Investigating the Role of Frugivorous Ungulates in Forest Nutrient Cycling

By

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Dedicated to my loving parents and to all the species whose existance we threaten.

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Introduction

Tropical forests are unique ecosystems characterized by high biodiversity (Dirzo et al., 2003) and medium to low soil nutrient content (Tanner et al., 1998; Vitousek, 1984). Within these systems, sunlight and precipitation are often abundant, leaving nitrogen to act as the limiting element to forest growth (Foster et al., 2006, Floate, 1981). The literature reveals that large ungulates (hoofed herbaceous mammals) play an important role in regulating ecosystem services (Hobbs, 1996), however their interaction with the nitrogen cycle is ecosystem and soil dependent. Large scale habitat fragmentation and destruction throughout the Neotropics have left large ungulates particularly vulnerable to local and regional extinctions (Altrichter et al., 2012; Jorge et al., 2013), and while conservation efforts for neotropical forests are on the rise (Cardoso Da Silva and Bates, 2002), a further understanding of the role ungulates play in maintaining ecosystem services will increase the effectiveness of any new regulation.

The majority of previous works have focused on the nitrogen-ungulate relationship within savannahs and grasslands (Ruess and McNaughton, 1987; Frank et al., 2000; Coetsee et al., 2011), leaving forests only marginally explored (Seagle, 2003; Pastor et al., 1993) and neotropical forests completely uncharted. Given the vastly different soil and plant compositions of forests systems, the ungulate-nitrogen cycle relationship within must be considered independently.

Further, previous studies have exclusively examined grazing ungulates, which often preferentially consume nitrogen rich plant tissue. Such consumption likely alters the nitrogen cycle within the ecosystem, and two competing theories have been postulated to explain this interaction: the first claims ungulate presence results in a deceleration of the nitrogen cycle (Pastor et al., 1993; Ruess and McNaughton, 1987), while the second claims such presence results in an acceleration of the nitrogen cycle (Figure 1) (Ritchie et al., 1998; Frank and Evans, 1997; Frank et al., 2000; McNaughton et al., 1988; Augustine et al., 2003).

In both theories, ungulates alter the nitrogen cycle through defecation. Ungulate excretion and urea contains nitrogen in the form of both ammonium and nitrate (hereby collectively referred to as available nitrogen). Available nitrogen is inorganic nitrogen that is readily available for plant assimilation (Floate, 1970) and thus, defecation acts similar to fertilizer and directly stimulates plant growth. In both theories, this addition of available nitrogen stimulates plant growth. Further, ungulate excretion and urea have a low C:N ratio (Ruess and McNaughton, 1987) and consequently the organic material within is less resistant to microbial breakdown. This once again increases the amount of available nitrogen relative to carbon and promotes further decomposition of the surrounding soil organic matter while increasing mineralization, the process of transforming organic nitrogen into ammonium (Floate, 1970).

The theories diverge when considering the consequences of preferential consumption.

While both claim that grazing ungulates actively seek plant tissue that is rich in nitrogen, as the element is necessary for their own growth, the deceleration theory states this preferential consumption creates an opportunity for plants with nitrogen poor tissues to out compete those with nitrogen rich tissues. The resulting leaf litter in the system is then also nitrogen poor. While the excretion of the ungulates will still stimulate decomposition to an extent, the organic matter that is broken down is nitrogen poor, meaning the return of available nitrogen to the system is decreased. This furthers the exclusion of nitrogen rich plants and creates a negative feedback that can be dramatically exacerbated in nitrogen poor systems (Ritchie et al., 1998; Tilman et al., 1998). The nitrogen acceleration theory, on the other hand, claims that the nitrogen rich plants that are grazed have developed compensations to combat this disruption, including quick growth and higher foliar nitrogen concentrations. These compensations increase the quality and quantity of the leaf litter in the systems. When the abundant, nitrogen rich leaf litter is decomposed, the soil is enriched with available nitrogen which helps further promote the growth of nitrogen rich plants (Hobbs, 1996; Ruess and McNaughton, 1987).

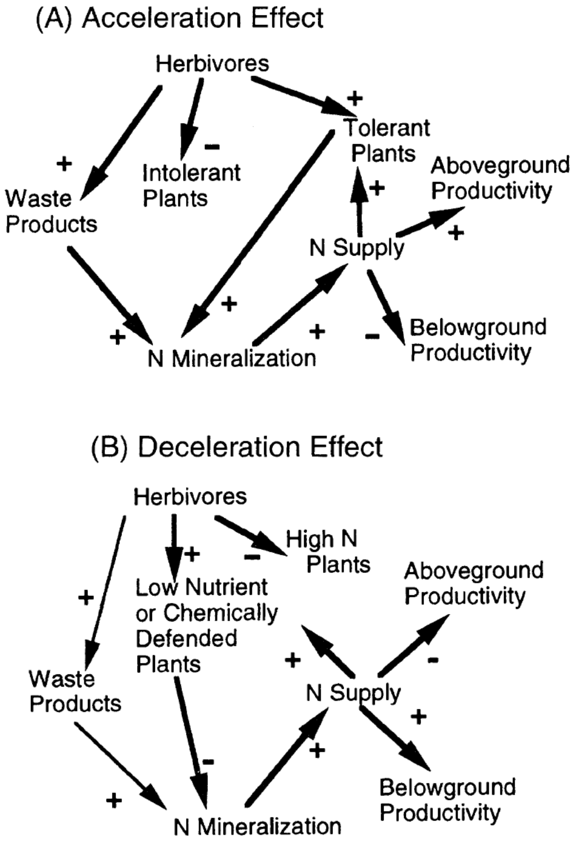

Figure 1: Nitrogen cycle positive feedbacks that describe A) the acceleration theory and B) the deceleration theory of herbivorous ungulates. The theories rest on whether the ungulates preferentially consume nitrogen rich material or nitrogen poor materials. Figure from Ritchie et al., 1998.

