Spring 5-13-2017

Precipitation Patterns and Fungal Community Succession in a Seasonally Dry Secondary Tropical Savanna

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PRECIPITATION PATTERNS AND FUNGAL COMMUNITY SUCCESSION IN A SEASONALLY DRY SECONDARY TROPICAL SAVANNA

by

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DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy

Biology

The University of New Mexico
Albuquerque, New Mexico

May, 2017

ACKNOWLEDGMENTS

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ABSTRACT

In the northwestern part of South America, seasonally dry forests have undergone extensive conversion to savannas to support ranching and agriculture. The combination of fire and vegetation removal employed for clearing, together with extensive agricultural practices, often lead to loss of soil fertility. This results in land that is no longer useful for agriculture and on which historical forests are unable to regenerate. This study examined the effects of common agricultural practices on soil fungal communities by simulating the effects of fire followed by plant removal. The goal was to determine whether common practices have rapid effects on soil communities, which in turn could signal a decline in soil quality.

To provide a climatological context for this study, I first performed an analysis (presented in Chapter 1) that employed a 40-year database of daily precipitation totals,

from a rain-gauge station located in the central portion of the Caribbean savannas of Colombia. The goal was to assess intra- and inter-annual variability in precipitation. I found that overall there is a tendency toward dryer years starting in the mid 1990´s. In addition, despite the documented relationship between El Niño and decreased precipitation, correlation between El Niño occurrence and total monthly precipitation was not statistically significant. Such results can be explained in part by local fluctuations in temperature and precipitation. Finally, results from this study highlight the importance of studying the distribution of precipitation at different time scales, which can provide insights into the pulse-driven nature of seasonally dry Tropical systems.

A second study (Chapter 2) examined changes in the composition of fungal communities in a Colombian seasonally dry savanna over a three-month period, under conditions simulating the local agricultural practices of vegetation burning and removal. Fungal community composition was estimated using next-generation pyrosequencing targeting the ribosomal RNA internal transcribed spacer (ITS) regions. The general fungal community was dominated by species of Ascomycota and Basidiomycota, many of which had been described previously from culture studies of tropical soils. Significant community changes occurred over time, but there were not dramatic differences between removal and non-removal experimental plots. The community at three months after vegetation cutting or removal differed in overall diversity from that observed at zero and thirty days. Several fungal taxa were identified as potential indicator species of either early or late sampling periods. Overall, it appears that the decline in soil health observed for sites subjected to long-term agricultural practices are not reflected in dramatic fungal community changes observed for a single season, even in the case of complete above-

ground vegetation removal. Additional studies will be required to document the time-course for changes in microbial community composition that accompany such decline.

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INTRODUCTION

This dissertation reports on two studies conducted in the "El Bajo," San Onofre municipality, State of Sucre in the Caribbean Region of Colombia. Throughout the Neotropics, seasonally dry davannas result from of the conversion of seasonally dry tropical forests as a result of efforts to support cattle ranching and agriculture (Murphy and Lugo, 1986). The current land-cover mosaic of this region includes savannas, gallery forests and isolated forest patches that form the remnants of the seasonally dry forests. The region is characterized by a marked rainfall seasonality with three or more dry months every year (precipitation < 100 mm/mo), a total annual precipitation between 700 and 2000 mm, and a mean annual temperature >25°C (Sánchez-Azofeifa et al., 2005).

The conversion of forests to pastures and agriculture induce changes in nutrient status and nutrient loss rates from these systems. Several studies have presented evidence of the shift in C and N dynamics following forest conversion, including a decrease in total soil organic carbon and changes in nitrogen status (Mass, 1995). The consequences of forest conversion on soil microbial processes are poorly understood. No studies have examined the composition, succession and responses of fungal communities in this region.

While the original goal of the current study was to study changes in the soil fungal community in response to simulated land-use practices of burning and vegetation removal, it became clear early in the study that there was a lack of information regarding long-term patterns of precipitation in the study area. Such information will be necessary for long-term efforts to understand the consequences of land-use practices and to predict changes in communities under climate change scenarios. Accordingly, I

undertook a study of intra- and inter-annual precipitation patterns using 40-year data from a rain-gauge station near my study sites. The results of this study are presented in Chapter 1.

Chapter 2 explores the question of whether fungal community composition changes rapidly (three month study period) in response to the common agricultural practices of burning and vegetation removal. This study also examined the effects of these practices on soil pH, nitrogen (NO_3 and NH_4) content and organic carbon. The combined results of the two studies suggest that despite the dramatic changes in precipitation during the course of each year in this region--long dry periods followed by periods of heavy rainfall--at least in the course of a single year, fungal communities are buffered against dramatic changes.

References

Maass, J., J. Vose, W. Swank, and A. Martinezyrizar. 1995. Seasonal changes of leaf area index (LAI) in a tropical deciduous forest in West Mexico. Forest Ecology and Management **74**:171-180.

Murphy, P., and A. Lugo. 1986. Ecology of tropical dry. Annual Review of Ecology and Systematics **17**:67-88.

Sanchez-Azofeifa, G., M. Kalacska, M. Quesada, J. Calvo-Alvarado, J. Nassar, and J. Rodriguez. 2005. Need for integrated research for a sustainable future in tropical dry forests. Conservation Biology **19**:285-286.

Chapter 1

Temporal analysis of precipitation in a seasonally dry savanna in Colombia (northern South America)

Abstract

Life in seasonally dry areas strongly depends on pulses of precipitation during certain portions of the year. This is particularly relevant for Tropical savannas on the Caribbean coast of Colombia that have been converted from Tropical Dry Forests and subjected to permanent agricultural and grazing practices. The annual cycle of Precipitation in the savannas is uni-modal due to the South-North oscillation of the Intertropical Convergence Zone (ITCZ), which imposes a five-month period of almost zero precipitation. Water stress is even more intense during the warm phase of El Niño Southern Oscillation (ENSO) that typically exacerbates high temperatures and reduced precipitation. A 40-year database of daily precipitation totals, from a rain-gauge station located in the central portion of the Caribbean savannas of Colombia, is used to describe intra- and inter-annual variability of precipitation. Using a series of attributes of the distributions of monthly and daily data I found that, overall, there is a tendency toward dryer years starting in the mid 1990´s. In addition, despite the documented relationship between El Niño and decreased precipitation, correlation between El Niño occurrence and total monthly precipitation was not statistically significant. Such results can be explained in part by local fluctuations in temperature and precipitation. Finally, results from this study highlight the importance of studying the distribution of precipitation at different time scales, which can provide insights into the pulse-driven nature of seasonally dry Tropical systems.

Introduction

Life in seasonally dry areas heavily depends upon short periods of high resource

availability caused by rainfall events (Noy-Meir, 1973; Schwinning and Sala, 2004). In

the tropics, where a high proportion of Tropical Dry Forests (Sánchez-Azofeifa, 2005)

have been converted to savannas that support agricultural and managed grazing systems

(Asner et al. 2004; Maass, 1995), water availability is critical. In converted savannas,

water availability is reduced even during the rainy season due to increased surface flow

and evaporation caused by a reduction in leaf area index and decreased root biomass

(Moreira et al. 1997; Giambelluca, 2002; Bruijnzeel, 1990). In addition, anomalies in

rainfall, soil moisture, and river flows experienced in Tropical South America during the

El Niño phase of ENSO (Poveda et al. 2006) affect ecosystem function (Mandujano,

2006; Ticktin, 2003; Borchert et al. 2002), alter the supply of rain-driven agricultural

commodities, and create increased food prices and inflation (Capa-Morocho, 2014; FAO,

2014). Such pressures on Tropical drylands, and the high dependence on seasonal rainfall,

call for a deeper knowledge of local conditions driven by climate. This information is

particularly important in the context of accurate Global Climate Change models, which

are needed to develop coherent water management policies in the areas affected by water

deficit.

It has also been recognized that there is a need for local information regarding

precipitation at monthly and daily temporal scales, given that the General Circulation

Models (GCM) often used as tools to predict effects of potential climate change offer

coarse-resolution results that are difficult to extrapolate to local scales (Giorgi and

Francisco, 2001, Easterling, 1999; Weltzin et al. 2003). This is especially relevant to

Tropical South America, where the annual hydroclimatological cycle is controlled by the

Inter Tropical Convergence Zone (ITCZ), which in turn is affected by El Niño Southern

Oscillation (ENSO). At the regional scale, geography also plays an important role

(Pulwarty et al. 1992). For example, in Panama, the precipitation regime differs between

the Caribbean and Pacific slopes of the central mountains. This is a consequence of

complex patterns of circulation of moisture-laden winds above both the Caribbean and the

Pacific, caused by the comparatively low relief of the mountains and the configuration of

the southern portion of the isthmus relative to prevailing easterly trade winds (Poveda,

2006).

The Caribbean coast of Colombia is very interesting in this context, because it

represents the geographical transition between Central America and the northernmost

portion of Eastern South America represented by Venezuela, Guyana and Brazil (Figure

1.1).

The central portion of the coast is representative of many seasonally dry savannas in the

Tropics that have been converted from Seasonally Dry Tropical Forests (Castellanos-

Castro and Newton, 2015). The "Sabanas" of the central portion of the Colombian

Caribbean [colloquial for savannas] were converted from Dry Forests in post-Hispanic

times (Etter et al. 2008) and have been subjected to slash and burn practices for the

establishment of grazing and agricultural systems. According to the National Assessment

of Water Resources (IDEAM, 2010), the "Sabanas" experience severe water stress even

during average precipitation conditions, thus threatening water supply during extreme

events like El Niño.

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Unfortunately, precipitation records for the "Sabanas" only include recent decades, and daily data are incomplete limiting options for data analysis. Nevertheless, there is potential for the analysis of precipitation data available from this region to inform regional climatic models.

The goal of the present study was to characterize intra- and inter-annual variability of monthly precipitation using data from a rain-gauge station in an agricultural region and near an experimental ecological site ("El Bajo") in the Colombian Caribbean (Figure 1.1). Four decades of data were available. My approach focused on daily precipitation totals, a time scale relevant for agriculture. I also explored the statistical distribution of daily totals in order to extract attributes useful in adjusting a model that explained the long-term variation. I relate the observed intra- and inter-annual patterns of precipitation to oscillations of the ITCZ and ENSO, which typically results in decreased precipitation in this region during El Niño phase (Córdoba-Machado et al., 2015).

Methods

Site

"El Bajo" (9°51'10.41" N; 75°25'29.54"W), belongs to a continuous system of hills of denudative origin ("lomerío") known as the "Sabanas de Mucacal" with an altitude ranging between ~50 and 200 meters above sea level. Average annual temperature at the Sabanas is 27°C and average relative humidity is 83.5% (IDEAM,

2010). Local geology is characterized by the presence of sedimentary rocks from

Continental and Transitional environments (quartz arenite sands to conglomerate

intercalated with limestone); soils are predominantly Entisols and Alfisols (IGAC, 2010).

In general, the soils in this area may be defined as hyperthermic, moderately fertile,

highly susceptible to erosion, pH ranging from mildly alkaline to acidic, and very poorly

to excessively drained.

Pastures were created by conversion of tropical dry forest and have been

continuously used for semi-extensive cattle ranching and agriculture for the last 30 years.

With the exception of scattered gallery forests, trees have been removed and extensively

replaced by forage vegetation like Mombasa Guinea grass (*Panicum maximum*) and the

perennial legume *Pueraria phaseoloides.*

The entire "Sabanas of Mucacal" system exhibits a uni-modal annual cycle of

precipitation (ranging form May to October with maximum precipitation occurring in

October) due to the oscillation of the ITCZ that affects the annual hydroclimatological

cycle of the Caribbean coast of Colombia (Hastenrath, 2002; Poveda et al. 2006). A slight

decrease of precipitation between July and August known as the "Veranillos of San Juan"

is also present, although its causes are less understood (Magaña et al. 1999).

I used total daily precipitation recorded by the closest hydro-metereological

station ["Sabanetica", 9°49'44.0004" N; 75°25'58.0002" W; IDEAM station code

13090010] (Figure 1.1) located ~1 km from "El Bajo". This station recorded data

between 1974 and 2014 but years 1974, 1981, 1982, 1991 and 1999 were excluded from analyses because >10% of daily precipitation values were missing from the record.

Analysis of precipitation

To determine the extent to which local precipitation matched the uni-modal hydro-climatological cycle, the distribution of daily precipitation totals per month was calculated for each year in the record. To visualize the inter-annual variability in monthly total precipitation, the years in the record were grouped using a hierarchical clustering with complete linkage method (Legendre and Legendre, 1998). The complete analysis of monthly precipitation totals per year were represented in a heat map using the R-project d3heatmap package (Cheng et al. 2016).

To examine the inter-annual behavior of precipitation I used two approaches. The first consisted of creating and analyzing a non-zero-values subset from the original data series. The character of this new dataset was assessed by exploring graphically the distribution of daily total precipitation for both the entire data set with no regard of time identity and for data pooled by year. In both cases, the mean, standard deviation and interquartile distribution were calculated and compared to those obtained for the original data. This procedure was applied because preliminary inspection of the data revealed that there was a high concentration of consecutive zero values between January and March for all years. Under such conditions, the zero values would hide the variability of wetter months, thus making it difficult to compare the distribution of precipitation among years. The mean and the 3rd quartile value from this new data set were used as predictors to

adjust linear and polynomial models of precipitation frequency. Both parameters were

used because although sensitive to extreme values the mean in this case is consistent and

sufficient, whereas the third quartile is expressing the behavior of 75% of the data in the

distribution of total daily precipitation per year. When adjusting the polynomial models,

two methods were used to transform the predictors in order to improve the fit. The first

consisted of adding polynomial terms (d) to the model $y = \beta_0 + \beta_1 x + \cdots + \beta_d x^d + \varepsilon$

to allow for a more flexible relationship. The second used the broken stick method as a

complementary approach and consisted of applying different linear regression models in

different regions of the data (Faraway, 2005).

The second approach to analysis of inter-annual variability employed further

exploration of variability with the aim of better observing long-term patterns. For this

purpose the non-zero total daily precipitation data were again subset into low and high

magnitude using as reference the 35 mm value corresponding to the 3rd quartile or the

upper limit of the distribution of total daily precipitation for all years. Ranking of monthly

precipitation values by percentiles with respect to the record for a given time interval is a

widely used method to establish the character of months as wet/dry (Grimm and

Tedeschi, 2009; Tedeschi et al., 2015). For the purpose of this study I used daily values

instead of monthly because of the high variability in daily precipitation for a single

month. Graphical analysis of the distribution of daily precipitation was used to identify

intra-decadal climatic phenomena like El Niño and the oscillation of the ITCZ. A

polynomial model was adjusted to explain the long-term structure of the data based on the

information provided by the previous graphical analyses. For this purpose, only low

magnitude values were used and the number of days with precipitation < 35 mm, along

with the mean value of total daily precipitation per year, were used as indicators of frequency and magnitude.

Results

Description of wet and dry seasons from 1975 to 2014

Total monthly precipitation from 1975 to 2014 exhibited a uni-modal intra-annual pattern with marked differences between wet and dry periods (Figure 1.2a). A severe dry season occurs from December to April. Monthly precipitation rarely exceeds 100 mm in December while average values drop to nearly 1 mm between January and March. This pattern translates into < 1mm of mean total daily precipitation and over 90% of days without any precipitation for the entire dry period. A steady rise in total monthly average precipitation occurs from April to September, reaching a peak in October. Finally, although monthly precipitation slightly decreases in July, it does not decline to values observed during the dry period.

The heat map of total monthly precipitation from 1975 to 2014 further supports the uni-modal pattern, showing the consistency within dry and rainy periods (Figure 1.2b). However, due to the great inter-annual variability April and December may be best considered as transitional months. In addition, although the highest monthly precipitation occurred in August and October, there was high variability among years and thus values lower than 200 mm were not uncommon during these months (Figure 1.2b). Similarly, in certain years (e.g. 1985 and 1992) the dry season extended through May. Cluster analysis

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pooled years into two major groups (Figure 1.2b). Group I included years comprising

nine of the highest annual precipitation values for the entire record, with October 2007

and August 2011 having the highest rainfall (566 and 517 mm, respectively). Group II

included the remaining years (75% of total record), and it was characterized by lower

values of monthly precipitation even during the rainy period. The two groups were

different in terms of monthly and daily precipitation. Mean values of total monthly

precipitation were 205 mm for group I and 140 mm for group II. Mean values of total

daily precipitation were 30 mm for group I and 25 for group II.

