2 kg.

On an areal basis, biomass in the three stands varied from 4.37 kg/m^2 to 9.56 kg/m^2 based on the Brown equation and from 5.87 kg/m^2 to 14.41 kg/m^2 based on the Padrón and Navarro (2004) specific equation for *Prosopis* (Table 1.6). Brandeis and Suarez-Rozo (2005) reported Puerto Rican dry forest biomass using Brown (1989) equation at 7.6 kg/m^2 and in PR moist forest at 11.32 kg/m^2. My mean values -for each stand (or pooled mean of 6.74 kg/m^2) using the Brown (1989) equation were comparable to the dry forest biomass. The values for RN, ENF and CNF stands range from below previously reported dry forest biomass to in between dry and moist forest biomass when using the Brown equation. Considering that the young age of the NFIT stands it seems unlikely that biomass would be greater in Laguna Cartagena than in mature Puerto Rican moist forest. The Padrón and Navarro equation apparently overestimates biomass since the CNF stands would have greater biomass than moist forest. Therefore I conclude that the Brown equation is more appropriate to use for Laguna Cartagena.

The area under my study tends to flood during the wet season, but is also really dry by the dry season. This can explain my values and the difference between natives and N-fixing tree biomass values. A year after the start of the experimental control trees had not grown based on annual diameter increment while 10% of the trees in the RN plots died (Table 1.8). Considering that the experimental year was an uncommon wet season (Personal communication with James Padilla), with higher precipitation, native trees encountered extremely wet conditions which could be responsible for both the lack of growth and mortality.

Vegetation structure changed over time in ENF. After removal, no tree had sprouted and species composition was different in areas with NFIT compared to non NFIT areas. The tall *Paspalum virtgatum* grass dominated ENF and CNF treatments; while RN remained almost 100% bare ground.

Cost and benefit of removal

The removal of invasive species has economic and ecological advantages and disadvantages. Invasive species removal can increase the area of production in an agricultural field and shift competition between agricultural crops and invasives. But management techniques in forest restoration or conservation of native species reflect costs and economic impact. One of the costs of bulldozed removal is significant changes in soil dynamics (Yelenik, Stock and Richardson 2004). Soil compaction results in decreases of soil porosity and filtration. Changes in these soil physical characteristics can result in more water retention, affecting seedling and sampling growth (Froehlich, 1979; Ampoorter, 2011).

In a more general perspective, invasive species management can cost up to $120 billion to the US Government (Pimentel, Zuniga and Morrison 2005). This includes control cost, grassland and forage losses, recreational use losses and crop yields losses. Invasive plants alone have a total cost of approximately $34,658 million and include 25 nonindigenous plants species. Pimentel et al. (2005) state that this is a conservative estimate because of the difficulty of estimating loss of ecosystem services and biodiversity. Ecosystem services are goods obtained from ecological functions for example cultural, recreation, gas regulation, climate regulation, waste treatment, nutrients cycling, pollination and erosion control. Some of these services are intangible so it is really difficult to obtain a cost. It has been estimated that ecosystem service values range from 16 to 54 trillion dollars per year for the entire biosphere (Costanza et al., 1997).

To estimate economic cost of invasive species removal by bulldozing in south west Puerto Rico one needs a medium bulldozer (D5-D6 machine) service. In the south area of PR the estimated cost per a day (8hrs) is $350 to $450, or $60 to $100 per hour. These costs include the operator and diesel fuel; however there is a $100 fee for the transportation of the machinery. Normally 3930 m^2 (1cuerda) can be cleared in one day, if there are few trees. So an area of 10 cuerdas will cost an average of $4,100 and take 10 days to clear. Eradication of the invasive is not possible, as most of the vegetation that reestablishes is invasive grass. This will require some post-bulldozing management (Zavaleta, Hobbs and Mooney 2001). Thus the estimate given above is low and one would need to add post-bulldozing management cost.

The mechanical elimination of herbaceous vegetation by bulldozing, by itself, will not produce the recovery of the ecosystem nor will it produce a reestablishment of the native species. To accomplish the reestablishment of native species, other mechanisms for land management are needed to help in the recovery of native species and to eliminate the dominance of grass species. After elimination by bulldozer, the first event to occur will be the establishment of grass. With its rapid and dense growth, grass will limit native species seeds ability to establish and grow, due to competition for light, space, water and nutrients. To cope with this, other issues need to be considered after the bulldozing. Care has to be taken not to eliminate all vegetation but there is a need to keep free and appropriate spaces that promote other native species and trees reestablish and grow on those spaces. Some of these management practices can be by controlled and selective use of herbicides after direct planting of seedlings, which reduce the competition created by the grass. This management, known as “chemical preparation", is generally effective and low cost as describe by North Carolina Forest Service, 2012. Even with this method hand labor is needed since the recommendations for this application are by stem injection, basal bark, basal soil, or foliar spray (Jackson and Finley 2011).