Ungulates of the neotropical forests, however, are typically frugivores (Altrichter et al., 2001; Galetti et al., 2015; Keuroghlian et al., 2009), though during times of fruit scarcity, will forage on additional foliage (Keuroghlian et al., 2009; Sowls, 1984). This provides a new interaction to explore; specifically the role of non-grazing ungulates in forest systems.

The white-lipped peccary *Tayassu pecari* provides a unique opportunity examine the role of non-grazing ungulates within forest ecosystems. This frugivorous ungulate has a significant presence in neotropical forests where they travel in large herds (40 to >200 individuals) and may make up to 34% of the non-flying biomass in areas where they are present (Kiltie, 1983; Keuroghlian, 2009). Given they do not preferentially consume nitrogen rich or nitrogen poor plants, they do not alter the nitrogen cycle through preferential consumption. They do, however, deposit excretion and urea, which both directly affect the amount of available nitrogen in the soil, and stimulate decomposition for a quick turnover of organic nitrogen to available nitrogen (Figure 2). Extensive habitat fragmentation and destruction, however, has led to a decline in populations, leaving the species endangered (Jorge et al., 2013) with a prognosis of local extinction in many areas (Altrichter et al., 2012). This study aims to help further elucidate the role this important species plays within its ecosystem as well as provide a better understanding of the nitrogen cycle within this unique system.

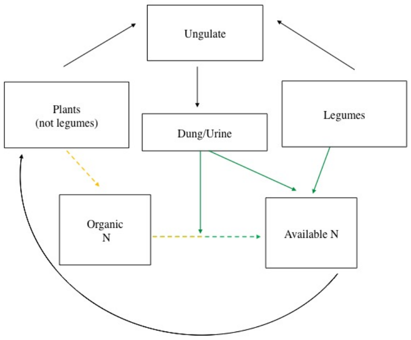

Figure 2: Theoretical accelerated nitrogen cycle by white-lipped peccaries; yellow arrows indicate slow processes, green arrows indicate fast processes.

A place of concern for local and regional extinction is the Brazilian Cerrado, a biome composed of a mosaic of landscapes within the Neotropics. While typically classified as savannah, the unique landscape contains many forest types (Bourliere and Hadley, 1983, Furley and Ratter, 1988). These include gallery forests and dry forests, which are particularly rich in diversity and endemic species (Cardoso Da Silva and Bates, 2002). In fact, these systems of the Cerrado are so diverse, Myers et al., (2000) named the biome one of the top 25 most important terrestrial hotspots. Despite this, the biome has seen more than 50% of its land transformed into agricultural and pastoral land in the last 35 years (Klink et al., 2005), making it an area of particular interest for conservation efforts.

Project Overview

Project Goals

This project aims to help elucidate the role of non-grazing ungulates in nitrogen cycling by:

- Investigating the impact ungulates have on the nitrogen cycle in forests, particularly when the primary food source is not nitrogen rich.

Further, this project aims to contibute information on nitrogen cycling within the Brazilian Cerrado by:

- Providing field-collected values for specific components of the nitrogen cycle within the forests of the Brazilian Cerrado system.

Study Species

The white-lipped peccary (Figure 5) is one of three species within the family Tayassuidae, accompanied by the slightly smaller collared peccary (*Pecari tajacu*) and the slightly larger Chacoan peccary (*Catagonus wagneri*) (Sowls, 1997). Found only in the Neotropics, the species' range is approximately 21°N to 30°S (Sowls, 1997) with populations estimated to inhabit a total of 11,177,435 km^2 (Altrichter et al., 2012). Within this range, the species can be found at elevations spanning from sea level to as high as 1900 m above sea level on the slopes of the Andes (Sowls, 1997). This range also includes areas with as little as 830 mm of precipitation a year (Paraguay) to areas with 3,234 mm of precipitation a year (Panama) (Sowls, 1997). The climatic range of the species has not been quantitatively examined, however one study conducted in the Cerrado of Brazil showed the species was present in areas where daytime temperatures can reach 40°C and nighttime temperatures can fall to -1.5°C (de Almeida Jcomo et al., 2013). While primarily a forest dweller, white-lipped peccaries can also be found in woodlands, xerophytic areas, and mangroves, and on occasion, in grasslands (Sowls, 1997).

Figure 3: Illustration of the white lipped peccary (*Tayassu pecari*) from Beck, 2006.

Characteristic of the white-lipped peccary is its social nature. The species lives in large herds, ranging from 40 to 400 individuals (Sowls, 1997). While the home range of the species is considered to be large as well, the exact size is highly dependent on the resources available in the region and herd size. In southern Mexico, home ranges were estimated to vary from 44 to 121 km^2 (Reyna-Hurtado and Tanner, 2009) while in Maraca Island of Brazil the home range size was estimated to be between 22 and 200 km^2 (Fragoso, 2004). In other areas, studies have reported much smaller home range sizes, including Costa Rica in which the mean annual home range was between 32 to 38 km^2 (Carrillo et al. 2002).

The energetic requirements of the white-lipped peccary have been investigated in the context of four captive male individuals, and provide a basis for understanding the needs of those in the wild (Nogueira-Filho et al., 2014). The study reported that for captive individuals, which weighed between 36 and 38.4 kg, the daily energetic requirements were approximately 557.7 $\pm$ 80.4 kJ/kg$^{\cdot 75}$ (Nogueira-Filho et al., 2014).

The white-lipped peccary is considered to be primarily frugivorous, but is known to consume roots, tubers, seeds, insects, fungi, and fish (Beck, 2005; Beck, 2006; Desbiez et al., 2009; Fragoso, 2004). In a rather recent literature review, Beck (2006) recorded the species consumes 144 different plant species throughout its range and that palm seeds could constitute up to 60% of the animals' diet. While it is agreed upon in the literature that the species primarily consumes fruits, the portion of its diet attributed to animal matter is somewhat controversial (Sowls, 1997). Some experts state consumption of animal tissue only occurs when foraging for other food (M.L. Jorge pers. comm.), however, a study by Bodmer (1989) showed animal matter made up to 11% of the stomach content in 46 samples. Nevertheless, it is generally agreed upon that white-lipped peccaries do not consume nitrogen rich plant material, and is thus not altering the nitrogen cycle through preferential consumption.