Inter-annual variability of total daily precipitation

Total annual precipitation varied between 556 and 2230 mm but graphical analysis

of total daily precipitation demonstrated that the number of days with very low or zero

precipitation constituted approximately 80% of the entire precipitation record. Zero-

precipitation days masked any inter-annual pattern because random variability was

observed around a very low mean (mean= 3.47, SD = ± 12.6). Moreover, such values also

underestimated both intra- and inter-annual variability of high values of total daily

precipitation (Figure 1.3a). The exclusion of days without precipitation increased the

resolution of the inter-annual variation (mean=25.73, SD = ± 24.3) (Figure 1.3b) shown

by an erratic pattern of 1st and 2nd quartile values (Figure 1.4)

Linear correlation of data showed no trend in third quartile and mean values as

expected from the distribution of data in Figure 1.4, but a non-linear pattern was

observed. A quadratic model showed clearer trends in mean precipitation and third quartile value (Figure 1.5), although in both cases the adjusted R square where low and in the second case the p value is only marginally significant According to this model, when days without precipitation were excluded, it was possible to observe a decadal variability with an increase in the mean magnitude of total daily precipitation starting around the mid seventies and extending to late nineties from where it started to decrease. These patterns where confirmed by the Broken stick regressions, indicating a peak in the mid nineties (mean and third quartile: 32.6 and 44.6 mm) and baseline values (21.4 and 26.8 mm, respectively) toward the extremes of the record. Third quartile values were excluded from subsequent analysis since the polynomial model adjusted better to the structure of the mean values.

Long-term patterns using low and high magnitude total daily precipitation

Graphical analysis of distribution of low magnitude precipitation (< 35 mm) using the lower 75% of each distribution as reference confirmed the results from the analysis presented in the previous section, showing a parabolic cycle from 1975 to 2014. There is a noticeable deviation from the overall fit between 1983 and 1985, and from 1992 to 1995 (Figure 1.6). The same analysis did not yield any noticeable long-term pattern for high magnitude precipitation (> 35mm).

According to the polynomial regression, there was a decline in the number of dry days (< 35 mm) per year between the mid 90's and early 2000's (ranging between 17 and 32 days) followed by a steady increase until 2014 (ranging between 31 and > 50 days).

Mean precipitation was lower than predicted by the least square fit for the period 1983-

1985 (unfortunately, data are lacking for 1981 and 1982) (Figure 1.7a). Mean

precipitation was also lower in 1995 but in this case the number of days < 35 mm

notoriously deviated from the model. The number of dry days in this year (52) is among

the highest in the entire record. This figure occurred in the previous decades only in 1975

and was not surpassed until 2010 (59 days) (Figure 1.7b).

Discussion

Seasonality

The observed intra-annual pattern in average total monthly precipitation for the

1975-2014 records in the study area responds to the oscillation of the ITCZ as it has been

extensively documented (Hastenrath and Lamb, 1977). The ITCZ has been defined in

different ways (Hartman, 1994; Gu and Zhang, 2001, Hastenrath, 2002), but the classical

approach defines it as the low pressure trough induced by a band of warm sea surface

temperature (SST), where the Northeast trade winds encounter the cross equatorial

airstream from the southern hemisphere. Oscillation of the ICTZ follows the sun

movements as it crosses the Equator twice a year causing a bi-modal pattern of

precipitation. However, the Colombian Caribbean and the Pacific flank of the Central

America Isthmus experience a uni-modal cycle (between May and October) that reflects

the northernmost position of the ITCZ (Hastenrath, 2002). This northward movement is

caused by an increase in Northern hemisphere insolation while Southward migration is

associated with cooling of the North Atlantic region due to enhanced high latitude ice

cover and a slowdown of the Atlantic meridional overturning circulation (Yan et al., 2015).

Comparisons of monthly vs daily data in the current study highlight the importance of considering a range of time scales. The analysis of monthly and daily precipitation (Figure 1.2b) during the rainy period from 1975 to 2014 indicated that examination of monthly totals might underestimate the magnitude and frequency of events, resulting in misleading conclusions about behavior of ecosystems. Results of comparisons between monthly averages for the two groups resulting from hierarchical clustering showed a noticeable difference of 65 mm. However, means differ in only 5 mm when comparison is based on total daily precipitation, thus suggesting that monthly differences emerge as a result of the increase in the number of rainy days instead of a mere increase in the magnitude of individual events.

Although several studies in Tropical and Temperate dry areas have pointed out that biological processes are highly synchronized with either dry or wet season, ultimately the magnitude and extent of ecological processes are closely related to the magnitude of precipitation pulses (Schwinning and Sala, 2004). For example, flowering of leafless trees in Tropical Dry Forests of Central America have been related, among other factors, to the occurrence of showers > 20 mm (Borchert et al., 2004), whereas Petrie et al. (2015) found that the occurrence of small rainfall events (< 3.8 mm) determined soil nutrient availability and ANPP response in desert grasslands in the Northern Chihuahuan desert. It is important to emphasize that the general seasonal pattern might be altered by physical mechanisms such as the increase in cyclonic activity in the Caribbean between June and

November (Poveda et al. 2006) that is responsible for an increase in precipitation, although the Colombian Caribbean is not located in the main cyclone track.

Tropical Dry Forests and by extension the areas converted to savannas experience a dry season during which <10% of annual precipitation occurs (Murphy and Lugo, 1986). Results from the present study show that zero precipitation days constitute 80% of the entire record for the study area but such values are not restricted to the dry months (Figure 1.3). Even though these observations only offer a partial view regarding the pulsing nature of the "Sabanas", a future approach will benefit from deeper knowledge of the distribution of precipitation at a sub-monthly scale and the identification of critical thresholds that trigger different processes in this ecosystem.

Inter-annual variation

Analysis of daily totals did not show any long-term (secular) trend when third quartile and mean values were used as parameters. The exclusion of zero precipitation days, however, uncovered an inter-annual variability in the mean magnitude and distribution of total daily precipitation during the rainy season [most of zero values are concentrated in the dry season between January and March] (Figure 1.4). Mean and 3rd quartile values of total daily precipitation exhibited a mild but statistically significant multi-decadal oscillation from 1975 to 2014. This is important because the pattern is significant despite the high inter-annual variability in both parameters responsible for large residuals in specific years. The highest fitted values occur between 1990 and 2000 and were lower toward the 70´s and 2010. These results are opposite to what has been reported by the Intergovernmental Panel on Climate Change [IPCC] (Hartmann et al.

2013) after analysis of annual precipitation anomalies averaged over all tropical land

areas (30º S to 30ºN). According to the IPCC, precipitation over tropical land areas has

increased during the last decade, in contrast with the period between the mid-1970s and

1990s that show the opposite behavior but such observations must be interpreted with

caution. Not only is the term "Tropical" used in a very broad sense, but also previous

conclusions tended to ignore the monthly and sub-monthly variability in precipitation

characteristic of seasonally dry areas. For example, Marengo (2004) found differences in

decadal and long term precipitation patterns in the Amazon basin when different

geographical scales were analyzed. There is a negative rainfall trend for the entire basin

but at the smaller regional scale the Southern Amazonia showed a positive trend. Feng et

al. (2013) analyzed monthly rainfall data from 2715 stations located between 20º N and

20º S to conclude that variability of seasonality has increased over many areas of the dry

tropics. Such results imply that the intensity, arrival and duration of seasonal rainfall were

increasingly uncertain over the 20th century. Furthermore, Knapp et al. (2015) who

analyzed 100-year precipitation records belonging to 144 sites worldwide at local and Eco

region scales did not find a statistically significant temporal pattern in annual

precipitation for most sites. They did find that extreme wet years have occurred more

frequently in recent decades relative to earlier decades, which is in accordance with my

results (Figure 1.2b). It is worth mentioning, however, that for seasonally dry areas like

the "Sabanas" the dry season remains highly distinct, and rainy days are heavily

clustered. Results from this study show that although 5 years of the most recent decade

(2001-2011) possessed the highest values of total precipitation for the entire record, it is

also true that ~23% of precipitation in the wettest month (October) of the wettest year

(2011) occurred in a single day, and rainy days were followed by an average of ~3 consecutive dry days (precipitation = 0 mm).

There is evidence indicating that dry and wet extremes will become more common across a range of timescales (Lintner, 2012). This is relevant for seasonally dry areas such as the "Sabanas" since "El Niño" episodes are associated with decreased levels of precipitation and therefore drier and more prolonged dry seasons (Garreaud et al. 2009, Poveda et al. 2006). Results from this study show that grouping days into low (<35) and high (>35 mm) magnitude precipitation supported the occurrence of a multi-decadal oscillation for low values, but overall this trend did not seem tied to the occurrence of El Niño events. Again, a general increase in magnitude was observed between 1990 and 2000, and this was accompanied by a reduction in the number of days with < 35 mm precipitation (Figures 1.6 and 1.7). Analysis of mean values of daily precipitation and < 35 mm showed a very strong signal only for the 1983 and 1995 El Niño periods, and of those only 1983 is ranked among the 7 strongest El Niño events since 1950 according to the Multivariate ENSO index [MEI] (Wolter and Timlin, 2011). Total precipitation per year seems to be a better indicator of El Niño conditions, since in general lower values are associated with its occurrence.

The results of daily precipitation data as affected by El Niño are in contrast with total annuals where a clearer association seems more evident. For instance, out of eight years with less than 1000 mm precipitation, five years (1976, 1977, 1983, 1997 and 2009; range: 556 – 881 mm year^{-1}) corresponded to El Niño. This apparent discrepancy may be explained by physical mechanisms associated with local changes in temperature and

precipitation. The "Sabanas" have been historically subjected to different vegetation covers depending on land use since conversion from Tropical Forests occurred. Change in vegetation type and architecture range from almost bare soil (after slash and burn) to tall grasses like Mombasa Grass and lately oil palm, that in turn can alter surface moisture, surface heat, momentum fluxes and gas exchange in the system (Mahmood et al. 2014). A recent review by Lawrence and Vandecar (2014) about the effects of tropical deforestation points out several studies reporting an increase in surface temperature accompanied by a decrease in evapotranspiration particularly during the dry season after forest conversion to pasture in the Amazon. In the same way, results from an experiment in Tropical semi-arid Africa (Burkina Faso) showed that the maximum amount of water percolating to 1.5 m soil (16 % of yearly rainfall) is reached close to tree canopies and tend to decrease in open areas (Ilstedt 2016).

Finally, monthly and event-specific increases in rainfall rate could be linked to differences in tropical cyclonic activity in the Caribbean. Although the Caribbean coast of Colombia is not strictly located on the main path of hurricanes that historically have affected the Antilles and the Southeastern coast of the United States, a total of 10 storms have significantly affected the continental portion of the Colombian Caribbean (Ortiz, 2007; Ortiz-Royero, 2012). For example, hurricane Joan caused significant damage to three coastal cities as it passed through the Guajira Peninsula in 1988. It has also been reported that tropical cyclones cause increased precipitation even if they do not hit land or if the eye is more than 150 km away, although more research about the subject is needed (Ortiz- Royero, 2012).

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Conclusions

According to the IPCC, "the latitudinal pattern of change in land precipitation and observed increases in heavy precipitation over the 20th century appear to be consistent with the anticipated response to anthropogenic forcing" (Bindoff et al. 2013). Consequently, it is important not only for global climatic models but also for policy makers to have local, reliable information about climatic conditions, particularly in seasonally dry areas. Although several studies have pointed out that the Caribbean coast of Colombia is subjected to a dry season between January and April, results from the current study actually show that even in the wet season, days with precipitation are followed by several days with no precipitation. The combination of information from both daily and monthly precipitation totals offer evidence of the highly pulse-driven nature of areas such as the "Sabanas". This pulse-driven nature not only imposes a very strict time window for human activities such as non-irrigation agriculture, but it also determines the period of highest activity for the biotic component of the ecosystem. Finally, because of these patterns of precipitation, systems like the "Sabanas" that have been converted from Tropical Dry Forests and heavily used for agriculture since conversion, offer a unique opportunity to explore further a wide array of questions about the relationships between land use change, the structure and functioning of soil microbial communities and climate change.

References

Asner, G.P., Elmore, J.A., Olander, L.P., Martin, R.E., Harris, T. 2004. Grazing systems, ecosystem responses and global change. Annual Review of Environment and Resources. 29, 261-99.

Bindoff, N.L., et al. 2013. Detection and Attribution of Climate Change: from Global to Regional. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Borchert R., Meyer S.A., Felger R.S., Porter-Bolland L. 2004. Environmental control of flowering periodicity in Costa Rican and Mexican tropical dry forests. Global Ecology and Biogeography. 13, 409–425.

Borchert, R., Rivera, G., Hagnauer, W. 2002. Modification of Vegetative Phenology in a Tropical Semi-deciduous Forest by Abnormal Drought and Rain. Biotropica. 34 (1), 27–39.

Bruijnzeel, LA. 1990. Hydrology of MoistForests and the Effects of Conversion: A State of Knowledge Review. Free University, Amsterdam.

Capa-Morocho, M., Rodríguez-Fonseca, B., Ruiz-Ramos, M. 2014. Crop yield as a bioclimatic index of El Niño impact in Europe: Crop forecast implications. Agricultural and Forest Meteorology 42-52.

Castellanos-Castro, C., Newton, A.C. 2015. Environmental heterogeneity influences successional trajectories in Colombian dry tropical forests. Biotropica. 47(6), 660-671.

Cheng, J., Galili, T., Bostock, M., Palmer, J. 2016. d3heatmap. Interactive Maps using 'htmlwidgets' and 'D3.js'. https://CRAN.R-project.org/package=d3heatmap

Córdoba-Machado S., Palomino-Lemus R., Gámiz-Fortis S.R., Castro-Díez Y., Esteban-Parra M.J. 2015. Influence of tropical Pacific SST on seasonal precipitation in Colombia: prediction using El Niño and El Niño Modoki. Climate Dynamics. 44, 1293–1310

Easterling, D. R., Diaz, H. F., Douglas, A. V., Hogg, W. D., Kunkel, K. E., Rogers, J. C., Wilkinson, J. F. 1999. Long-term observations for monitoring extremes in the Americas. Climatic Change. 42(1), 285-308.

Etter, A., McAlpine, C., Possingham, H. 2008. Historical patterns and drivers of

    landscape change in Colombia since 1500: a regionalized spatial approach.

    Annals of the Association of American Geographers. 98(1), 2-23.

Faraway, J. 2005. Linear models with R. Chapman & Hall, Boca Ratón.

Feng, X., Porporato, A., Rodriguez-Iturbe, I. 2013. Changes in rainfall seasonality in the

    tropics. Nature Climate Change. 3, 811-815.

Garreaud, R. D., Vuille, M., Compagnucci, R., Marengo, J. 2009. Present-day south

    american climate. Palaeogeography, Palaeoclimatology, Palaeoecology, 281(3),

    180-195.

Giambelluca, TW. 2002. Hydrology of altered tropical forest. Hydrological Processes. 16,

    1665–1669

Giorgi, F., Fracisco, R. 2001. Uncertainties in the prediction of regional climate change,

    in: Visconti et al. (Eds.), Global and Protected Areas. Kluwer Academic

    Publishers, pp. 127-139.

Grimm A.M., Tedeschi R.G. 2009. ENSO and extreme rainfall events in South America.

    Journal of Climate. 22, 1589–1609.

Gu, G., Zhang, C. 2001. A spectrum analysis of synoptic-scale disturbances in the ITCZ. Journal of Climate. 14, 2725-2739.

Hastenrath, S. 2002. The Intertropical Convergence Zone of the Eastern Pacific revisited. International Journal of Climatology. 22, 347-356.

Hastenrath, S., Lamb. 1977. Climatic Atlas of the Tropical Atlantic and Eastern Pacific Oceans. University of Wisconsin Press, Madison.

Hartmann, D.L., 1994. Global Physical Climatology Academic Press, San Diego.