Another practice that can be used to decrease competition and establishment of native species is mowing and trimming. Mowing and trimming can be used around seedlings to ensure they are not overgrown by grass. In the use of installation of tree shelters, more hand labor and time will be necessary.

Elimination of trees by bulldozer at this site will not lead to establishment of native tree species unless they are planted. Reforestation with natives has been successful however, as evidenced by persistence of the trees in the RN plots that were planted 20 years ago. Their establishment took a lot of effort as the USFW service had to water the trees for many years (James Padilla, personal communication). It has been shown that native trees will eventually establish in the understory of non-native dominated forests in abandoned agricultural lands elsewhere on the island; however, it takes 60-80 years to occur (Lugo 2004). Many managers do not want to wait that long and the areas to be re-established with natives are often too far from native forest fragments for establishment to occur (Chinea and Helmer 2003).

The NIFT present here could be used as nurse trees. The closed canopy of the NFIT could decrease grass cover and thus competition with native tree seedlings that would be planted (Santiago-García, et al. 2008). This might reduce the need for mowing or trimming the grass as well as reduce the need for watering, decreasing the overall management costs.

Invasive species also provide ecological services and can be used in different scenarios to benefit the system. Ostertag et al. (2009), states that removal also can lead to alterations in microclimate, slow decomposition and lower total litterfall mass. In forests, keeping invasive trees species can prevent erosion and also helps with regulation of gas (CO_2, O_2 and SO levels), climate (greenhouse gas regulation, clouds formation) and water (regulation of hydrological flows, supply storage and retention) (Costanza, et al. 1997). Accumulation of organic matter can help in formation of the soil and can provide habitat for fauna. Tree removal will lead to C losses in the system. This can be detrimental for the entire ecosystem, especially in deforested tropical forests which are considered a major source of CO_2 emissions to the atmosphere (Grau, et al. 2003). Keeping trees will avoid CO_2 emissions by conservation of this carbon stocks, and planting natives under its canopy will increase carbon storage due to mechanisms of trees to convert carbon dioxide into sugar molecules and eventually biomass.

Besides these potential ecological benefits there are also a few social ones as well. Recreation and cultural activities are often more enjoyable if trees are present in the landscape, despite whether the tree is invasive or not. Even in agricultural lands, trees can provide shade for both cattle and employees.

Management plans should be linked with a post-removal and revegetation plan, because the disturbed space left by bulldozing could favor dominance of more and maybe worse invasive species. Thaxton et al. (2012) recommends a combination of grass removal and shading as management in an approach to restore degraded tropical dry forest. It states that bulldozing grasses is particularly effective to increase seedling performance and soil water availability. The NFIT of Puerto Rico might be able to provide the shade needed to establish the native trees (Santiago-García, et al. 2008) so that bulldozing might not be necessary. The idea of the novel forest could be a good management tool, especially since many of the invasive species in Puerto Rico have been around for many years with some of them considered naturalized (Lugo 2004)

Recommendations

1. In abandoned agricultural sites instead of removal, NFIT will be better to use as a nurse tree so that native trees can establish under the invasive canopy and increase carbon stocks.
2. Bulldozing should not be considered as a management tool on its own.
3. If removal by bulldozing is to be used, season should be considered and the best timing seems to be the dry season, as it decreases impacts to soil and nutrient leaching occurring during rainy season.
4. Management driven to restoration should be an integrated plan. Post-removal attention and control, as herbicides, mowing, trimming and native tree planting, must be added.

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Appendices

Appendix A. Mean monthly values for ammonia (NH3--N), nitrate (NO3--N) and nitrite (NO2 --N) from soil samples at Laguna Cartagena, Lajas, Puerto Rico. Values were used to calculate N mineralization and nitrification rates. Samples analyzed by Laboratorio Central Analítico, Estación Experimental Agrícola, Recinto Universitario de Mayagüez, Universidad de Puerto Rico.

Month	Treatment	NO_3^--N (mg/gsoil)		NH_4^--N (mg/soil)		NO_2^--N (mg/gsoil)	
November	ENF	0.7 (0.16)	A	0.21 (0.06)	c	-1.8E-04 (2.6E-04)	a
November	CNF	1.0 (0.29)	ab	0.20 (0.08)	c	-2.0E-04 (3.3E-04)	a
November	RN	0.94 (0.40)	ab	0.08 (0.03)	a	-3.8E-04 (2.3E-04)	a
December	ENF	0.78 (0.43)	ab	0.20 (0.07)	bc	-7.9E-05 (3.7E-04)	a
December	CNF	1.7 (0.14)	C	0.10 (0.07)	bc	-3.6E-04 (2.9E-04)	a
December	RN	1.1 (0.30)	B	0.07 (0.02)	a	-1.1E-04 (2.3E-04)	a

Means with the same letter have no significant differences p<=0.05.