The diverse set of regions and ecosystems types occupied by the white-lipped peccary mean the exact composition of its diet likely varies between regions, however the dependence on sources with relatively low crude protein is pervasive (Beck, 2006). In fact, the fruit of two of the plants the white-lipped peccary regularly consumes, the *Astrocaryu*m sp. and the *Mauritia flexuosa* have crude protein compositions of just 6.0% and 7.4%, respectively (Kiltie, 1981). While the nutrient requirement of the white-lipped peccary is generally understudied, several studies have closely examined the anatomy and physiology of the species in an attempt to explain the species ability to thrive on such food sources. The forestomach of individuals of the species represents up to 85% of the total digestive track volume, and within, fermentation of dietary fiber occurs (Cavalcante-Filho et al., 1997). Thus, while the species does not ruminate, it is able to digest as if it did (Cavalcante-Filho et al., 1997).

Nitrogen is an important crude protein for animal growth and health where it acts as a building block for amino acids. Given the animals' rumanat body structure, they are especially adept at processing ingested nutrients, which likely accounts for increased nitrogen retention and recycling throughout the body (Reynolds and Kristensen, 2008). Therefore their excretion contains relatively high levels of nitrogen (Carl and Brown, 1985). Nogueira-Filho et al. (2014) estimated that captive individuals require a daily nitrogen intake of 336.5 mg N/kg$^{.75}$. In addition, the study calculated the daily urea and fecal excretion of nitrogen, which ranged between 215.5-452.2 mg/kg$^{.75}$/day and 48-81.2 mg/kg$^{.75}$/day, respectively. It should be noted, the individuals that excreted lower values of nitrogen in fecal matter excreted higher values in their urea and visa versa (Nogueira-Filho et al., 2014). This is not an insignificant amount of nitrogen to be excreted daily. For example, a herd of 100 peccaries, which weigh on average 37 kg, could excrete between .45 and .75 kg of N per day.

$$37^{.75}kg * \frac{(81.2+215.5)mg}{1000000\ mg/kg} * 100\ individuals/herd = .45kg/herd/day$$

$$37^{.75}kg * \frac{(48+452.2)mg}{1000000\ mg/kg} * 100\ individuals/herd = .75kg/herd/day$$

Throughout its range, the white lipped peccary faces many threats, including habitat destruction and fragmentation, hunting, and zoonotic disease (Altrichter et al., 2012; Jorge et al., 2013). The species is especially vulnerable to habitat fragmentation, as large, connected tracts of lands are needed to sustain such large herds (Keuroghlian and Eaton, 2008; Altrichter et al., 2012; Jorge et al. submitted). The Cerrado ecosystem is already highly fragmented due to land use change and increased agricultural practices (Carvalho et al., 2009). A recent study on the status of the white-lipped peccary noted that the such fragmentation has isolated populations and has resulted in low to medium population survival probabilities within the Cerrado (Altrichter et al., 2012). In fact, currently only 18% of the land within the Cerrado is fit for the white-lipped peccary to inhabit (Altrichter et al., 2012).

Within the Cerrado systems the white-lipped peccary still inhabits, the species plays important roles in maintaining ecosystem functioning. This includes several vital interactions with plants, including seed dispersing (Fragoso, 1997; Keuroghlian and Eaton, 2009), seed predators (Beck, 2005; Wyatt and Silman, 2004), and seedling trampling (Beck, 2006; Wyatt and Silman, 2004). They also create and maintain wallows, which may be important in maintaining anuran diversity, a role that may classify the species as an ecosystem engineer (Beck et al., 2010). In addition, they serve as an important food source for large predators, specifically the puma (*Puma concolor*) and the jaguar (*Panthera onca*) (Altrichter et al., 2012; Sowls, 1997; Taber et al., 1997). Given other large ungulates play important roles in nutrient cycling within their respective ecosystems (Hobbs, 1996; Ruess and McNaughton, 1987; Frank et al., 2000; Coetsee et al., 2011) it is possible the white-lipped peccary may do the same. This study aims in elucidate how the white-lipped peccary may be impacting the nitrogen as understanding the role of the species within its ecosystem may help inform better conservation practices within the Cerrado.

Study Region

The Cerrado is the largest savannah of the world, and the second largest biome in South America, falling short of only the Amazonia (Cardoso Da Silva and Bates, 2002). In Brazil, the Cerrado covers over 2 million km^2, 21% of the countrys land coverage (Furley and Ratter, 1988; Ratter et al., 1997). While typically classified as tropical savannah, the region actually includes a diverse set of ecosystems. These include classical savannahs, riparian forests, patches of semi deciduous woodlands, and areas of swamps and marshes, and closed canopy forests (Bourliere and Hadley, 1983, Furley and Ratter, 1988). Considered a biodiversity hotspot (Myers et al., 2000), the biome has a large percentage of endemic species including: vascular plants (44%), amphibians (30%), reptiles (20%), mammals (11.8%), and birds (1.4%) (Silva, 1995; Myers et al., 2000; Cardoso Da Silva, 2002).