Hartmann, D.L., Klein, A., Rusticucci, M., Alexander, L.,Brönnimann, S., Rahman, Y.Dentener, Frank., Dlugokencky, E., Easterling, D.R., Kaplan, A., Soden, B.J., Thorne, P., Wild, M., Zhai, P. 2013. Observations: Atmosphere and Surface. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Ilstedt, U. 2016. Intermediate tree cover can maximize groundwater recharge in the seasonally dry tropics. Nature Scientific Reports. 6, 21930.

Instituto de Hidrología, Metereología y Estudios Ambientales. 1993. Precipitation records for the Sabanetica station [13090010] http://institucional.ideam.gov.co/ (accessed, 15.03.11)

Instituto de Hidrología, meteorología y estudios Ambientales (IDEAM). 2010. Estudio Nacional del Agua 2010. Instituto de Hidrología, Meteorología y Estudios Ambientales. Bogotá D.C., pp. 171-227.

Instituto Geográfico Agustín Codazzi (IGAC). 2010. Mapa Geo-pedológico de Colombia. http://geoportal.igac.gov.co/ (accessed, 15.03.11)

Knapp, A.K., Hoover, D.L., Wilcox, K.R., Avolio, M.L., Koerner, S.E., La Pierre, K.J., Loik, M.E., Luo, Y., Sala, O.E., Smith, M. 2015. Characterizing differences in precipitation regimes of extreme wet and dry areas: implications for climate change experiments. Global Change Biology. 21, 2624-2633.

Lawrence, D., Vandecar, K. 2015. Effects of tropical deforestation on climate and agriculture. Nature climate change. 5, 27-36.

Legendre, P., Legendre, L. 1998. Numerical Ecology, second English ed. Elsevier,

Amsterdam.

Lintner, B.R. 2012. Amplification of wet and dry month occurrence over tropical land

regions in response to global warming. Journal of Geophysical Research. 117,

D11106.

Maass, J.M. 1995. Conversion of tropical dry forest to pasture and agriculture. In

Seasonally Dry Tropical Forests, ed. S.H. Bullock, H.A. Mooney, and E.

Medina, 399– 422. Cambridge: Cambridge University Press.

Magaña, V., Amador, J.A., Medina, S., 1999. The midsummer drought over Mexico and

Central America. Journal of Climate. 12, 1577-1588.

Mahmood, R. ,Pielke, R.A.Sr., Hubbard, K.G.,Niyogi,D., Dirmeyer. P.A., McAlpine, C.,

Carleton, A.M., Hale, R., Gameda, S., Beltrán-Przekurat, A., Baker, B.,

McNider, R., Legates, D.R., Shepherd, M., Du,J., Blanken, P.D., Frauenfeld,

O.W., Nair, U.S., Fall, S.. 2014. Land cover changes and their biogeophysical

effects on climate. International Journal of Climatology. 34, 929-953.

Mandujano, S. 2006. Preliminary Evidence of the Importance of ENSO in Modifying

Food Availability for White-tailed Deer in a Mexican Tropical Dry Forest.

Biotropica. 38(5), 695–699.

Marengo, J.A. 2004. Interdecadal variability and trends of rainfall across the Amazon basin. Theoretical and Applied Climatology. 78(1-3), 79-96.

Moreira, M., Sternberg, L., Martinelli, L., Victoria, R., Barbosa, E., Bonates, L., Nepstad, D. 1997. Contribution of transpiration to forest ambient vapour based on isotopic measurements. Global Change Biology. 3(5), 439-450.

Murphy, P.G., Lugo, A.E. 1986. Ecology of Tropical Dry Forest. Annual Review of Ecology and Systematics. 17, 67-88

Noy-Meir, I. 1973. Desert ecosystems: environment and producers. Annual Review of Ecology and Systematics. 25-51.

Ortiz, J.C. 2007. Hurricanes and storms in the Colombian Caribbean since 1900. Scientific Bulletin, CIOH. 25, 54-60

Ortiz-Royero, J.C. 2011. Exposure of the Colombian Caribbean coast, including San Andrés Island, to tropical storms and hurricanes 1900-2010. Natural Hazards. 61, 815-827.

Petrie, M.D., Collins, S.L., Litvak, M.E. 2015. The ecological role of small rainfall events in a desert grassland. Ecohydrology. 8, 1614-1622.

Poveda, G. Waylen, P.R. Pulwarty, R. 2006. Annual and inter-annual variability of the present climate in northern South America and Southern Mesoamerica. Paleogeography. Palaeoclimatology and Palaeoecology. 234, 3-27.

Pulwarty, R.S., Barry, R.G., Riehl. 1992. Annual and seasonal patterns of rainfall variability over Venezuela. Erdkunde. 46, 273-289.

Schwinning, S., O. E. Sala, M. Loik, and J. Ehleringer. 2004. Thresholds, memory, and seasonality: understanding pulse dynamics in arid/semiarid ecosystems. Oecologia. 141, 191-193.

Tedeschi, R.G., Grimm, A.M., Cavalcanti, I. 2015. Influence of Central and East ENSO on extreme events of precipitation in South America during austral spring and summer. International Journal of Climatology. 35(8), 2045-2064.

Ticktin, T. 2003. Relationships between El Niño Southern Oscillation and Demographic Patterns in a Substitute Food for Collared Peccaries in Panama. Biotropica. 35(2), 189–197.

Weltzin et al. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. Bioscience. 53(10), 941-952.

Wolter, K., and M. S. Timlin, 2011. El Niño/Southern Oscillation behaviour since 1871

as diagnosed in an extended multivariate ENSO index (MEI.ext). International Journal of Climatology. 31, 1074-1087.

Yan et al. 2015. Dynamics of the intertropical convergence zone over the Western Pacific during the Little Ice Age. Nature Geoscience. 8, 315-320.

Tables and Figures

Figure 1.1 Location of the Colombian Caribbean as a transitional area between Central America and the northernmost portion of Eastern South America. The enlarged area is showing the location of the Sabanetica hydro-metereological station (Black dot inside the highlighted circle).
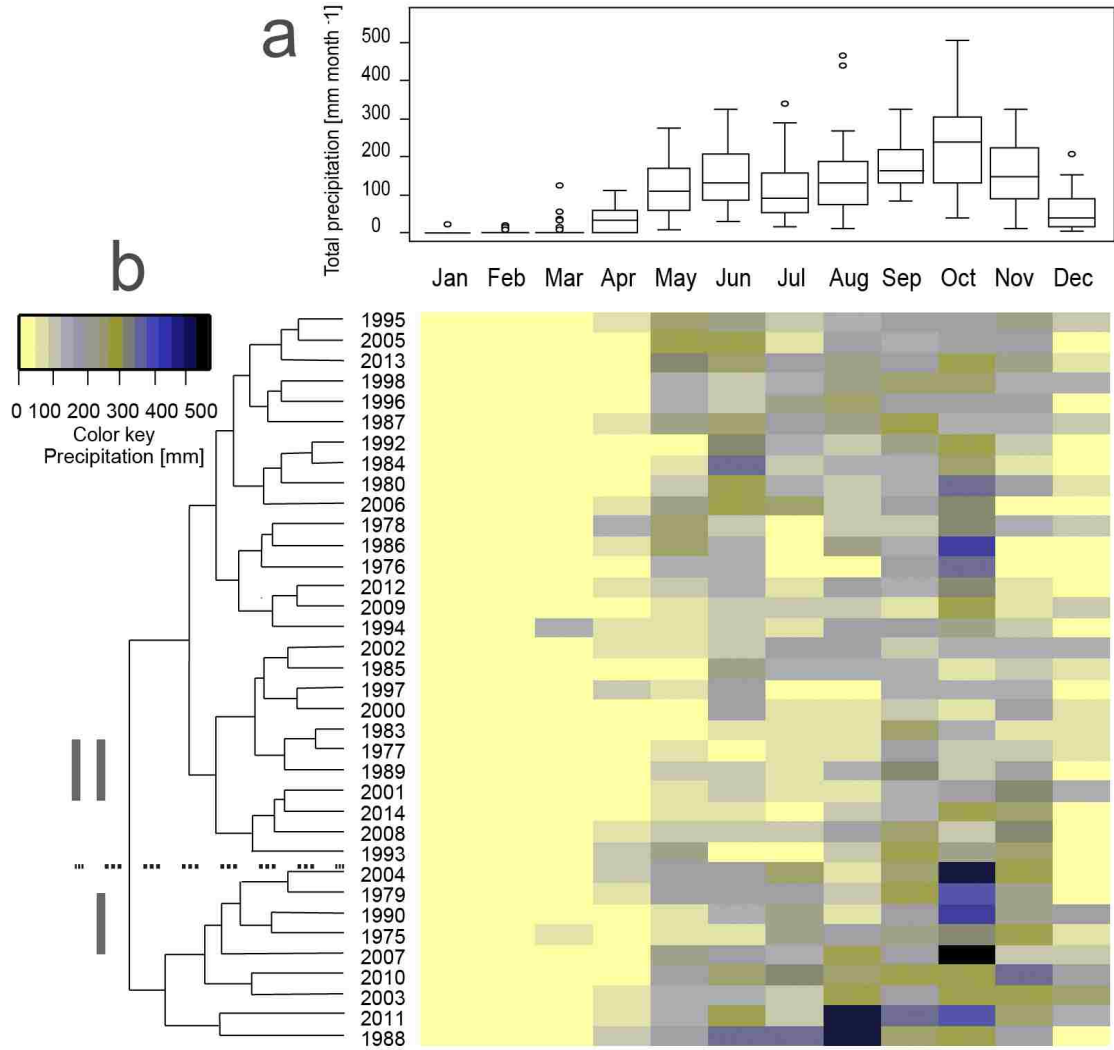
Figure 1.2 (a) Distribution of total monthly precipitation at Sabanetica station in the San Onofre

municipality from 1975 to 2014. The bottom and top of the box represent the 25th and 75th

percentile while the whiskers extend up and down to 1.5 times the interquartile range. (b)

Hierarchical clustering of years based on total monthly precipitation (mm) for the same record.
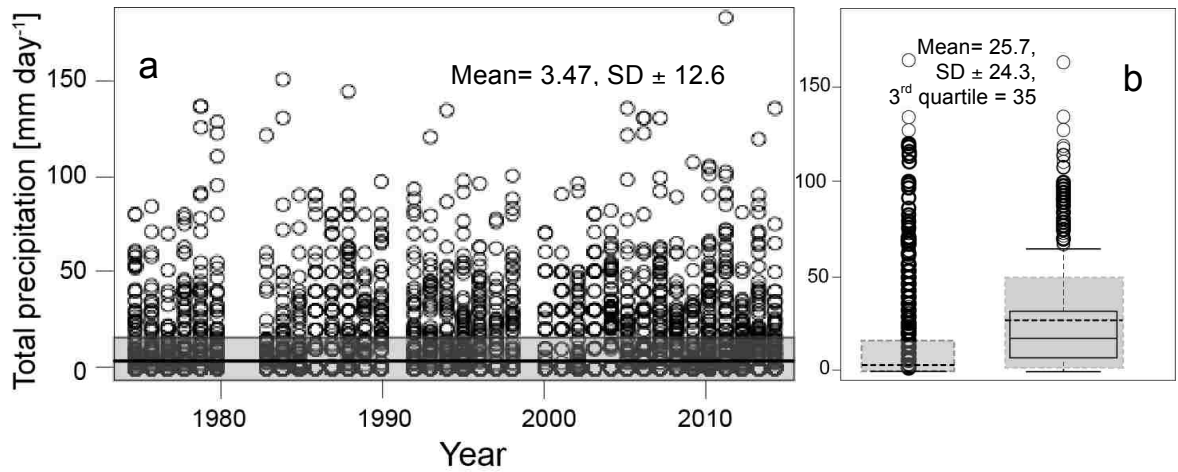
Figure 1.3. Yearly variation of total daily precipitation; (a) Time series including days with zero precipitation. The black line and shaded area indicate the mean value ± 1 SD. (b) Summary of data for the entire time series. The boxplot on the right shows the unbiased distribution when zero values are excluded from the record (boxplot on the left). The dotted black lines and shaded areas indicate the mean value ± 1 SD. Note the differences in central trend and dispersion between the two boxplots. Dots are outliers.
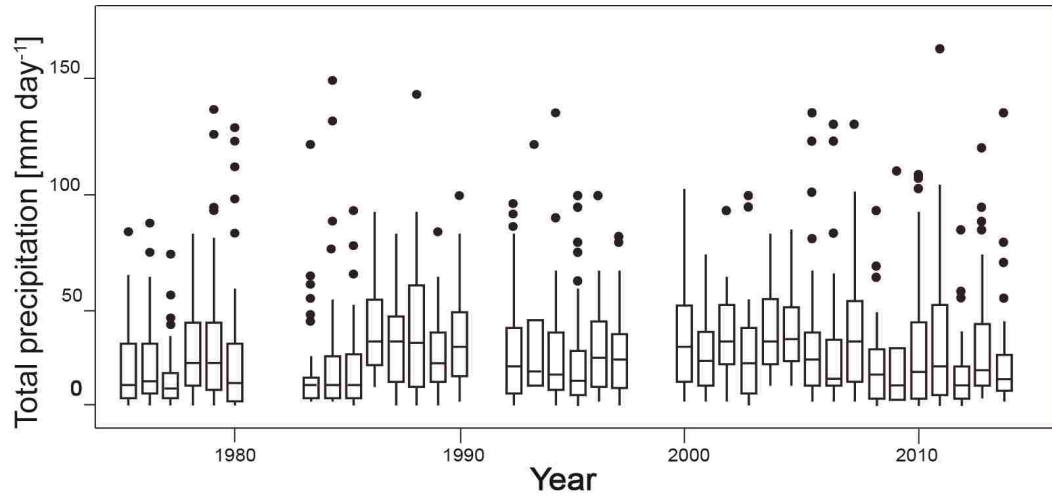
Figure 1.4. Yearly variation of total daily precipitation excluding zeroes. The bottom and top of the box represent the 25th and 75th percentile while the whiskers extend up and down to 1.5 times the interquartile range. Dots are outliers.
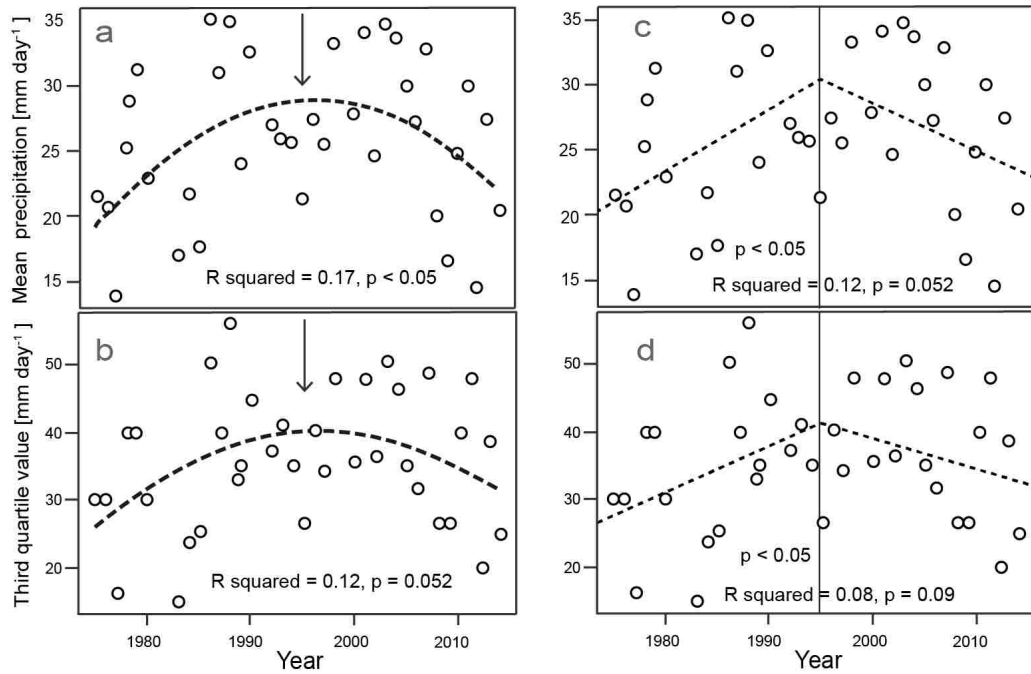
Figure 1.5. Long-term trend of total daily precipitation. (a and b) Polynomial regression using a quadratic term. Arrows indicate years that may be affecting the fit. (a) Model adjusted using mean values. (b) Model adjusted using the third quartile value. (c and d) Broken stick regression using 1995 as knot. Overall adjustment of Broken stick regression to the data structure seems very poor, particularly in the case of the third quartile (d). Yet, in both cases the regression serves its purpose in supporting the idea of an appreciable change in slope around the mid 90's (c and d). Probability values for the ascending portion of the regression are also indicated.
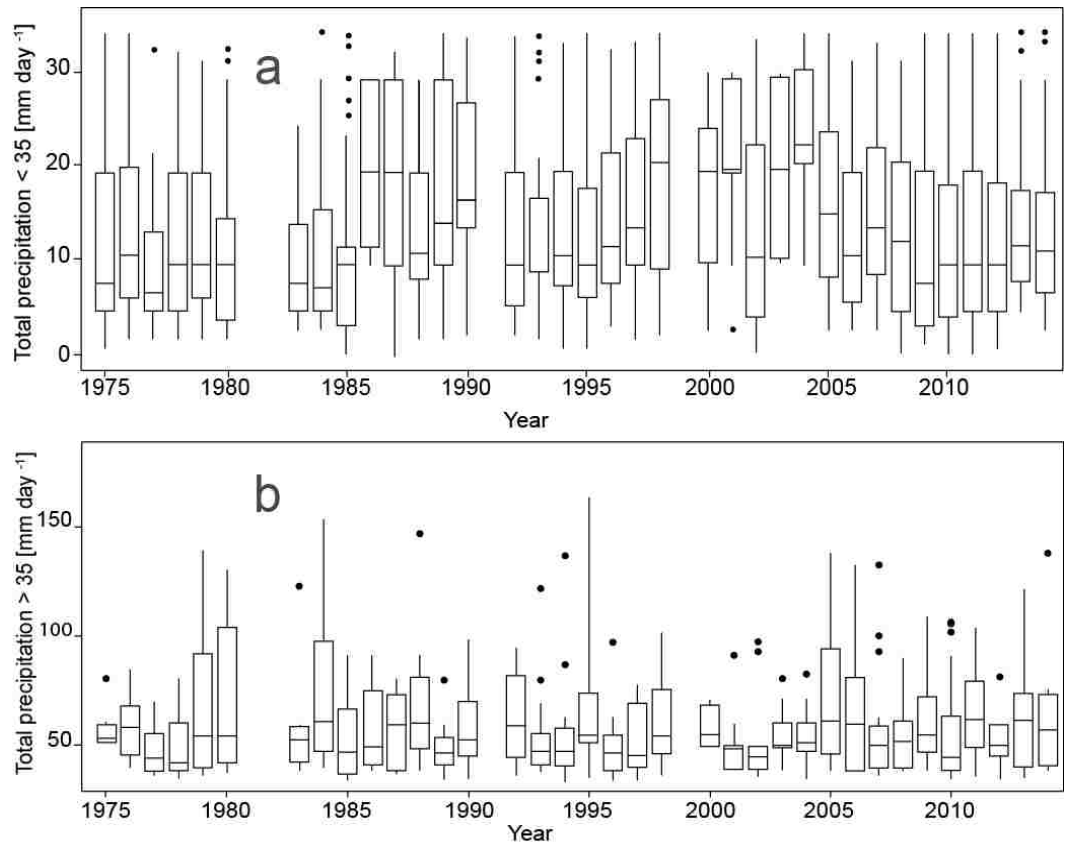
Figure 1.6. Long-term dynamics of total daily precipitation from 1975 to 2014, independently