Appendix B. Allometric equation used to calculate tree biomass.

Brown (1989):

$Y= \exp[-3.1141 + 0.9719 \ln (D^2H)$

Where: D is dbh in centimeters
H is total height

Padrón and Navarro (2004) were used for *Prosopis juliflora* trees:

Above-ground fresh biomass

$= 75.1691+ 0.08732 [(\text{diameter at the base})^2 \times (\text{total height})]$

Above-ground dry biomass

$= 66.5541 + 0.05796 [(\text{diameter at the base})^2 \times (\text{total height})]$

Appendix C. InfoStat (version 2012e) output for tree measurements.

Tree biomass

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
AGB kg/tree	77	0.36	0.35	159.77

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	1356736.07	2	678368.03	21.13	<0.0001
Treatment	1356736.07	2	678368.03	21.13	<0.0001
Error	2375824.15	74	32105.73		
Total	3732560.22	76			

Test:LSD Fisher Alfa=0.05 DMS=108.06391

Error: 32105.7317 gl: 74

Treatment	Medias	n	E.E.		
Reference-N	55.65	34	30.73	A	
Exp-NN	72.24	34	30.73	A	
Control-NN	476.39	9	59.73		B

Medias con una letra común no son significativamente diferentes (p<= 0.05)

Variable	N	R^2	R^2 Aj	CV
AGB kg/m2	9	0.59	0.45	45.66

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	138.89	2	69.44	4.24	0.0711
Treatment	138.89	2	69.44	4.24	0.0711
Error	98.22	6	16.37		
Total	237.11	8			

Test:LSD Fisher Alfa=0.05 DMS=8.08350

Error: 16.3702 gl: 6

Treatment	Medias	n	E.E.		
ENF	5.87	3	2.34	A	
RN	6.31	3	2.34	A	
CNF	14.41	3	2.34		B

Medias con una letra común no son significativamente diferentes (p<= 0.05)

Appendix D. SAS program and output for forest vegetation data (4 months of biomass for ENF and CNF). Split plot design with month as the split.

```
ods rtf;
data forest;
input plot rep trt $ month $ weight biomass;
datalines;
proc glm data= forest;
class trt month rep;
model biomass = trt rep(trt) month trt*month;
test h=trt e=rep(trt);
means month;
means trt/ tukey;
means month /tukey;
run;
ods rtf close;
```

SAS Program output for herbaceous vegetation biomass

R-Square	Coeff Var	Root MSE	biomass Mean
0.274784	55.36934	689.0703	1244.498

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	1	1522322.4	1522322.436	3.21	0.0802
rep(trt)	4	1017193.6	254298.395	0.54	0.7103
month	2	3640280.8	1820140.379	3.83	0.0292
trt*month	2	1736156	868077.994	1.83	0.1727

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	1	1522322.4	1522322.436	3.21	0.0802
rep(trt)	4	1017193.6	254298.395	0.54	0.7103
month	2	3640280.8	1820140.379	3.83	0.0292
trt*month	2	1736156	868077.994	1.83	0.1727

Tests of Hypotheses Using the Type III MS for rep(trt) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	1	1522322.4	1522322.436	5.99	0.0707

Month Minimum Significant Difference	377.97	**Treatment Minimum Significant Difference**	557.11

Appendix E. SAS program for forest soil data (2 months). Split plot design with month as the split.

```
ods rtf;
data forest;
input plot rep trt $ date $ month $ Al Ca Fe K Mg Mn Na P C Npercent LOI CN N
NP;
datalines;
proc glm data= forest;
class trt month rep;
model NP = trt rep(trt) month trt*month;
test h=trt e=rep(trt);
means month;
means trt/ tukey;
means month /tukey;
run;
ods rtf close;
```

SAS Program example output for soil nutrient soil data.

Total Nitrogen

R-Square	Coeff Var	Root MSE	N Mean
0.466916	25.5582	0.591578	2.31463

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	0.49707037	0.24853519	0.71	0.4974
rep(trt)	6	1.82815556	0.30469259	0.87	0.5244
month	1	9.16782407	9.16782407	26.2	<.0001
trt*month	2	1.3810037	0.69050185	1.97	0.1517

Tests of Hypotheses Using the Type III MS for rep(trt) as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	0.49707037	0.24853519	0.82	0.486

Month Minimum Significant Difference	0.4791	**Treatment Minimum Significant Difference**	0.3249