The ecosystems that constitute the transition from savannah to forest are well defined and are broken up into distinct vegetation classifications (Furley et al., 1988; Cardoso Da Silva, 2002; Schwieder et al., 2016) (Table 1 and Figure 4). About 24% of the biome is considered part of the savannah-forest transition (campo sujo-cerrado denso) while only 4% of the system is considered strictly forest (cerrado and mata de galeria) (Cardoso Da Silva, 2002).

campo limpo	grasslands with few or no shrubs (canopy $>$ 1 m)
campo sujo	grasslands with scattered shrubs (canopy 2-3 m)
campo cerrado	open scrubland with few trees (canopy 3-6m)
cerrado sensu stricto	woodland with closed scrub and scattered trees (canopy 5-8m)
cerrado denso	woodland with closed scrub and many trees (canopy 8-16m)
cerradão	dense dry semi-deciduous forest, completely closed canopy (canopy 8-16m)
Mata de galeria	gallery forests: evergreen forest, at river banks

Table 1: Vegetation classifications for the savanna-forest transition within the Cerrado of Brazil (Cardoso Da Silva, 2002)

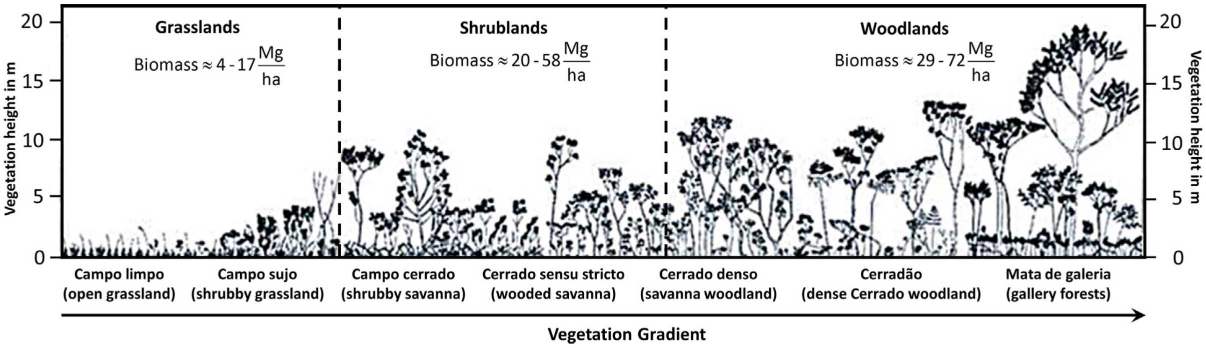

Figure 4: Gradients of Cerrado vegetation from Schwieder et al., 2016 and adapted from Mesquita Jr, H. N., (2000).

Underlying this suite of systems are well-drained, oxisol soils with relatively low pH, low calcium concentrations and high aluminum concentrations (Furley and Ratter, 1988). Found primarily in tropical regions, oxisol soils typically do not have clearly defined horizons and are composed of a mixture of quartz, kaolin, free oxides, and organic matter (U.S. Department of Agriculture). Soil characteristics and nutrient concentrations tend to determine the vegetation within the Cerrado (Haridasan, 2001) with forest systems (dense Cerrado woodlands and gallery forests) associated with dark red latosols (Haridasan, 2001). Forest patches also tend to be associated with limestone outcrops, which often have calcium and magnesium rich soils (Ratter, 1992).

Forest patches are particularly important as these systems account for a large portion of the diversity of the Cerrado biome. In fact, 38% of plant species (Mendona et al., 1998), 83% of bird species (Silva, 1995), and 56% of the non-flying non-volant mammal species (Redford et al., 1986) rely on these ecosystems in some capacity. While the Cerrado has been subject to habitat fragmentation and large-scale deforestation (Ward et al., 1992; Furley and Ratter, 1988), patches of natural vegetation still occur in many regions throughout the biome (Furley and Ratter, 1988).

This work took place within the southwest region of the Cerrado in Bodoquena and Corguinho counties, Mato Grosso do Sul, Brazil. The Corguinho region is the more northern region, within the Maracaju Mountain Ridge, while Bodoquena is located 100 km to the south, within the Bodoquena Mountain Ridge. Both ridges belong to the highlands of the Paraguay River Basin, and their waterways feed into the lowlands to the West (Figure 5). Precipitation in this region of Brazil ranges from 1300-1700 mm/year and mean annual temperatures range between 20- 22°C (Alvarenga et al., 1982). Soils in the region are well drained, acidic Oxisols with high levels of aluminum saturation and typically low nutrient content. Such characteristics are considered typical of the Cerrado (Furley et al., 1988).

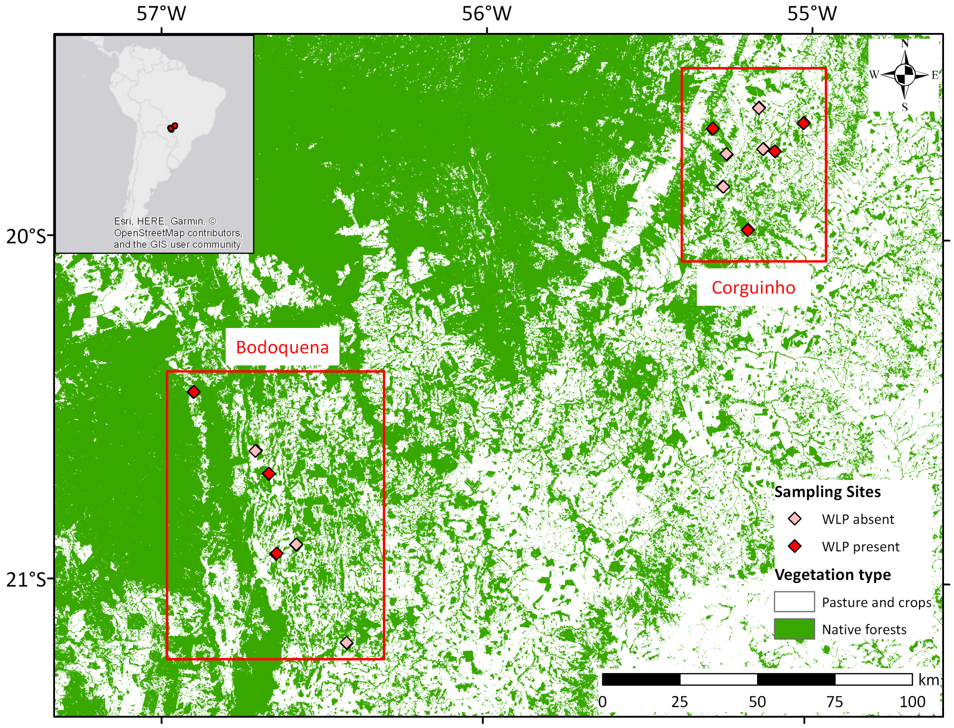

Figure 5: Study region within the highly fragmented Cerrado of Brazil. Samples were taken from patches of native forest on farms in the Bodoquena (n=6) and Corguinho counties (n=8), Mato Grosso do Sul, Brazil. A total of 14 forest patch were sampled from different farms farms, representing white-lipped peccary presence (n=7) and absence (n=7).