showing the variation for values a) < 35 and b) >35 [mm day -1].
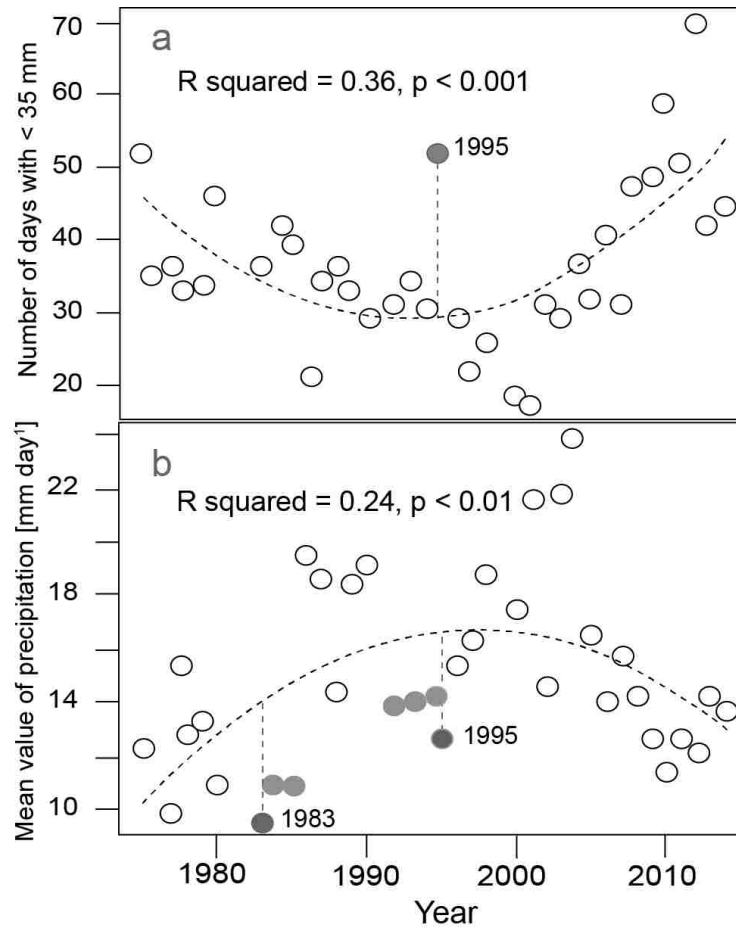
Figure 1.7. Frequency and magnitude dynamics for daily precipitation < 35mm. (a) Number of days < 35 mm. (b) Mean value of days < 35 mm. Very dry conditions predominated in the period 1983-1985 and 1992-1995. Dark grey dots correspond to the greatest deviations consistent with extremes in 1983 and 1995. Light grey dots represent secondary deviations either in previous or posterior years. Fit improves when only days < 35 mm of precipitation are considered in the polynomial model.

Chapter 2

Soil fungal community succession in a seasonally dry savanna in Colombia

(northern South America).

Abstract

In the northwestern part of South America, seasonally dry forests have undergone

extensive conversion to savannas to support ranching and agriculture. The combination of

fire and vegetation removal employed for clearing, together with extensive agricultural

practices, often lead to loss of soil fertility. This results in land that is no longer useful for

agriculture and on which historical forests are unable to regenerate. This study examined

the effects of common agricultural practices on soil fungal communities by simulating the

effects of fire followed by plant removal. The goal was to determine whether common

practices have rapid effects on soil communities, which in turn could signal a decline in

soil quality. The fungal community was estimated by next-generation sequencing

targeting the ribosomal internal transcribed spacer (ITS). Changes in the composition of

the community were monitored over a three-month period. To my knowledge this

represents the first such study in a seasonally dry tropical savanna of the Western portion

of the South American Caribbean. The general fungal community was dominated by

species of Ascomycota and Basidiomycota, many of which had been described previously

from culture studies of tropical soils. Significant community changes occurred over time,

but there were not dramatic differences between removal and non-removal experimental

plots. The community at three months after vegetation cutting or removal differed in

overall diversity from that observed at zero and thirty days. Several fungal taxa were identified as potential indicator species of either early or late sampling periods. Overall, it appears that the decline in soil health observed for sites subjected to long-term agricultural practices are not reflected in dramatic fungal community changes observed for a single season, even in the case of complete above-ground vegetation removal. Additional studies will be required to document the time-course for changes in microbial community composition that accompany such decline.

Introduction

Throughout the Neotropics, Secondary Seasonally Dry Savannas (SSDs) result from the conversion of Seasonally Dry Tropical Forests (SDTF) due to clearing for cattle ranching and crop establishment (Murphy and Lugo, 1986). The consequences of such conversion on soil processes are poorly understood in the Caribbean Region of Colombia (Fajardo et al., 2005; Sánchez-Azofeifa et al., 2005). A large portion of lowland SSDs in this region has appeared since pre-Columbian times (Etter et al., 2008). Currently, the land-cover mosaic that includes SSDs, gallery forests and isolated SDTF forest patches is characterized by a marked rainfall seasonality with three or more dry months every year (precipitation < 100 mm/month), a total annual precipitation between 700 and 2000 mm, a mean annual temperature > 25°C, and the dominance by deciduous trees (Sánchez-Azofeifa et al., 2005).

Conversion of SDTF to pastures and agriculture induces changes in nutrient status and causes increased nutrient loss (Maass, 1995). Nutrient loss can be partially explained by changes in vegetation architecture induced by forest conversion/clearing that affects water and nutrient retention due to increased runoff (Likens et al., 1970). On the other hand, conversion imposes a change in species composition that affects litter quality, decomposition rates and ultimately nutrient turnover rates and soil fertility (Swift et al., 1979; Vitousek et al., 1987; Vitousek and Matson, 1988; Vitousek, 1988). Several studies carried out in SDTF in Mexico provide information regarding the shift in C and N dynamics following forest conversion. For example, García-Oliva et al. (1999) evaluated the effect of forest conversion under the slash and burn practice in the Chamela region

and found a decrease in Total Soil Organic Carbon (TSOC) during forest to pasture transition and also during long term pasture management. Comparison of TSOC content in a 10-year-old pasture and a control forest in the same study showed that the pasture had a 31% lower C concentration than the control forest at a depth of 0 - 2 cm and 28% lower C concentration at 2 - 5 cm. In addition, Ellingson et al. (2000) found that slashing alone dramatically increased the soil mineral N pool in dry areas from ≈10 kg/ha in a reference forest to 57 kg/ha in disturbed sites.

Plant litter chemistry and environmental factors interact to determine the composition and activity of soil microbial biomass. Moorhead and Sinsabaugh (2006) conducted a series of simulations to assess the interactions among microbial activity, litter chemistry and litter decay and concluded that these relationships are dynamic. Their results suggest that the potential litter decay rate increases with microbial biomass, and a high respiration coefficient limits the size of the microbial pool. In contrast, a lower respiration coefficient allows microorganisms to colonize fresh litter during the early stages of litter decay. This study also found that the response of different guilds of microorganisms to nitrogen enrichment varied depending on the type of litter compound each guild consumes (intermediate metabolites and soluble polymers, cellulose and lignocellulose, and humidified organic matter).

Saynes et al. (2005) measured soil C and N pools as well as potential transformations of N to determine how seasonal rainfall affected microbial biomass in seasonally dry primary and secondary forests in Central Mexico. They found that microbial N pools of all forests were largest during the dry season regardless of

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successional stage, reflecting nutrient immobilization during the period of no rain. On the contrary, both soil NO_3 and NH_4 content, as well as potential N mineralization and nitrification rates, were different depending on forest type, which in turn was related to the differential abundance of leguminous vegetation producing litter with a lower C:N ratio. Similar results were found by Marrs et al. (1991) in a seasonally dry forest-savanna transition in Northern Brazil, where the more deciduous portion of the forest showed the highest mineralization and nitrification rates during the onset of the rainy season, contrary to the savanna, where values for the same processes declined substantially regardless of precipitation amount.

Fungi are key components of the Soil Organic Matter (SOM) decomposer community. Several studies indicate that they are particularly relevant under water deficit conditions due to their extended survival rates when compared with bacteria. High resistance against harsh environments (particularly UV radiation) is conferred by the presence of complex polymeric melanin pigments synthesized from phenolic or indolic monomers (Butler and Day, 1998). These substances are highly stable, insoluble and resistant, and they have been associated with increased virulence in pathogenic fungi Jacobson, 2000) and protection against hydrolytic attack from antagonists (Bell and Wheeler, 1986). In seasonally dry areas, the expected shift in soil microbial composition during the rainless period from bacterial to fungal dominated, may also cause a shift in mineralization rates due to different N-use efficiency. Fungi produce biomass with a higher C:N ratio than bacteria (Paustian and Schnürer, 1987a, 1987b) and thus immobilize less N per unit of C assimilated, which in turn may reduce rates of net N mineralization, as observed in many ecosystems during dry seasons (Austin et al., 2004).

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The interaction between SOM and fungi goes beyond decomposition since hyphal growth promotes the formation of macro aggregates, and fungal exudates help in the stabilization of such aggregates (Six et al., 2002). Glomalin, a recalcitrant glycoprotein produced by arbuscular mycorrhizal fungi (AMF), is positively correlated with soil aggregate stability (Wright and Upadhyaya, 1998), and it is also a significant component of total soil C and N pools in soils of tropical forests (Lovelock et al., 2004). Dead fungal material (i.e recalcitrant cell wall components) accumulates during decomposition and becomes part of the SOM pool (Pausian and Schnürer, 1987), which in turn could help to explain the increase in polyphenolic compounds during the early stages of litter decomposition (Aber et al., 1990; Moorhead and Sinsabaugh, 2000, 2006)

From a land management point of view, soil fungal populations have also proven to be sensitive to human induced disturbance, and this constitutes a powerful tool to detect the consequences of land conversion (Acosta-Martinez et al., 2010). Vallejo et al. (2012), who analyzed the effects of three land management systems in South Western Colombia [Conventional Pastures (CP), Intensive Silvopastoral Systems (ISS) and Seasonally Dry Forest (SDF)] on microbial biomass, found that the fungal:bacterial biomass ratio and abundance of fungal biomarkers (total and AMF) were higher in SDF and ISS than in CP. This was attributed to monoculture of the native grass *Cynodon plectostachyus* and disruption of fungal hyphae by agricultural machinery and overgrazing in CP.

Recent evidence from temperate semi-arid regions supports the idea that fungal networks strongly influence the cycling of nutritional resources by integrating islands of

fertility and promoting water movement (Herrera et al., 2011; Green et al., 2008). This is contrary to mesic areas where soil temperature and humidity (Raich and Schlesinger, 1992) favor a different pathway in which there is significant formation of SOM mediated by decomposers. To my knowledge, there are no studies that have explicitly addressed the effects of land management and forest conversion on fungal communities in SDS, where high soil temperatures and long periods of low soil moisture content might favor a pulse regulated system where fungal communities could play a relevant role.

In this study I investigated the effects of land use on fungal community dynamics of secondary savannas in the Colombian Caribbean. I specifically aimed to explore the composition of fungal communities during land abandonment after exposure to fire and above ground biomass removal. For this, I designed a repeated measures experiment that resembled the traditional practices of landowners in this region, by establishing 36 (2 m^2) plots in May of 2014 on private land that had been submitted to fire earlier that same year. The plots were fenced and left to rest until the start of the rainy season when the above ground vegetation was trimmed and removed from 18 plots. The fungal communities from removal and non-removal plots were then compared during the wet and wet-dry transition periods. Fungal communities were characterized using a metabarcoding approach (454 pyrosequencing of the ribosomal ITS region). The main objectives were to 1) test if there was a significant deviation from the original composition of the fungal community after removal of the vegetation, and 2) to characterize fungal community changes across seasons. My study describes, for the first time, an inventory of fungal communities associated with soils of seasonally dry savannas of the Caribbean coast of Colombia. In this study, I found that time and not treatment was

the main factor controlling community composition.

Materials and Methods

Soil sampling

The soil samples used in this study were obtained from "El Bajo" experimental pastures (9°51'10.41" N; 75°25'29.54"W), with an altitude of **50** meters above sea level. Average annual temperature at this savanna is 27°C and average relative humidity is 83.5% (Figure 2.1). Local geology is characterized by the presence of sedimentary rocks from Continental and Transitional environments (quartz arenite sands to conglomerate intercalated with limestone); soils are predominantly Entisols and Alfisols. In general, the soils in this area may be defined as hyperthermic, moderately fertile, and highly susceptible to erosion with a pH ranging between 7.5 and 6 (IGAC, 2010). Pastures were created by conversion of tropical dry forest and have been continuously used for semi-extensive cattle ranching and agriculture for the last 30 years. The pastures were submitted to burning every year for at least 15 years prior to this study.