Appendix F. SAS Program repeated measures for PRS™-Probes samples. Treatment numbers are (1) experimental N-fixing, (2) control N-fixing and (3) reference native.

```
ods rtf;
data forest;
input plot trt $ month sample TN NO3 NH4 Ca Mg K P Fe Mn Cu Zn B S Pb Al Cd;
datalines;

proc mixed data=forest;
class trt month plot sample;
model P = trt month trt*month;
repeated month/ subject = sample(plot) type= AR(1);
random plot;
contrast 'CNF vs. RN' trt 0 1 -1;
contrast 'ENF vs. CNF' trt 1 -1 0;
lsmeans trt*month / slice=month slice=trt;
run;
ods rtf close;
```

SAS Program output for total nitrogen flux.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Trt	2	257	110.04	<.0001
Month	10	257	36.04	<.0001
trt*month	20	257	7.79	<.0001

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
CNF vs. RN	1	257	194.79	<.0001
ENF vs. CNF	1	257	128.9	<.0001

Tests of Effect Slices

Effect	Trt	month	Num DF	Den DF	F Value	Pr > F
trt*month		1	2	257	10.77	<.0001
trt*month		2	2	257	69.21	<.0001
trt*month		3	2	257	14.74	<.0001
trt*month		4	2	257	8.85	0.0002
trt*month		5	2	257	18.35	<.0001
trt*month		6	2	257	6.71	0.0014
trt*month		7	2	257	1.15	0.3188
trt*month		8	2	257	1.73	0.1786
trt*month		9	2	257	20.68	<.0001
trt*month		10	2	257	34.91	<.0001
trt*month		11	2	257	57.74	<.0001
trt*month	1		10	257	17.93	<.0001
trt*month	2		10	257	31.71	<.0001
trt*month	3		10	257	1.57	0.1152

Appendix G. SAS Program repeated measures output for phosphorus flux.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
trt	2	138	9.36	0.0002
month	5	138	24.41	<.0001
trt*month	10	138	6.95	<.0001

Contrasts

Label	Num DF	Den DF	F Value	Pr > F
CNF vs. RN	1	138	4.03	0.0468
ENF vs. CNF	1	138	5.36	0.022

Tests of Effect Slices

Effect	trt	month	Num DF	Den DF	F Value	Pr > F
trt*month		1	2	138	4.18	0.0173
trt*month		2	2	138	17.94	<.0001
trt*month		3	2	138	28.64	<.0001
trt*month		4	2	138	1.61	0.204
trt*month		9	2	138	0.16	0.8543
trt*month		10	2	138	0.39	0.68
trt*month	1		5	138	2.67	0.0244
trt*month	2		5	138	6.95	<.0001
trt*month	3		5	138	28.69	<.0001

Appendix H. InfoStat output for light measurements data by treatment

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Measurement (µmol/m2/s)	324	0.27	0.27	109.59

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	7773554.86	2	3886777.43	60.76	<0.0001
Treatment	7773554.86	2	3886777.43	60.76	<0.0001
Error	20533257.68	321	63966.53		
Total	28306812.53	323			

Test:LSD Fisher Alfa=0.05 DMS=67.71238

Error: 63966.5348 gl: 321

Treatment	Medias	n	E.E.		
Native	91.05	108	24.34	A	
Control	154.55	108	24.34	A	
Bulldozed	446.75	108	24.34		B

Medias con una letra común no son significativamente diferentes(p<= 0.05)

Appendix I. Herbaceous vegetation cover estimation InfoStat output.

Time= August

Frecuencias absolutas
En columnas:Category

Time	Treatment	0-25	25-50	75-100	Total
August	CNF	0	0	9	9
August	ENF	0	0	9	9
August	RN	2	7	0	9
August	Total	2	7	18	27

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	27.00	4	<0.0001
Chi Cuadrado MV-G2	34.37	4	<0.0001
Coef.Conting.Cramer	0.58		
Coef.Conting.Pearson	0.71		

Time= December

Frecuencias absolutas
En columnas:Category

Time	Treatment	0	0-25	25-50	50-75	75-100	Total
December	CNF	0	1	1	1	6	9
December	ENF	0	4	3	2	0	9
December	RN	2	2	4	0	1	9
December	Total	2	7	8	3	7	27

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	18.61	8	0.0171
Chi Cuadrado MV-G2	20.80	8	0.0077
Coef.Conting.Cramer	0.48		
Coef.Conting.Pearson	0.64		

Time= June

Frecuencias absolutas
En columnas:Category

Time	Treatment	0	0-25	25-50	50-75	75-100	Total
June	CNF	0	1	1	1	6	9
June	ENF	1	4	3	1	0	9
June	RN	2	2	4	0	1	9
June	Total	3	7	8	2	7	27

Estadístico	Valor	gl	p
Chi Cuadrado Pearson	15.61	8	0.0484
Chi Cuadrado MV-G2	18.02	8	0.0211
Coef.Conting.Cramer	0.44		
Coef.Conting.Pearson	0.61		