Main factor

White-lipped peccary presence and absense

While only 2% of the Cerrado is legally protected reserves (Klink et al., 2005) Brazilian Environmental Legislation requires 20% of all farmland within the Cerrado to be left intact (Klink et al., 2005), resulting in native forest patches on farms throughout the biome. An evaluation of white-lipped peccary occurrence and movement patterns from 2009 to 2018 in the Corguinho and Bodoquena regions revealed some forest patches on farms are occupied by the species while others are not (Jorge, unpublished data). All patches were, however, likely inhabited with the species prior to extensive fragmentation (Jorge, unpublished data). Such local variation of white-lipped peccary presence and absence creates a natural experiment to test the effects of white-lipped peccaries on nutrient deposition in the soil. In order to do so, an equal number of forest patches from various farms representing white-lipped peccary presence and absence were sampled from each of the two regions (Figure 5 and Table 2).

Name	Abbreviation	Region	Peccary Presence	Area (km^2)
Mato do Celso	MC	Bodoquena	No	~ 7
St. Peter's Farm	SP	Bodoquena	No	~ 0.5
Unnamed Farm	UF	Bodoquena	No	~ 1
Cachoeira	CA	Bodoquena	Yes	~ 35
Novo Horizonte	NH	Bodoquena	Yes	~ 4
Primavera	PV	Bodoquena	Yes	> 600
Bidi	BD	Corguinho	No	~ 1
Jatoba	JT	Corguinho	No	> 30
Lambria	LA	Corguinho	No	~ 4
Santa Adelaide	SD	Corguinho	No	~ 5.5
Colorado	CO	Corguinho	Yes	> 30
Jacobina	JA	Corguinho	Yes	> 25
Lagoa	LG	Corguinho	Yes	~ 6.5
Safira	SA	Corguinho	Yes	~ 7.5

Table 2: Farms sampled, identification code, and white-lipped peccary presence or absence

Previous studies that investigated the role of ungulates in soil dynamics have relied on exclosure plots (Cotesee et al., 2011; Frank et al., 2000, etc.). These are typically fenced areas set up within the ecosystem of interest to exclude large ungulates. This allows for direct comparison between study areas without ungulate presence and control areas with ungulate presence. The resources and time frame for this project did not allow for exclosure plots to be built, however forest fragmentation has led to a the natural exclosure of white-lipped peccaries in some forest patches. The use of GPS data (Jorge Research Lab ongoing project), which has tracked many of the herds within the two studied regions allowed for confidence in selecting farms where white-lipped peccaries were undoubtedly present. Further, in each farm of white-lipped peccary present, evidence of use such as footprints and feces, were recorded. Given the large herds the white-lipped peccary travels in, and the rather small size of the forest patches, evidence for presence was often abundant. Absence was ensured using a combination of information from camera-trap studies (Keuroghlian, et al. unpublished; Roque et al. unpublished), detailed conversation with local residents (with questions such as: "are there white-lipped peccaries in this patch?", if not, "since when have you not seen the species here?", "for how long have you lived in the area?", "are there other species here, such as collared peccaries, tapirs and deer?"), and careful investigation of indirect evidence of white-lipped peccary presence in the forest (footprints and feces) while walking through the forest to sample the soil.

To further support the hypothesis that only white-lipped peccaries were not present in the absence forest patches, physical evidence of other large ungulates (namely the tapir and deer) was recorded and during the field campaign and supplemented by camera trap data (Keuroghlian, et al. unpublished; Roque et al. unpublished). Results showed evidence of these species in all "absence" forest patches except St. Peters Farm, which only had deer tracks entering the forest. Of course, lack of evidence in our limited sampling does necessitate they were not present in the forests, and again conversations with locals indicate that it is likely these solitary yet abundant species are indeed present.

Co-Variables

Plant Composition

While all locations fell within the same floristic region of the Cerrado (Ratter et al., 2003), in order to ensure the forest patches sampled had comparable plant compositions, a plant survey was conducted. The aim was to sample only forest patches with plant compositions distinctive of the tall, closed canopy dense savannah woodland (cerradão) system mentioned above. In addition, species indicative of mesotrophic facies cerrado, a set of geological characteristics common and specific to dense cerrado woodland, were identified. This facies also has an indicative type of floral composition that occurs on soils with relatively higher calcium content (Furley and Ratter, 1988; Ratter, 1971). These systems also often have relatively higher pH, ranging from 4.5 to 6.5 and several studies have shown %N ranges from .14% to .28% (Ratter et al., 1977; Furley and Ratter, 1988).

Soil Abiotic Characteristics - pH and clay content

Both pH and soil texture were analyzed at each forest patch. In tropical soils, the soil pH is linearly related to rates of nitrification, the biological processes of transforming ammonium into nitrate, (Sahrawat, 1982). In fact, loe pH can inhibit the process altogether

(Sahrawat, 1982). In a series of lab tests using tropical soils, Sahrawat (1982) found that in pH lower that 4.4, nitrification is impeded. While still in a form that is available for plant assimilation, nitrate is also susceptible to denitrification, the transformation of nitrate into gaseous nitrogen. This nitrogen is then lost from the system. If, however, there is a low pH in the system, nitrogen loss via this pathway may not be significant, as there is little material nitrified in the first place. (Sahrawat, 1982). Therefore an understanding of the pH at any given farm may help untangle the relative rates of the nitrification and denitrification processes in the nitrogen cycle.