In these pastures, 36 plots (2 m^2) were established in 2014, at the start of the growing season, three months after the land was submitted to annual burning. By this time, spontaneous regrowth appeared and runoff had cleared the excess of ashes and burned material. Vegetation was allowed to grow for 100 days and was then trimmed in late August before the peak of the rainy season. By that time, a mix of mostly native grasses and shrubs approximately one meter tall covered the plots. All plant material on

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each plot was trimmed at ground level. The plant material was removed immediately

after trimming from 18 randomly selected plots (R = removal treatment), but it was

returned to the remaining plots and left on the ground after being weighed and

fractionated in situ (NR = non-removal treatment). The NR plots were covered with

plastic mesh (pore size 3 mm) to prevent losses due to water runoff or wind action and

were left undisturbed for the rest of the experiment. All NR plots received between 7 and

9 kg of biomass. The above manipulation recreates actual land management practices

where on one hand there is vegetation removed by cattle grazing or post harvest removal,

while on the other, plant residues are abandoned in the field.

The sample regime consisted of collecting soil samples at three times during 2014:

1) immediately before trimming the vegetation (T0, time zero) on August 28, 2) one

month after trimming, in the second half of the rainy season on September 26 (T1, 30

days), and 3) after three months, near the beginning of the dry season on December 27

(T2, 90 days) (Figures 2.2 and 2.3). Each plot was divided into ten 20x50 cm sections,

and two core samples (2.5 cm diameter $\times$ 5 depth cm depth) were collected from the

central point of each of five sections chosen randomly. Half the cores were used for

molecular analysis and the other half for soil chemical analyses. The former were kept at -

20 ºC until processed whereas the latter were kept refrigerated at 4 ºC.

Environmental variables

Soil nitrogen and soil organic matter (SOM) were measured to evaluate the effect

of the treatments on the soil nutrient pool. Ammonium N was determined by Flow

Injection Analysis (Maynard and Kalra, 1993), and Nitrate N was measured in soil water extracts after reaction with phenoldisulphonic acid (Maynard and Kalra, 1993). SOM content was determined by mass loss on ignition (LOI). For the latter procedure, samples were air dried for 24 h to eliminate excess moisture, then roots were removed, and root-free soil samples were oven-dried at 105 ºC for 24 h. Five grams of soil were placed in porcelain crucibles and heated overnight in a furnace muffle (Fisher Scientific, Philadelphia Pennsylvania, USA) at 375 ºC. Samples were removed from the furnace, placed in a desiccator and weighted. Organic matter content was measured as the difference between pre and post ignition mass. Soil water content was measured gravimetrically after drying samples overnight at 105ºC. Soil pH was measured in a 1:1 mixture of soil:water using an YSI pH meter.

The differences in the levels of the factors for all environmental variables were analyzed using a complete mixed model ANOVA in two ways using 36 samples: first in a crossed design where the effects of treatment was tested independently of sampling time, and second with the factor treatment nested in each sampling time

DNA extraction, PCR amplification and pyrosequencing

Approximately 1 cm^3 of root-free soil was taken from each of the five subsamples and pooled to obtain a representative sample for each plot. Soil DNA was extracted using the Norgen Soil DNA isolation kit (NORGEN Biotek Corp. Thorold, ON, Canada) following the manufacturer´s instructions. Quality of DNA samples was assessed with the aid of a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Fungal PCR and next-generation sequencing were performed targeting the ribosomal RNA internal transcribed spacer (ITS) using primers ITS4 and ITS1F (Gardes and Bruns, 1993).

PCR amplification employed a 30-cycle procedure using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA). The thermal cycling program was: 94 ºC for 3 minutes, followed by 28 cycles of 94 ºC for 30 seconds; 53 ºC for 40 seconds and 72 ºC for 1 minute; after which a final elongation step at 72 ºC for 5 minutes was performed. Following PCR, all amplicon products from different samples were mixed in equimolar concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Samples were sequenced using a Roche Sequencer GS FLX Titanium series (454 Life Sciences, Brandford, CT) at a private facility (Molecular Research LP, Shallowater, Texas, USA), following manufacturer's guidelines.

Pyrosequencing data analysis

Raw fungal sequences were processed using an UPARSE-OTU analysis pipeline for 454 reads (Edgar, 2013). In summary, sequencing errors were reduced using the expected error filtering method included in USEARCH v.8.1 (Edgar and Flyvbjerg, 2015). Low quality sequences were removed by allowing a minimum length of 250 base pairs (bp) and discarding reads with expected error > 0.5. Reads (already trimmed and dereplicated) were clustered into operational taxonomic units (OTUs) at a 3% dissimilarity distance using the UPARSE-OTU algorithm. Chimeric OTUs were detected and discarded.

Taxonomy was assigned to each OTU by using the RDP Warcup Fungal ITS

training set 2 included in the Ribosomal Data Project (RDP) classifier tool (Cole et al.,

2014) at a 50% bootstrap confidence level. Data were pre-processed before downstream

analysis by excluding outlying samples and filtering noisy variables, i.e. non-abundant

and spurious OTUs. To do this, total abundance data were converted to relative

abundances and OTUs below the technical reproducibility threshold of 0.005% were

eliminated. Further filtering was done by dividing OTUs into persistent (core) and

occasional (satellite) OTUs by using the method reported by Magurran and Henderson

(2003). According to this procedure, the discontinuity observed in the persistence-

abundance plot sets a threshold below which OTUs are classified as occasional. Data

were further transformed by standardizing the relative abundance of each OTU to the

median sequencing depth. I used the R extension Phyloseq (McMurdie and Holmes,

2013) for data transforming and filtering, and for other analyses.

To determine if there were differences in the community richness and diversity

among treatments, a set of diversity metrics was calculated. Calculation of such metrics

was done using the original samples including singletons, since the estimators Chao1

(Chao, 1984; Gotelli and Colwell, 2011), and the Shannon-Weaver diversity index rely on

the presence of rare species (OTUs). The Calculation of these indices was followed by an

Analysis of Covariance (ANCOVA) to evaluate if there were significant differences

across treatments using time as a covariate.

The taxonomic identity of the 71 most abundant taxa was confirmed using the

BLAST tool after exploration of the rank-abundance curves. The differences in fungal

composition of samples were graphically assessed using Nonmetric Multidimensional

Scaling (NMDS) (Legendre and Legendre, 2012). This method is particularly

advantageous since it is not limited to Euclidean distance matrices and can better

summarize distances in fewer dimensions than other similar ordination methods. The

graphical results obtained from NMDS were validated using a permutational multivariate

ANOVA (PERMANOVA). This analysis was followed by an Indicator Species Analysis

with the purpose to evaluate the strength of association of indicator species with groups of

sites (sites groups). Indicator species are defined as those that occur in specific habitat

types or at specific time points, and are therefore considered as better ecological

indicators of environmental changes than generalist species (De Cáceres and Legendre,

2009). Dufrêne and Legendre (1997) developed the IndVal index to measure the

association between a species and a site group; this index is the result of the combination

of two components "A" and "B". "A" is called the *specificity* or *positive predictive value*

of the species and is related to the probability of a site belonging to a target site group

(group of interest). The second component "B" is called the *fidelity* or *sensitivity* of the

species and expresses the sensitivity of the species as an indicator. Strictly speaking "B"

is the probability of finding the species in sites that belong to the site group.

The analysis was carried out using the R package indicspecies ver. 1.7.1 (De

Cáceres, 2013). Attention was paid not only to the indicator species of individual site

groups but also to combinations of site groups (De Cáceres et al., 2010). In summary, this

analysis combines the input clusters and compares each of the resulting combinations

with the species in the input matrix. The combination with the highest association value

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for each species is tested for statistical significance using a permutational test. A significance level of 0.05 and 999 permutations were used for all the analyses.

Results

Community attributes

In general, the ANCOVA did not show any significant differences in richness or diversity between treatments. Only marginal differences were found for the Chao1 values across sampling dates ($F_{(2, 20)} = 3.35$, $p = 0.055$). However, a displacement of the entire distribution of data can be observed among sampling dates (Figure 2.4). In the case of species richness ("Observed") and Chao1, the distribution moves from higher to lower values, whereas Shannon´s diversity shows the opposite behavior. Richness values were found to fluctuate between 135 and 90 species at the beginning of the study and between 114 and 63 species 120 days later.

The resulting pattern from the rank abundance curve indicates a highly diverse environment with a few dominant species and a long "tail" that corresponds to rare OTUs (Figure 2.5a). The threshold between abundant (i.e. "persistent" or "core"), and rare (i.e. "satellite") species was set at 12 samples according to the prevalence diagram (Figure 2.5b); further analysis of the shape of the two resulting distributions corroborated the validity of such a grouping. The group of abundant species comprised 70 OTUs distributed among 19 orders, 17 families and 45 genera according to the best BLAST hit (Table 2.1).

The fungal composition of samples was quite constant between treatments and with time, although there was a large gap between the most and less abundant orders (Figure 2.6). The orders Pleosporales, Sordariales, Hypocreales and Eurotiales were the most abundant across all samples, containing 33.4 %, 19%, 12.9%, and 12.9% of all OTUs respectively. The remaining orders did not surpass 6% of abundance, and most of them stayed below 2%. The rank order of taxa presented in this section differs somewhat from the order presented in Table 2.1, because the results presented in Table 2.1 represent sequence counts obtained before the final filtering used for the analysis presented in Figure 2.6.

The most abundant families were Chaetomiaceaceae (16.4%), Nectriaceae (15.7%), Lasiosphaeriaceae (14.5%) and Trichocomaceae (12.5%). The most abundant genera were *Fusarium* (15.2%), *Podospora* (9.5%), *Penicillium* (7.2%) and *Curvularia* (5.5%), *Chaetomium* (8.8%), *Humicola* (7.6%) and *Zygopleurage* (5.1%).

Environmental variables

As expected, at the beginning of the study there were no statistically significant differences among treatments with respect to environmental variables (Figure 2.7). Moreover, SOM and soil pH did not show statistically significant temporal trends or response to treatment. The content of SOM varied between 4.1 and 9.1 percent, and pH varied between 6.7 and 5.

Among the environmental variables examined, only NO_3 and NH_4 were affected by treatment, with differences in both cases noticeable after 30 days but not 90 days. After 30 days, the mean value of NH_4 was significantly higher under the non-removal treatment than under removal (39 vs 21 mg/kg dry mass, respectively; $F_{(1,10)} = 4.59$, $p<0.05$), whereas the concentration values for NO_3 showed the inverse pattern (7 vs 25.5 mg/kg dry mass; $F_{(1,10)} = 6.85$, $p<0.05$).

With both treatments NH_4 was significantly lower after 90 days relative to the beginning of the experimental period ($F_{(1,22)}= 32.06$, $p<0.0001$) (Figure 2.7). Although NO_3 in the removal plots was noticeably higher at 30 days, the difference with respect to other time points was not statistically significant at the 0.05 level.

Community dynamics

The two dimensional representation resulting from NMDS analysis clearly separated three different groups corresponding to sampling dates in the second dimension (Figure 2.8a). This separation was particularly strong with respect to sampling at 90 days. PERMANOVA analysis supported this arrangement and shows that most of the variation can be attributed to the time after treatment when comparisons are made either within each treatment or across all treatments ($F_{(1,22)} = 2.507$; $p < 0.001$ and $F_{(1,20)} = 2.43$; $p < 0.001$, respectively). Although the entire set of environmental (ecosystem) variables covered in the previous section was fit into the ordination, none of the variables were statiscally significant after a permutation test.

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The indicator value analysis using combinations of site groups (Table 2.2) showed that five species can be considered good indicators of the combination of ordination groups A and B (Figure 2.9). Of those, *Massarina* sp. was strongly and significantly associated with this combined group ($p < 0.001$), although this species did not appear in all the sites (samples) in this group, nor was it exclusive to it (specificity = 0.9565, fidelity = 0.9167). Another good indicator species is *Lasiodiplodia parva,* since it appeared in all the sites of this combined ordination group, altough it is not exclusive to this group (specificity = 0.8862, fidelity = 1.000). Visual inspection of the distribution of abundances between treatments and across sampling dates (Figure 2.9) suggested a steady decrease in the abundance of *Podospora fimiseda*, whereas the abundance of *Didymella vitalbina* was lower at 0 and 90 days, but the trends were not statiscally significant. *Zygopleurage zygospora* and an unknown fungus were good indicators of group C (Table 2.2) ($p < 0.05$ and $p < 0.001$, respectively), although only the second occurs in all group C (90 days) samples (fidelity = 1.000), and none appears only in that group. In this case, the abundance of both species increased 90 days after treatment regardless of treatment ($F_{(1,14)} = 0.441$, $p < 0.05$; $F_{(1,14)} = 8.0213$, $p < 0.05$, respectively) (Figure 2.9).

A different approach to consider the species that were generally more abundant in each ordination group showed that *Westerdykella ornata* and *Candida gosingica* were more abundant 90 days after treatment. The first one was significantly more abundant regardless of treatment ($F_{(1,14)} = 11.43$, $p<0.05$) (Figure 2.9, Table 2.1), whereas the second seemed to respond to treatment, since it was significantly more abundant under the removal condition ($F_{(1,14)} = 5.96$, $p<0.05$) (Figure 2.9).

Discussion

Diversity values

Soil microbiome studies offer the opportunity to characterize unknown or untargeted diversity that can help in better understanding the effect of transient or long-term disturbances on soil processes such as decomposition, plant-microbe interactions and microbial succession (Barreiro et al., 2016; Soares et al., 2016). In this study, the abundance (richness) and diversity of fungal species was not affected by the removal of biomass, but rather both varied with time. Species abundance declined with time, whereas the distribution of diversity values increased during the first 30 days and the variance of values increased substantially by 90 days after biomass removal, suggesting that other factors overrode the removal treatment. This finding agrees with results obtained by McGuire and colleagues (2012) who found that increased precipitation and not increased species richness or soil nutrient status was positively correlated with fungal diversity in tropical soils. Similar results were obtained by Pereira de Castro et al. (2016) in highly seasonal tropical savannas of Brazil (*Cerrado*).

Changes in abundance and diversity with time were likely due in part to periods of rainfall, which varied substantially during the course of the experiment. Most of the experimental period coincided with the rainy season, with the dry season beginning approximately 30 days prior to the final sampling (Figure 2.3). Soil moisture was highest at the 30-day sampling period but did not vary substantially during the course of the experiment. Even during the growing season, the number of dry days was quite high

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(more that 15 days per month), and the days with high precipitation were few (only eight days with more than 35 mm/day for the duration of the study between May and December, see Chapter 1 for more details). This is a pattern typical of the seasonally dry savannas.

The second sampling (at 30 days) occurred after 10 non-consecutive days with low precipitation. In contrast, there were only 11 dry days in the 61 days between the second and third sampling dates. This is potentially important given that even small rainfall events (< 3.8 mm) can influence soil nutrient availability and ANPP responses in desert grasslands in the Northern Chihuahuan desert (Petrie et al., 2015). It was 30 days after treatment application, during the wettest period, that NO_3^- and NH_4^+ showed statistically significant differences (but opposite trends), between treatments (Figure 2.7).

The decrease in the total number of species and the high variation in diversity values 90 days after treatment might have resulted from a combination of factors that included an increase in rhizodeposition and the resulting microbial interactions, as roots were not removed and vegetation was allowed to resprout. Several studies have found that SOM mineralization rates are higher under living plants when compared to bare controls, because the labile pool of organic C contained in root exudates, mucilage and sloughed-off border cells stimulates soil microbial growth (Fontaine et al., 2003; Nguyen, 2009; Shahzad et al., 2015). Exuded C is used preferentially for the synthesis and release of extracellular enzymes that mineralize SOM. This in turn stimulates the presence of the so-called *k-strategists* (organisms that can degrade SOM) such as saprotrophic fungi in the presence of root litter (Shahzad et al., 2015). In addition, some studies have suggested

that the cellulolytic activity of fungi is actually carried out by two different groups, one consuming fresh C and another mining SOM (Fontaine et al., 2003; Fontaine et al., 2011; Moorhead and Sinsabaugh, 2006). The observed decrease in species richness could have been caused in part by a decrease in the abundance of SOM decomposers as vegetation recovered, while at the same time diversity increased as a more diverse array of C sources became available.

Fungistasis, defined as the inhibition of spore germination or hyphal growth, constitutes another factor affecting the abundance and diversity of fungal groups. It is plausible that as vegetation recovered and microbial groups became more abundant and diverse, certain bacteria inhibited fungal growth, as has been observed for species of *Bacillus* and *Pseudomonas* (Garbeva et al., 2006, 2011).

Finally, it has been suggested that soil microbial communities inhabiting highly fluctuating environments become relatively resistant and resilient to such fluctuations. The proposed explanation is that after several cycles of disturbance the communities evolve to inhabit and persist in this kind of habitat and therefore land use has a disproportionally stronger effect on community composition than elevation or precipitation regime (Ng, 2015).

In this study, the effects of the removal treatment could have been more dramatic if the roots had been removed, but at the same time my results suggest that leaving the root biomass could be enough to maintain soil fungal diversity in the short term. Furthermore, this implies that under certain circumstances moderate grazing and seasonal

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crops could be sustainable as long as fire is not applied or crop harvest does not involve the removal of roots, as is the practice for cassava or rice crops.

Community dynamics

This study was the first to employ next-generation sequence analysis toward the goal of characterizing soil fungal communities in a seasonally dry tropical ecosystem. Among the fungal sequences obtained, some were derived from genera or species that are nearly cosmopolitan and that are not necessarily associated with tropical ecosystems, while others have been reported primarily or exclusively from the tropics. The following section examines the taxa associated with the most abundant OTUs as well as those that were identified as potential indicator species by the Indicator Species Analysis (De Cáceres and Legendre, 2009).

Ascomycota and Basidiomycota dominated the fungal communities as has been reported previously for a wide variety of habitats, including culture studies of fungi in seasonally dry Neotropical savannas (Pereira de Castro, 2016). *Fusarium* (Hypocreales) was the most abundant genus (Table 2.1) in the present study. Members of this genus include common and important plant pathogens (including *F. oxysporum* and *F. solani*, both present here) as well as species considered to be saprotrophs that degrade lignin (Sutherland et al., 1983) and other macromolecules characteristic of litter and plant debris (Starke at al., 2016, España et al., 2011).

Second in abundance to sequences that matched *Fusarium*, were sequences from well-represented genera in the Sordariales, including*Chaetomium, Sordaria,*

Zygopleurage and *Podospora*, genera which include well-known saprotrophs including coprophilous fungi. In fact, among fungal orders the Sordariales dominated in terms of sequence numbers (Figure 2.6, Table 2.1).

The most abundant sequences assigned to a single OTU from the Basidiomycota hit *Macrolepiota fuliginosa* in BLAST searches. Species in the genus *Macrolepiota* ("parasol mushrooms") constitute a widely-distributed group of saprotrophs (Vellinga et al., 2003).

NMDS analysis (Figure 2.8) supported the conclusion that time more than treatment drove the abundance and diversity patterns of fungal taxa. After 90 days the fungal community deviated significantly from the pattern exhibited at 0 and 30days. The indicator species analysis showed that five species were good indicators for the two early sampling periods: *Massarina* sp. *Lasidioplodia parva*, *Podospora fimiseda*, *Didimella vitalbina* and *Cochliobolus lunatus,* whereas *Zygopleurage zygospora* and an unknown fungus were the best indicators for the final sampling period (90 days).

Massarina has been classified as a cosmopolitan genus that is found in marine (Hyde, 1989), freshwater and terrestrial habitats (Wong and Hyde, 2001). In tropical terrestrial habitats, *Massarina* has been identified as a decomposer of wood (Kodsueb et al., 2016) and grass material (Wong and Hyde, 2001).

Lasiodiplodia parva, has been reported from the eastern "Llanos" of Colombia (natural savannas) in cassava field soils and also on *Theobroma cacao* (Alves et al.,

2008). A closely realated species, *L. theobromae,* is common in the tropics and sub-tropics where it has been reported to be an endophyte and plant pathogen (Wenham, 1995; Alves et al., 2008). As an endophyte, its hosts include the mangrove associated coastal swamp species, *Barringtonia racemosa* (Osorio et al., 2016).

The genus *Didymella* is in the Didymellaceae family (De Gruyter et al., 2009), which contains several plant endophytic, pathogenic and saprobic fungi associated with diverse hosts. *D. vitalbina* is a pathogen of plants in the genus *Clematis* (probably introduced) (Woudenberg et al., 2012), which is present in Colombia as a pioneer in dry forests.

Species in the genus *Cochliobolus* are common as endophytes of several herbaceous plants present in semi-arid grasslands of Northern Venezuela, where *C. lunatus* specifically has been identified as an endophyte of the herb *Cenchrus echinatus* (Loro et al., 2012). *C. lunatus* is also known to be an aggressive pathogen of a wide range of economically important plants worldwide, including rice (*Oryza sativa*), maize (*Zea mays*) and potato (*Solanum tuberosum*) (Louis et al., 2015).

Z. zygospora, identified as an indicator species for the final sampling period (90 days), is a coprophilous fungus on herbivore dung (Jones, 2006). This species possesses ascospores with mucilaginous appendages that aid in attachment to vegetation. It is interesting that its abundance increased dramatically during the last part of the study (Table 2.1, Figure 2.9). Although large herbivores were excluded from study plots, smaller herbivores such as rabbits would have had access. It is therefore possible that the

increase in abundance of sequences from this species represents normal succession on herbivore droppings.

A separate analysis (Figure 2.9) suggested that two additional species, *Westerdykella ornata* and *Candida gosingica*, while not identified as indicator species in the Indicator Species Analysis, showed an increase in abundance with time, being most abundant 90 days after treatment. *C. gosingica* is a recently-described species (Chang et al., 2011) identified during research on the yeast community in soils in Taiwan and Thailand. Member of the genus *Candida* were found by Soares et al. (2016) as endophytes of sugarcane stalks in Brazil, where it presented the highest relative abundance of all the genera in the stalks fungal community.

W. ornata, sequences of which were abundant at 90 days (Table 2.1), was first isolated from mangrove mud (Stolk 1955). Although members of the genus *Westerdykella* are found worldwide in diverse substrates including mud, dung and plant material (Ebead et al., 2012), the family to which it belongs, the Sporomiaceae, is recognized as the largest coprophilous family of Pleosporales (Zhang et al., 2009). As with *Z. zygospora*, its presence at 90 days could therefore represent succession on herbivore droppings deposited by small mammals.

Conclusions

This work showed that the soils from secondary seasonally dry savannas of the Colombian Caribbean support a high diversity of fungal species. The application of the

method proposed by Magurran and Henderson (2003) proved to be a powerful tool since the separation of species into persistent (core) and occasional (satellite) added more significance to downstream analyses. The use of the persistent species showed that time after treatment and not treatment alone affected the species composition of the community. The effects of time appeared to be complex and related to a combination of factors derived of the interaction between precipitation and the type of carbon sources available during the decomposition process. Further analysis of the species composition revealed that only a few species of all the 71 comprising the core could be considered as indicators of the community at different time points, which in turn supports the hypothesis of a highly resistant and resilient fungal community inhabiting highly perturbed soils at this savanna. This study represents one of only a few that attempts to characterize the soil fungal community composition in relation to agricultural practices, but the lack of an immediate, strong response from the fungal community calls for more detailed studies tracking the effects of one source of disturbance at a time.

References

Aber, J., Melillo, and McClaugherty. 1990. Predicting long-term patterns of mass-loss, nitrogen dynamics, and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. Canadian Journal of Botany-Revue Canadienne De Botanique **68**:2201-2208.

Acosta-Martinez, V., C. Bell, B. Morris, J. Zak, and V. Allen. 2010. Long-term soil microbial community and enzyme activity responses to an integrated cropping-livestock system in a semi-arid region. Agriculture Ecosystems & Environment **137**:231-240.

Alves, A., P. Crous, A. Correia, and A. Phillips. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Diversity **28**:1-13.

Austin, A., L. Yahdjian, J. Stark, J. Belnap, A. Porporato, U. Norton, D. Ravetta, and S. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia **141**:221-235.

Barreiro, A., E. Baath, and M. Diaz-Ravina. 2016. Bacterial and fungal growth in burnt acid soils amended with different high C/N mulch materials. Soil Biology & Biochemistry **97**:102-111.

Bell, A., and M. Wheeler. 1986. Biosynthesis and fuctions of fungal melanins. Annual Review of Phytopathology **24**:411-451.

Butler, M., and A. Day. 1998. Fungal melanins: a review. Canadian Journal of Microbiology **44**:1115-1136.

Chang, C., C. Yao, S. Young, S. Limtong, R. Kaewwichian, N. Srisuk, and C. Lee. 2011. *Candida gosingica* sp. nov., an anamorphic ascomycetous yeast closely related to *Scheffersomyces spartinae*. International Journal of Systematic and Evolutionary Microbiology **61**:690-694.

Chao, A. 1984. Nonparametric-estimation of the number of classes in a population. Scandinavian Journal of Statistics **11**:265-270.

Cole, J., Q. Wang, J. Fish, B. Chai, D. McGarrell, Y. Sun, C. Brown, A. Porras-Alfaro, C. Kuske, and J. Tiedje. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Research **42**:D633-D642.

De Caceres, M., and P. Legendre. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology **90**:3566-3574.

De Caceres, M., P. Legendre, and F. He. 2013. Dissimilarity measurements and the size structure of ecological communities. Methods in Ecology and Evolution **4**:1167-1177.

De Caceres, M., P. Legendre, and M. Moretti. 2010. Improving indicator species analysis

by combining groups of sites. Oikos **119**:1674-1684.

De Castro, A., M. da Silva, B. Quirino, M. Bustamante, and R. Kruger. 2016. Microbial

Diversity in Cerrado Biome (Neotropical Savanna) Soils. Plos One **11**.

De Gruyter, J., M. Aveskamp, J. Woudenberg, G. Verkley, J. Groenewald, and P. Crous.

2009. Molecular phylogeny of *Phoma* and allied anamorph genera: Towards a

reclassification of the *Phoma* complex. Mycological Research **113**:508-519.

De Souza, R., V. Okura, J. Armanhi, B. Jorrin, N. Lozano, M. da Silva, M. Gonzalez-

Guerrero, L. de Araujo, N. Verza, H. Bagheri, J. Imperial, and P. Arruda. 2016.

Unlocking the bacterial and fungal communities assemblages of sugarcane

microbiome. Scientific Reports **6**.

Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: The

need for a flexible asymmetrical approach. Ecological Monographs **67**:345-366.

Ebead, G. A., D. P. Overy, F. Berrue, and R. G. Kerr. 2012. Westerdykella reniformis sp.

nov., producing the antibiotic metabolites melinacidin IV and chetracin B. IMA

Fungus **3**:189-201.

Edgar, R. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon

reads. Nature Methods **10**:996.

Edgar, R., and H. Flyvbjerg. 2015. Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics **31**:3476-3482.

Ellingson, L., J. Kauffman, D. Cummings, R. Sanford, and V. Jaramillo. 2000. Soil N dynamics associated with deforestation, biomass burning, and pasture conversion in a Mexican tropical dry forest. Forest Ecology and Management **137**:41-51.

España, M., F. Rasche, E. Kandeler, T. Brune, B. Rodriguez, G. Bending, and G. Cadisch. 2011. Assessing the effect of organic residue quality on active decomposing fungi in a tropical Vertisol using N-15-DNA stable isotope probing. Fungal Ecology **4**:115-119.

Etter, A., C. McAlpine, and H. Possingham. 2008. Historical patterns and drivers of landscape change in Colombia since 1500: A regionalized spatial approach. Annals of the Association of American Geographers **98**:2-23.

Fajardo, L., V. Gonzalez, J. Nassar, P. Lacabana, C. Portillo, F. Carrasquel, and J. Rodriguez. 2005. Tropical dry forests of Venezuela: Characterization and current conservation status. Biotropica **37**:531-546.

Fontaine, S., C. Henault, A. Aamor, N. Bdioui, J. Bloor, V. Maire, B. Mary, S. Revaillot, and P. Maron. 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biology & Biochemistry **43**:86-96.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry **35**:837-843.

Garbeva, P., J. Postma, J. van Veen, and J. van Elsas. 2006. Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. Environmental Microbiology **8**:233-246.

Garbeva, P., O. Tyc, M. Remus-Emsermann, A. van der Wal, M. Vos, M. Silby, and W. de Boer. 2011. No Apparent Costs for Facultative Antibiotic Production by the Soil Bacterium *Pseudomonas fluorescens* Pf0-1. Plos One **6**.

Garcia-Oliva, F., R. Sanford, and E. Kelly. 1999a. Effect of burning of tropical deciduous forest soil in Mexico on the microbial degradation of organic matter. Plant and Soil **206**:29-36.

Garcia-Oliva, F., R. Sanford, and E. Kelly. 1999b. Effects of slash-and-burn management on soil aggregate organic C and N in a tropical deciduous forest. Geoderma **88**:1-12.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for Basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology. **2**:113–118.

Gotelli, N., and R. Colwell. 2001. Quantifying biodiversity: procedures and pitfalls in the

measurement and comparison of species richness. Ecology Letters **4**:379-391.

Green, L., A. Porras-Alfaro, and R. Sinsabaugh. 2008. Translocation of nitrogen and carbon integrates biotic crust and grass production in desert grassland. Journal of Ecology **96**:1076-1085.

Herrera, J., R. Poudel, K. Nebel, and S. Collins. 2011. Precipitation increases the abundance of some groups of root-associated fungal endophytes in a semiarid grassland. Ecosphere **2**.

Instituto Geográfico Agustín Codazzi (IGAC). 2010. Mapa Geo-pedológico de Colombia. http://geoportal.igac.gov.co/ (accessed, 15.03.11)

Jones, E. B. G. 2006. Form and function of fungal spore appendages. Mycoscience **47**:167.

Kodsueb, R., S. Lumyong, E. Mckenzie, A. Bahkali, and K. Hyde. 2016. Relationships between terrestrial and freshwater lignicolous fungi. Fungal Ecology **19**:155-168.

Legendre, P., and L. F. Legendre. 2012. Numerical ecology (Vol. 24). Elsevier.

Likens, G., F. Bormann, N. Johnson, D. Fisher, and Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in Hubbard Brook watershed-ecosystem. Ecological Monographs **40**:23.

Loro, M., C. Valero-Jimenez, S. Nozawa, and L. Marquez. 2012. Diversity and composition of fungal endophytes in semiarid Northwest Venezuela. Journal of Arid Environments **85**:46-55.

Louis, B., S. Waikhom, R. Jose, S. Goyari, N. Talukdar, and P. Roy. 2015. Cochliobolus lunatus colonizes potato by adopting different invasion strategies on cultivars: New insights on temperature dependent-virulence. Microbial Pathogenesis **87**:30-39.

Lovelock, C., S. Wright, D. Clark, and R. Ruess. 2004. Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. Journal of Ecology **92**:278-287.

Maass, J., J. Vose, W. Swank, and A. Martinezyrizar. 1995. Seasonal changes of leaf area index (LAI) in a tropical deciduous forest in West Mexico. Forest Ecology and Management **74**:171-180.

Magurran, A., and P. Henderson. 2003. Explaining the excess of rare species in natural species abundance distributions. Nature **422**:714-716.

Marrs, R., J. Thompson, D. Scott, and J. Proctor. 1991. Nitrogen Mineralization and nitrification in terra-firme forest and savanna soils on Ilha-de-Maraca, Roraima, Brazil. Journal of Tropical Ecology **7**:123-137.

Matson, P., P. Vitousek, J. Ewel, M. Mazzarino, and G. Robertson. 1987. Nitrogen

transformations following tropical forest felling and burning on a volcanic soil.

Ecology **68**:491-502.

Maynard, D. G., and Y.P. Kalra. 1993. Nitrate and exchangeable ammonium nitrogen.

Soil sampling and methods of analysis, 1.

McGuire, K., N. Fierer, C. Bateman, K. Treseder, and B. Turner. 2012. Fungal

Community Composition in Neotropical Rain Forests: the Influence of Tree

Diversity and Precipitation. Microbial Ecology **63**:804-812.