Due to the negative charge of clay minerals, clay has the ability to fix ammonium. This process is one in which ammonium (NH_4^+) ions are absorbed by the mineral or organic fraction in soil in a manner that they are relatively unexchangeable by the usual method of cation exchange (Osborne, 1976). Studies have shown this fixed ammonium can be released and used by plants (Marschner, 1995; Saha et al., 1986) and with decreasing available NH_4^+, an increasing amount of fixed NH_4^+ is released from clays, or adsorbed, to become available NH_4^+ (Nieder et al., 2011). Therefore, Nieder et al. (2011) suggest that by having an increased amount of fixed ammonium in a system, there may be in increased pool of available nitrogen in the system. Further, they suggest there may be less loss of nitrogen in the system as NH_4^+ ions do not undergo nitrification and are therefore less susceptible to leaching. While this study does not directly measure fixed NH_4^+, it is expected that higher clay content will be correlated with higher NH_4^+ values, more available nitrogen, and possibly a faster nitrogen cycle (Nieder et al., 2011).

Investigated Proxies

Nutrient Analysis

Based on previously established methods developed to explore the impact of ungulates on grasslands (Frank et al., 2000), major nitrogen cycle components were analysed. This included the concentration of nitrate (NO_3^-), ammonium (NH_4^+), the total available nitrogen (the sum of the two), total soil nitrogen, and total soil carbon. Nitrate and ammonium constitute the majority of nitrogen supply used for plant uptake, and therefore, the quantity of these ions from soil samples are often used as an index of available nitrogen for plant use (Binkley and Hart, 1989). Ungulates have been shown to increase available nitrogen (Frank and Groffman, 1998) as well as increase the rate of soil nitrogen transformations that result in more available nitrogen (Frank et al., 2000).

Measuring the total soil carbon and soil nitrogen could help bring to light if there is a faster nitrogen cycle as well. While most studies show similar total soil nitrogen concentrations between farms of native ungulate presence and absence (McNaughton et al., 1988; Augustine et al., 2003) such measurements allow for the calculation of a C:N ratio, which can be used to better understand nutrient turnover in the system (Seagle et al., 2002; Singer and Schoenecker, 2003; Pastor et al., 1993). Several studies have shown that through the deposition of available nitrogen in excretion, surface soil C:N ratios are lowered. With more nitrogen in the soil system, the microbial demand for nitrogen is more easily met, which in turn accelerates decomposition rates and nutrient turnover (Frank and Groffman, 1998; Seagle et al., 2002; Singer and Schoenecker, 2003; Pastor et al., 1993).

Isotope Analysis

The use of natural abundance of ^{15}N in soil and plant material to investigate the role of ungulates in nitrogen cycling is widely applied, though only in the context of grazers in grasslands (Frank and Evans, 1997; Frank et al., 2000; Craine et al., 2009a; Coetsee et al., 2011). Studies have shown within the nitrogen cycle, processes that are associated with nitrogen loss tend to be depleted in ^{15}N. In other words, systems become enriched with ^{15}N when molecules that are depleted of ^{15}N, such as NH_3, NO_3^-, N_2O and N_2, are lost through soil nitrogen transformation (Nadelhoffer and Fry, 1994). Therefore, an accelerated nitrogen cycle leads to an ecosystem enrichment in ^{15}N, often seen in plant and soil matter (Nadelhoffer and Fry, 1994; Schulze et al., 1998; Craine et al., 2009b; Coetsee et al., 2011). For example, ungulate presence in the grasslands of Yellowstone has been identified as a control on soil δ^{15}N due to the loss of ammonia from urine and dung. This loss through volatilization favors the lighter ^{14}N isotope and therefore resulted in a heavier δ^{15}N signal in the soils of ungulate presence (Frank and Evans, 1997). While this relationship has yet to be explored in the context of ungulates in forests, the same theories apply. Further, the processes of mineralization and nitrification leave the soil enriched in ^{15}N, and an enriched signal may indicate faster turnover of organic nitrogen into available nitrogen (Dawson et al., 2002). Therefore, this study uses δ^{15}N to elucidate the role of ungulates on δ^{15}N processes in a forested ecosystem.

Methods

Data Collection

Forest patches of interest were determined by evaluating white-lipped peccary movement data from 2013-2018 (Jorge, unpublished data) as well as through interviews with local farm owners and farm managers, and camera-trap information (Keuroghlian, unpublished data). Experts of the land, locals' knowledge was invaluable to ensure absence of the white-lipped peccary in many cases. Patches were of similar plant composition and forest type (dense savannah woodland or cerradão). By determining forest patches with and without white-lipped peccary presence, natural enclosure and enclosure plots were established. This was done in both the Bodoquena region (n=6) and the Corguinho region (n=8).

Within each forest patch, three sampling locations were established along a 300 m transect with each sampling location at least 100 m away from the edge of the forest and 100 m away from the previous sampling location. This ensured that there was no edge-effect in the samples and plant and soil composition was similar. At each sampling location, three soil samples were taken 1 m apart from each other using a 5 cm x 10 cm punch soil corer (Frank and Groffman, 1998, Feeley, 2005) (Figure 6). This method captures the top 10 cm of the soil. This is the typical depth sampled as generally, the nitrogen on the forest floor and within 0-15 cm depth is responsible for half, or more than half, of the nitrogen mineralized (Binkley and Hart, 1989). Surface litter was cleared before any samples were taken. The samples were clearly labeled and stored in a plastic bag until they could be dried in a field oven (maximum storage time of 6 hours before drying). Soil samples samples were weighed and then dried for 48 hours at 60°C. The dry samples were weighed again to determine water weight lost. After drying, 100 grams of each sample were placed in a soil bag and stored in a dry Styrofoam box to be shipped for δ^{15}N and δ^{13}C analysis. For NH_4^+ and NO_3^- analyses, 30 grams of dry soil from each soil core at a given sampling location (n=3) were mixed together in a bag, in order to create a homogenous sample that represented each sampling location. Bags were also stored in a dry Styrofoam box to be shipped for laboratory analysis.