McMurdie, P., and S. Holmes. 2013. Phyloseq: An R Package for Reproducible

Interactive Analysis and Graphics of Microbiome Census Data. Plos One **8**.

Moorhead, D., and R. Sinsabaugh. 2000. Simulated patterns of litter decay predict

patterns of extracellular enzyme activities. Applied Soil Ecology **14**:71-79.

Moorhead, D., and R. Sinsabaugh. 2006. A theoretical model of litter decay and

microbial interaction. Ecological Monographs **76**:151-174.

Murphy, P., and A. Lugo. 1986. Ecology of tropical dry. Annual Review of Ecology and

Systematics **17**:67-88.

Ng, E., A. Patti, M. Rose, C. Schefe, R. Smernik, and T. Cavagnaro. 2015. Do organic inputs alter resistance and resilience of soil microbial community to drying? Soil Biology & Biochemistry **81**:58-66.

Nguyen, C., E. Lichtfouse, M. Navarrete, P. Debaeke, S. V, and C. Alberola. 2009. Rhizodeposition of Organic C by Plant: Mechanisms and Controls. Sustainable Agriculture 97-123.

Osorio, J., M. Wingfield, and J. Roux. 2016. A review of factors associated with decline and death of mangroves, with particular reference to fungal pathogens. South African Journal of Botany **103**:295-301.

Paustian, K., and J. Schnurer. 1987a. Fungal growth-response to carbon and nitrogen limitation – A theoretical-model. Soil Biology & Biochemistry **19**:613-620.

Paustian, K., and J. Schnurer. 1987b. Fungal growth-response to carbon and nitrogen limitation – Application of a model to laboratory and field. Soil Biology & Biochemistry **19**:621-629.

Petrie, M., S. Collins, and M. Litvak. 2015. The ecological role of small rainfall events in a desert grassland. Ecohydrology **8**:1614-1622.

Raich, J., and W. Schlesinger. 1992. The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus Series B-Chemical and

Physical Meteorology **44**:81-99.

Sanchez-Azofeifa, G., M. Kalacska, M. Quesada, J. Calvo-Alvarado, J. Nassar, and J. Rodriguez. 2005. Need for integrated research for a sustainable future in tropical dry forests. Conservation Biology **19**:285-286.

Saynes, V., C. Hidalgo, J. Etchevers, and J. Campo. 2005. Soil C and N dynamics in primary and secondary seasonally dry tropical forests in Mexico. Applied Soil Ecology **29**:282-289.

Shahzad, T., C. Chenu, P. Genet, S. Barot, N. Perveen, C. Mougin, and S. Fontaine. 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biology & Biochemistry **80**:146-155.

Six, J., R. Conant, E. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. Plant and Soil **241**:155-176.

Starke, R., R. Kermer, L. Ullmann-Zeunert, I. Baldwin, J. Seifert, F. Bastida, M. von Bergen, and N. Jehmlich. 2016. Bacteria dominate the short-term assimilation of plant-derived N in soil. Soil Biology & Biochemistry **96**:30-38.

Stolk, A. C. 1955. *Emericellopsis minima* sp. nov. and *Westerdykella ornata* gen. nov., sp. nov.. Transactions of the British Mycological Society **38**:419-424.

Sutherland, J., A. Pometto, and D. Crawford. 1983. Lignocellulose degradation by *Fusarium* species. Canadian Journal of Botany-Revue Canadienne De Botanique **61**:1194-1198.

Swift, M. J., Heal, O. W., and J. M Anderson. 1979. Decomposition in terrestrial ecosystems (Vol. 5). Univ of California Press.

Vallejo, V., Z. Arbeli, W. Teran, N. Lorenz, R. Dick, and F. Roldan. 2012. Effect of land management and *Prosopis juliflora* (Sw.) DC trees on soil microbial community and enzymatic activities in intensive silvopastoral systems of Colombia. Agriculture Ecosystems & Environment **150**:139-148.

Vellinga, E., R. de Kok, and T. Bruns. 2003. Phylogeny and taxonomy of Macrolepiota (Agaricaceae). Mycologia **95**:442-456.

Vitousek, P., and J. Denslow. 1987. Differences in extractable phosphorus among soils of the La Selva Biological Station, Costa Rica. Biotropica **19**:167-170.

Vitousek, P., T. Fahey, D. Johnson, and M. Swift. 1988. Element interactions in forest ecosystems – succession, allometry and input-output. Biogeochemistry **5**:7-34.

Vitousek, P., and P. Matson. 1988. Nitrogen transformations in a range of tropical forest soils. Soil Biology & Biochemistry **20**:361-367.

Wenham, J.E. 1995. Post-harvest deterioration of cassava. A biotechnology perspective. FAO Plant Production and Protection Paper 130. NRI/FAO. Rome, 90 p.

Wong, M., and K. Hyde. 2001. Diversity of fungi on six species of Gramineae and one species of Cyperaceae in Hong Kong. Mycological Research **105**:1485-1491.

Woudenberg, J., J. De Gruyter, P. Crous, and L. Zwiers. 2012. Analysis of the mating-type loci of co-occurring and phylogenetically related species of *Ascochyta* and *Phoma*. Molecular Plant Pathology **13**:350-362.

Wright, S., A. Upadhyaya, and J. Buyer. 1998. Comparison of N-linked oligosaccharides of glomalin from arbuscular mycorrhizal fungi and soils by capillary electrophoresis. Soil Biology & Biochemistry **30**:1853-1857.

Zhang, Y., C. Schoch, J. Fournier, P. Crous, J. de Gruyter, J. Woudenberg, K. Hirayama, K. Tanaka, S. Pointing, J. Spatafora, and K. Hyde. 2009. Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. Studies in Mycology 85-102.

Tables and figures

Table 2.1 Most common taxa detected by 454 Next Generation Sequencing based on best

BLAST hit at GenBank.

Organism	Counts Day Zero	Counts 30 days	Counts 90 days
Fusarium solani	1075	1964	965
Chaetomium globosum	1652	688	429
Zygopleurage zygospora	54	51	2041
Podospora fimiseda	1693	301	83
Humicola grisea	570	689	721
Penicillium pinophilum	770	154	964
Lasiodiplodia parva	608	680	96
Macrolepiota fuliginosa	0	198	962
Podospora anserina	232	690	162
Cochliobolus geniculatus	459	411	139
Cyberlindnera fabianii	78	52	868
Eupenicillium javanicum	460	169	220
Nigrospora oryzae	265	316	193
Aspergillus awamori	608	4	0
Microdiplodia miyakei	318	153	64
Elmerina caryae	493	12	0
Gibellulopsis sp.	413	29	5
Gliocephalotrichum longibrachium	122	292	19
Fusarium oxysporum	159	153	85
Penicillium pimiteouiense	169	131	93
Pyrenochaeta sp.	64	139	137
Rhizophydium haynaldii	33	200	104
Westerdykella ornata	62	23	225
Aspergillus alabamensis	88	104	118
Curvularia gladiol	106	103	80
Neurospora pannonica	138	83	48
Rosellinia buxi	131	89	46
Archaeorhizomyces finlayi	3	1	259
Cladorrhinum phialophoroides	28	53	179
Rhizopus microsporus	87	2	160

Table 2.2 Taxonomy and significance of indicator species for NMDS groups A, B, C. "i.s" = *incertae sedis*, *p < 0.05, **p < 0.001.

| Phylum	Class	Order	Family	Species	Group	Specificity	Fidelity
Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae	*Lasiodiplodia parva* *	A - B	0.8862	1
Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Podospora fimiseda* *	A - B	0.9293	0.9167
Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	*Massarina* sp. ***	A - B	0.9565	0.9167
Ascomycota	Dothideomycetes	Pleosporales	Pleosporales_i.e.	*Didymella vitalbina* *	A - B	0.8627	0.9167
Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Cochliobolus lunatus* *	A - B	0.8070	0.9167
Ascomycota	Sordariomycetes	Sordariales	Lasiosphaeriaceae	*Zygopleurage zygospora* *	C	0.944	0.667
Unknown	Unknown	Unknown	Unknown	Fungus sp **	C	0.944	0.667
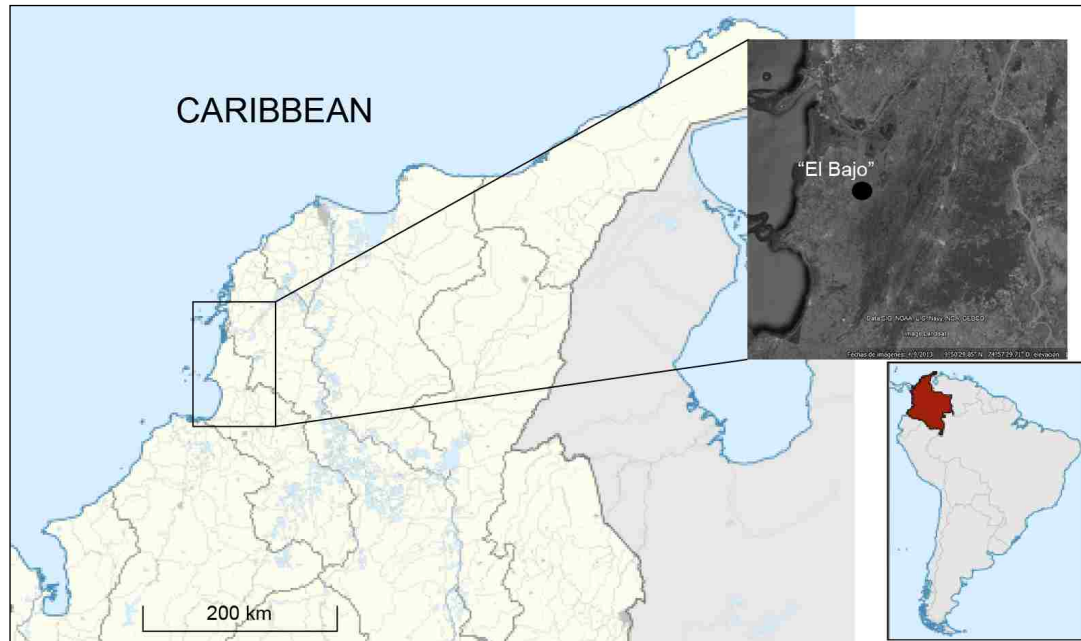
Figure 2.1. Location of "El Bajo" experimental pastures.