The soil profile was recorded at the first sampling location of each forest patch to determine root depth, soil type, and clay content. Clay content was categorized using the ribbon test (Brown, 2018). If a slope was detected at any sampling location, a general classification was made and its direction recorded.

A plant survey was conducted at each sampling location (Figure 6). The survey was taken along four transects in each of the cardinal directions. The starting point for each transect was be the northernmost point of the soil sample triangle. Each transect was 10 m in each cardinal direction and 2 m wide. All trees with a circumference of 10 cm or more were identified to the genus level with the help of a local botanist.

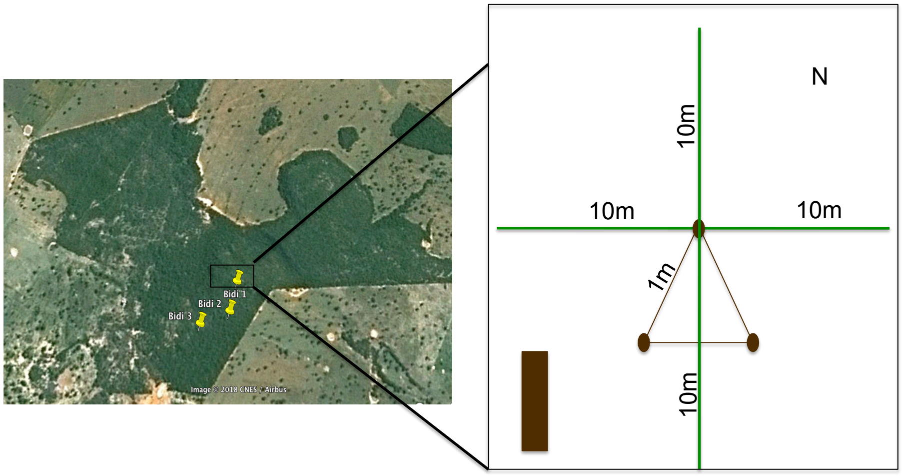

Figure 6: Schematic of sampling at a forest patch. Green lines represent the plant survey, brown circles represent the soil cores take and the brown rectangle represents the soil profile taken.

Laboratory Analysis

To analyze NH_4^+ and NO_3^- concentrations, as well as pH, samples were shipped to the Oregon State University Soil and Crop Central Analytical Laboratory. There, available nitrogen was extracted using 2 ml KCl. The filtered extracts were then analyzed using a Lachat 8500 Series 2 Flow Injection Analyzer. pH was determined using a Hanna HI5522 with appropriate probes.

Samples for isotope analysis were finely ground using a ball mill grinder at the... Isotope analysis, as well as total carbon and nitrogen analysis was performed at the University of California-Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Nitrogen isotope results were reported in relation to atmospheric N_2 while carbon isotope results were reported in relation to Vienna PeeDee Belemnite. They are expressed in standard delta notation where

$$\delta = [\frac{R_{sample}/R_{standard}}{R_{standard}} - 1] * 1000$$

resulting in a δ value which is the isotope ratio of the sample relative to the respective standard.

Statistical Analysis

Because isotopic data, as well as total soil nitrogen and total soil carbon, values were collected at a higher resolution than the rest of the data (three samples per sampling location), the medians of the values for each sampling location were taken when analyzing those variables.

To determine whether parametric or non-parametric tests should be used, a Kolmogorov-Smirnov test was employed to examine the normality of the distribution of the data collected. All of the data showed non-normal distribution, and therefore nonparametric tests were used. To statistically evaluate if the chosen forest patches were of similar plant composition, a detrended correspondence analysis (DCA) was used. A Kendalls rank test was used to evaluate possible correlations among soil variables (pH, clay content, NH_4^+, NO_3^-, total available nitrogen, total soil nitrogen, and total soil carbon). Because the two regions (Bodoquena and Corguinho) were 100 km apart, a principal component analysis and a Mann-Whitney U test were used to investigate regional differences in both soil and nutrient characteristics. Finally, in order to evaluate the effect of white-lipped peccaries on soil and nutrient variables, farms with and without white-lipped peccaries were compared using a Mann-Whitney U test and a linear discriminant analysis. Throughout, α=0.05 was used to reflect statistical significance.

All statistical analyses were performed in RStudio 1.1.423 (R Core Team, 2017) using the packages: EnvStats (Millard, S.P., 2013), extrafont (Chang, W., 2014), Factoextra (Kassambara, A. et al., 2017), FactoMineR (Le, S., et al., 2008), ggplot (Wickham, H., 2009), ggpubr (Kassambara, A., 2017), Kendall (McLeod, A.I., 2011), klaR (Weihs, C. et al., 2005), MASS (Venables, W. N. et al., 2002), nortest, (Gross, J. et al., 2015), pspearman (Savicky, P., 2014), raster (Hijmans, R.J., 2017), and vegan (Oksanen, J.F. et al., 2018).

Results

General Characteristics of the Forest Patches

Plant Characteristics

All forests sampled were tall closed canopy patches, typically classified as dense savannah woodland (cerradão). Of the 14 forest patches, nine had indicator species which classify the soils within as mesotrophic facies cerrado (Table 3). While the sampling did not reveal the other five forest patches to have plant indicator species for a mesotrophic facies cerradão, they still had soil characteristics and tall canopies typically of a dense savannah woodland (cerradão) system. Further, a detrended correspondence analysis (DCA) evaluation using plant genera showed no distinction between those classified as mesotrophic facies cerrado and those not (Figure 7). Such analysis confirms the forest patches chosen were similar in plant composition.