Figure 2.2. General aspect of "El Bajo" experimental pastures (a), cattle grazing on re-sprouting vegetation in a non-experimental pasture (b), cleared sampling plots at the beginning of the experiment (c), plot with vegetation trimmed, fractionated and replaced in an addition plot (d), plant material in addition plots covered with plastic mesh to prevent loss of material (e).
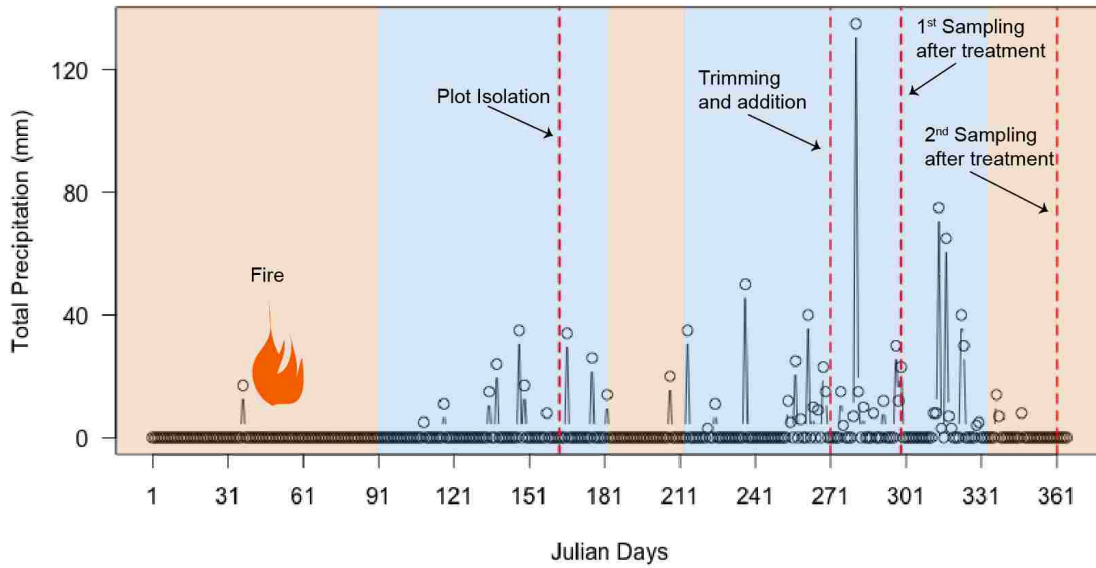
Figure 2.3. Sampling scheme. Orange and blue areas indicate dry and rainy seasons respectively. The areas between dotted red lines represent sampling intervals (0, 30 and 90 days post fire).
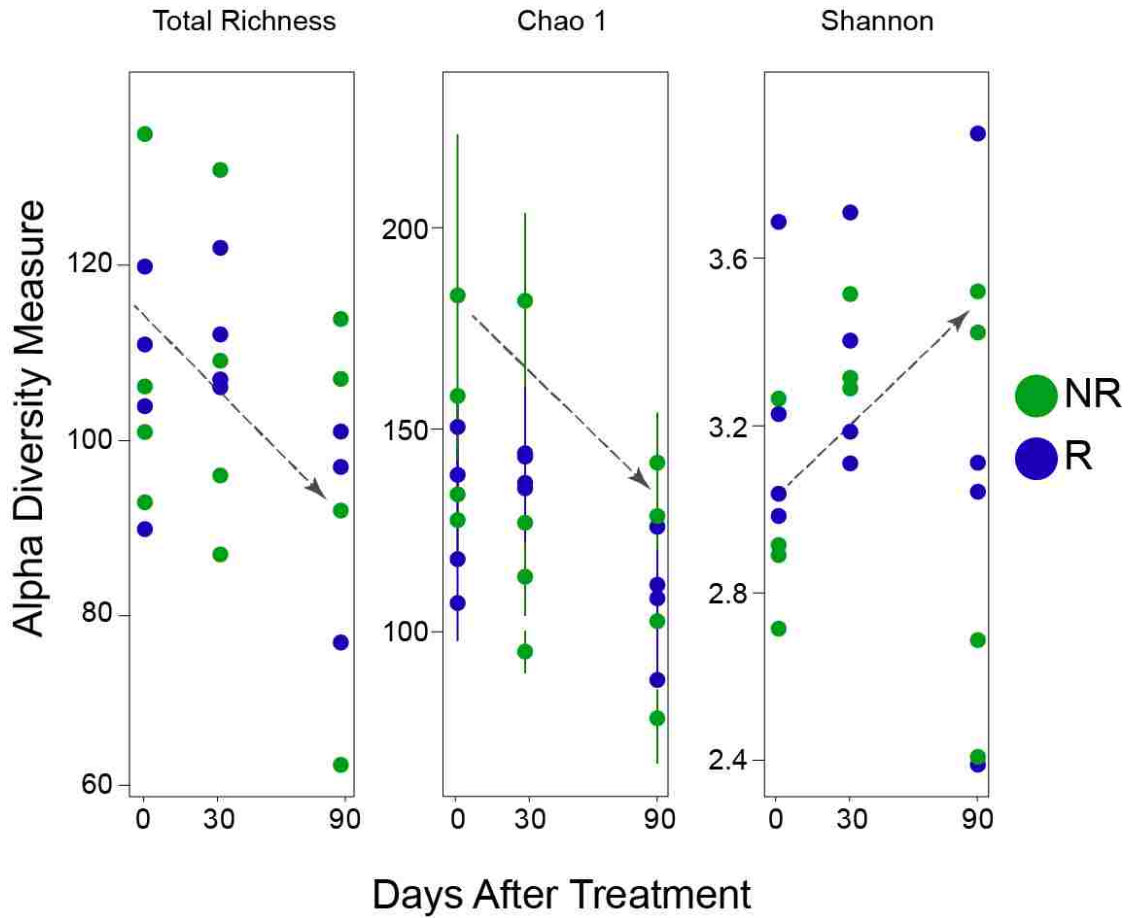
Figure 2.4. Distribution of values of richness and diversity for each sample after 0, 30, and 90 days of treatment. Blue dots indicate biomass removal, while green dots indicate non-removal. Grey arrows indicate the direction of displacement for the entire distribution of data. In the case of Shannon´s diversity, there is a noticeable shrinkage of the distribution 30 days after treatment followed by an expansion 90 days later. Error bars in the Chao1 index correspond to the standard error.
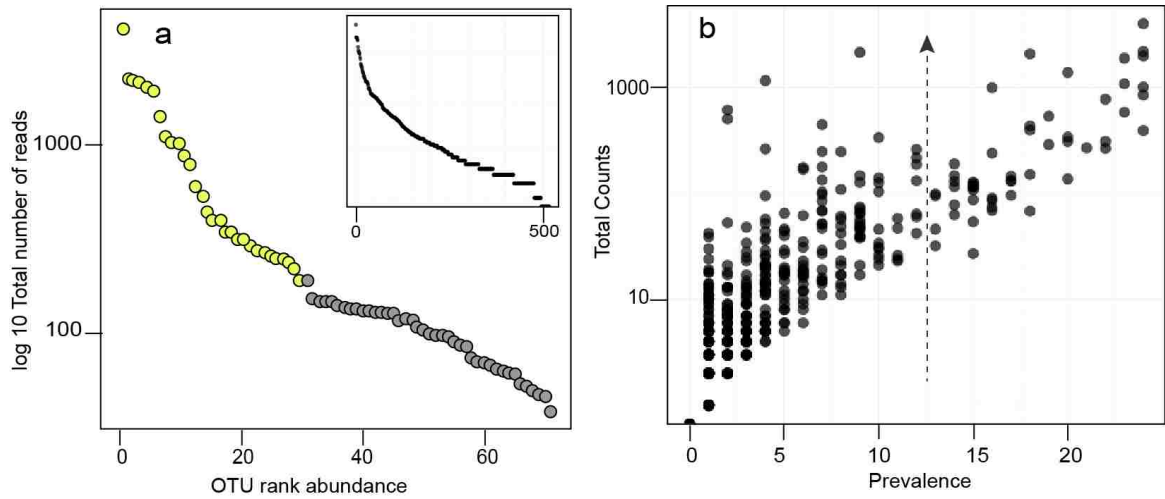
Figure 2.5. Rank-abundance curve for the most abundant OTUs once rare species have been removed. The highlighted dots are indicating the 30 most abundant OTUs (a). The graph at the top right corner of (a) is showing the curve for all OTUs in the original dataset. Classification of OTUs into abundant and rare (b) was achieved by plotting the number of samples for which each OTU was observed against the maximum abundance in any sample. The discontinuity indicated by the arrow sets the threshold between abundant and rare species as those present in >12 and <12 samples.
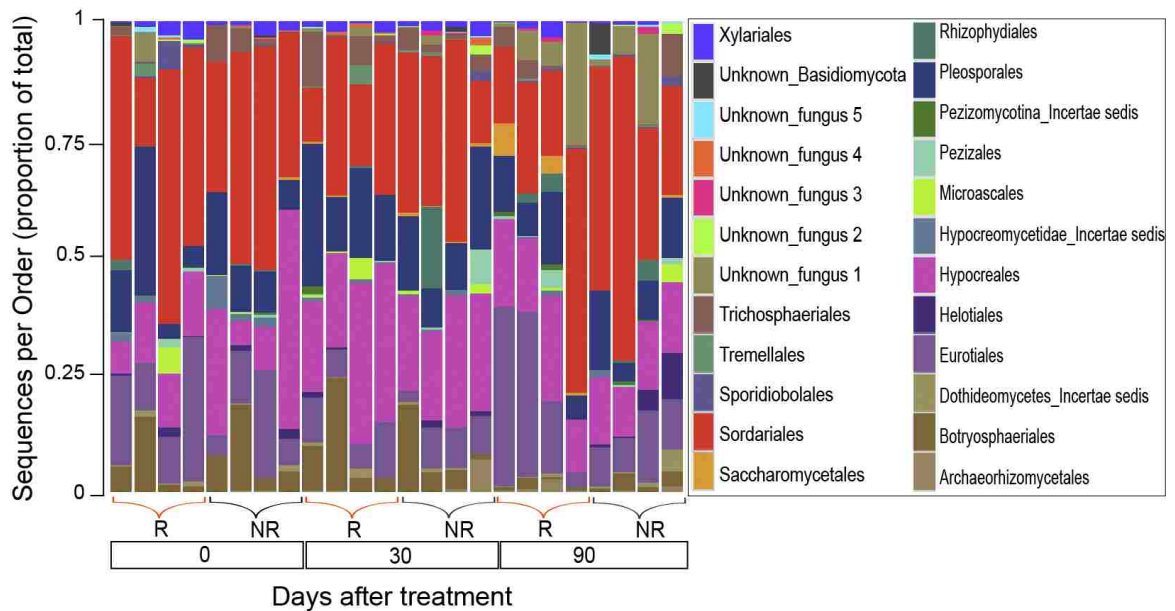
Figure 2.6. Abundance of sequences per order after 0, 30 and 90 days of treatment. The Brackets indicate samples grouped by treatment: R=Removal, NR=non-removal. There were 5 OTUs that could not be assigned to any fungal group. None of these had an occurrence greater than 5% but were included in this analysis because at least one of them was consistently present in several samples and also became relevant after 90 days. The color key must be read top-down and left to right as orders follow the same arrangement in the bars.
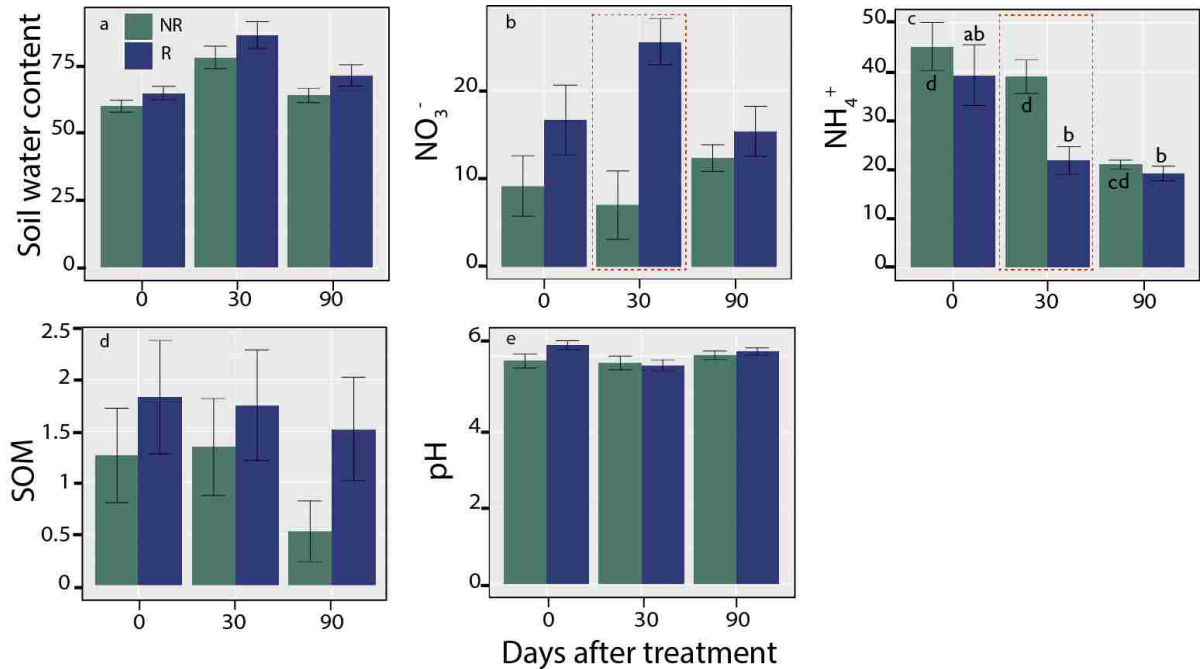
Figure 2.7. Dynamics of ecosystem variables with respect to treatment (blue = **NR** non-removal, green = **R**emoval) and time. The variables are represented as mean values for soil water content (gravimetric %), nitrate (mg/kg dry mass), ammonium (mg/kg dry mass), SOM (percentage of mass loss after ignition), pH and fungal biomass (percentage content of 18:2w6c per sample). Bars with different letters represent statistically significant differences inside each treatment (Tukeys multiple comparison test at $p < 0.05$). The dotted squares indicate the occurrence of statistically significant differences between treatments ($p<0.05$). Only NH_4 showed a statistically significant temporal trend, while both NO_3 and NH_4 were affected by treatment. Vertical lines represent the standard error of the mean
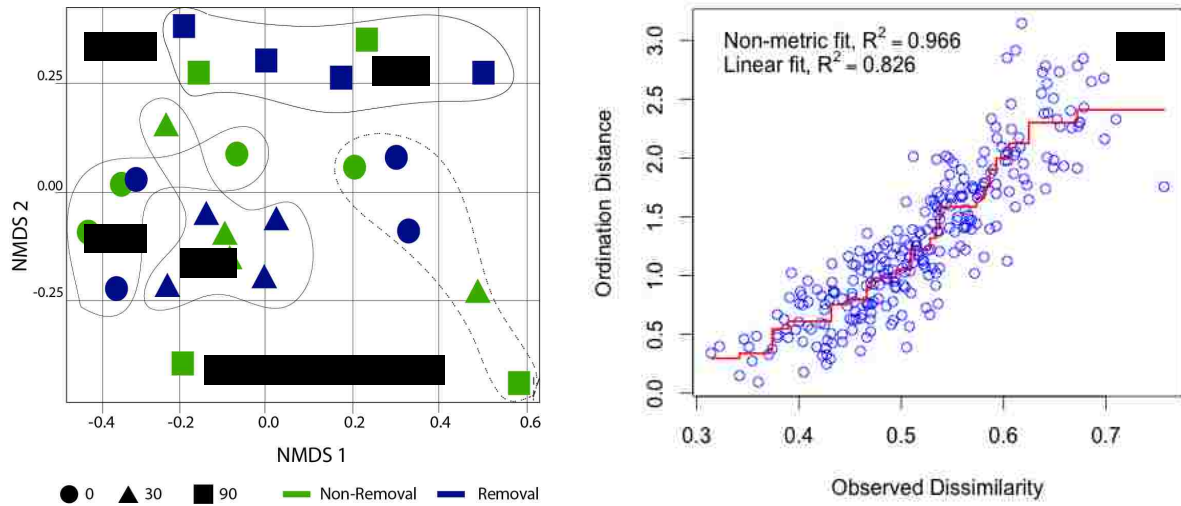
Figure 2.8. (a) Ordination of samples according to Bray-Curtis dissimilarity based on OTU (97% DNA identity) composition. The graph is showing the samples discriminated according to the level of treatment and sampling date. The groups labeled with capital letters correspond to the different sampling dates; a fourth group delimited by the dotted line shows a suspected plot-specific response different from the one exhibited by the other groups. Data were square root transformed and then submitted to a Winsconsin double standardization. (b) The Shepard diagram supports the validity of the ordination as it shows a good adjustment between the observed dissimilarity and the ordination distance. The "linear fit" represents the correlation between the step line and the points.
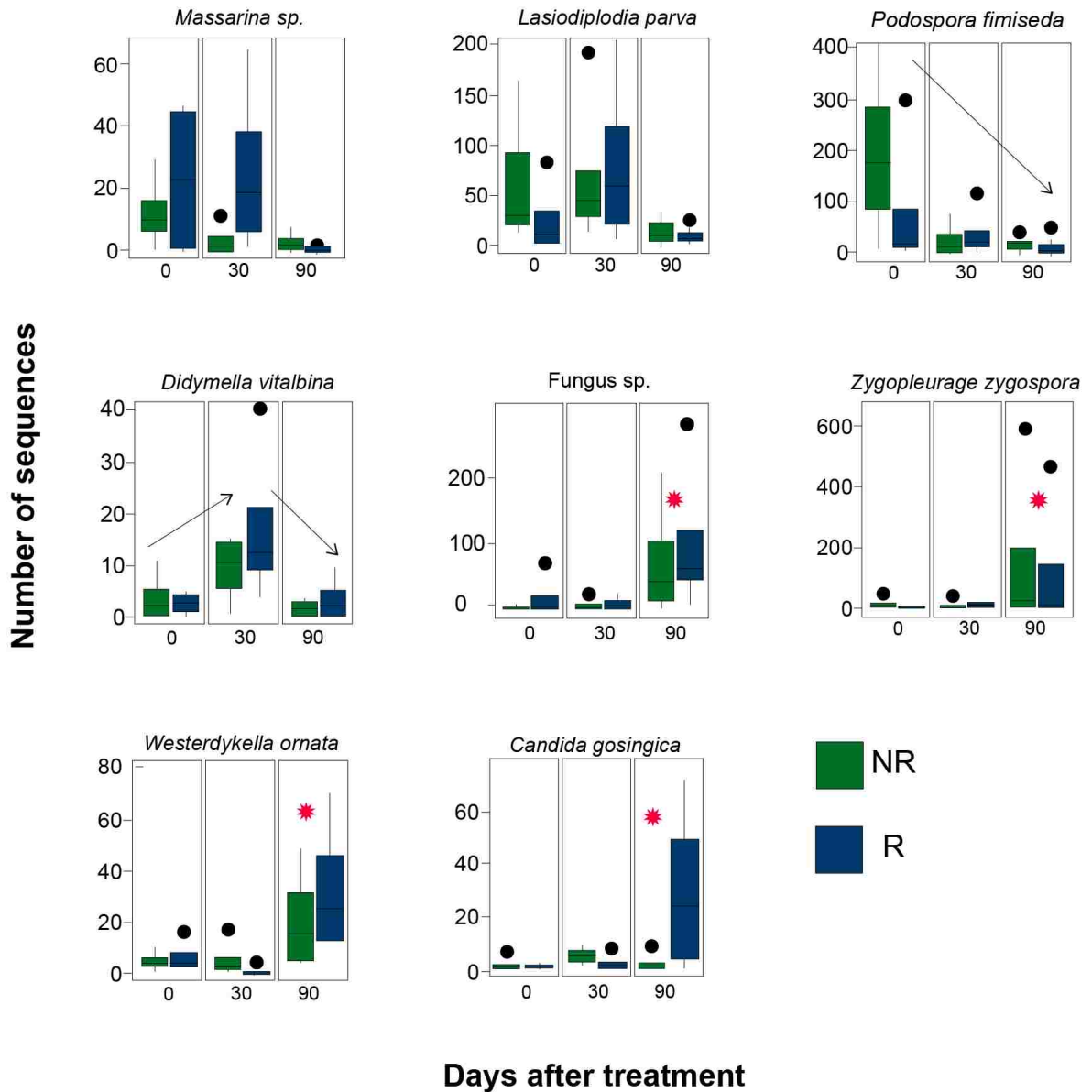
Figure 2.9. Distribution and significance of indicator species abundances according to the groups formed by the NMDS. Arrows indicate a suspected temporal trend. The species *Westerdykella ornata* and *Candida gosingica* are not considered indicator species by the Indicator Species Analysis, but they were found to be more abundant 90 days after treatment. Red asterisks indicate p < 0.05.

CONCLUSION

The present study provides a description of the most dominant fungal species in an agricultural soil previously submitted to repeated cycles of fire. It also offers insights into the climatic constraints that rainfall regimes impose on fungal soil communities. My analysis of monthly and daily rainfall totals indicate that "Sabanas" can be classified as pulse driven ecosystems, because dry conditions predominate even in the wet season, when days with precipitation are followed by several days with no precipitation.

This work showed that time after treatment and not treatment alone affected the species composition of the community. The results from the analysis of the 71 core species revealed that different species were present at certain time points, which in turn supports the hypothesis of a highly resistant and resilient fungal community inhabiting highly perturbed soils in this savanna. The dynamics of the composition and abundance of this fungal community seems to obey to a combination of factors derived from the interactions between precipitation and the types of carbon sources available during the decomposition process.

Finally, the lack of an immediate, strong response from the fungal community in this study suggests the effects of disturbance in this system should be tracked over long periods.