Farm	Region	Speices
CA	Bodoquena	*Anadenanthera* sp., *Callisthene fasciculata*, *Terminalia argentea*
UF	Bodoquena	*Anadenanthera* sp.,
LA	Corguinho	*Anadenanthera* sp., *Pseudobombax tomento*
SD	Corguinho	*Anadenanthera* sp.,
SP	Bodoquena	*Aspidosperma* sp.,
JA	Corguinho	*Aspidosperma* sp., *Pseudobombax tomento*
CO	Corguinho	*Dipteryx alata*, *Pseudobombax tomento*
MC	Bodoquena	*Astronium fraxinifolium*, *Terminalia argentea*
SA	Corguinho	*Callisthene fasciculata*

Table 3: Indicator plants of mesotrophic facies cerradão in each forest patch as indicated by Furley et al., 1988

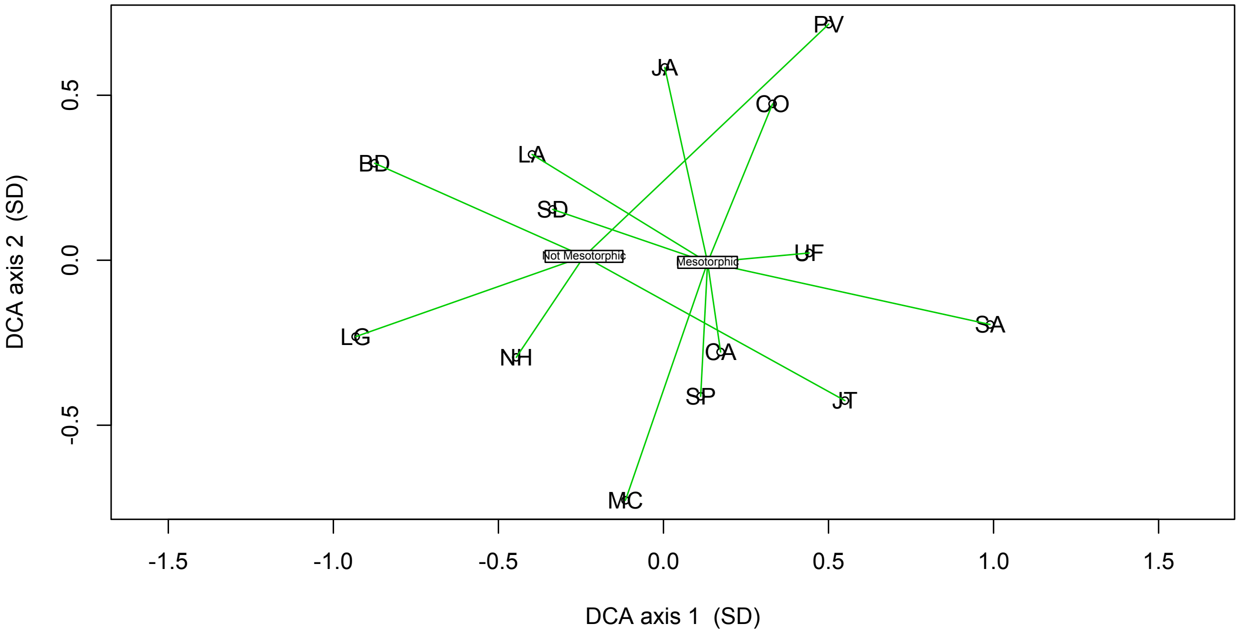

Figure 7: Detrended correspondence analysis (DCA) of plant genus which show no difference in plant composition between those with mesotrophic facies indicator species and those without.

Soil Abiotic Characteristics

Of the samples analyzed for available nitrogen, two from Mato do Celso (MC2 and MC3) returned nitrate values below the quantifiable limit, and thus were assigned the lowest quantifiable limit of .05 NO_3^--N/g dry soil for analysis. Of the samples analyzed for isotopes, one sample, from the third sampling site at Saint Peters Farm, did not return a value due to instrument error.

All soils sampled fell within the general description of Cerrado soils and were reddish in color with deep profiles (Table 4). Clay content, however, varied between each forest patch, ranging from 5 to 45% (N = 14). When considering all forest patches, clay content was significantly positively correlated with pH (Kendalls rank p = 0.0002; $\tau = 0.425$), ammonium (Kendalls rank p = 0.0005; $\tau = 0.406$), total available nitrogen (Kendalls rank $p << 0.0001$; $\tau = 0.581$), total soil nitrogen (Kendalls rank p = 0.0006; $\tau = 0.401$), and total soil carbon (Kendalls rank p = 0.003; $\tau = 0.423$) (Figure 8). Clay was also significantly positively correlated with more positive δ^{15}N values (Kendalls rank p = 0.0006; $\tau = 0.404$) and less negative δ^{13}C values (Kendalls rank $p << 0.0001$; $\tau = 0.584$) (Figure 9). pH was significantly positively correlated with nitrate (Kendalls rank p =0.002; $\tau = 0.326$), total available nitrogen (Kendalls rank p = 0.002; $\tau = 0.320$), total soil nitrogen (Kendalls rank $p << 0.0001$; $\tau = 0.567$) and total soil carbon (Kendalls rank $p << 0.0001$; $\tau = 0.502$) and significantly negatively correlated with C:N ratio (Kendalls rank p = 0.0002; τ = -0.397) (Figure 10). pH was also significantly positively correlated with more positive δ^{15}N values (Kendalls rank p $<<$ 0.0001; τ = 0.539) and less negative δ^{13}C values (Kendalls rank p =0.0005; τ = 0.373) (Figure 11).

Farm	Region	pH Range	Median pH	Clay Content (%)
BD	Corguinho	5.02-5.25	5.15	35
CA	Bodoquena	4.81-6.6	6.11	45
CO	Corguinho	4.67-5.24	5.06	25
JA	Corguinho	5.84-6.12	6.02	30
JT	Corguinho	5.41-6.39	6.18	5
LA	Corguinho	4.6-4.96	4.76	10
LG	Corguinho	4.66-4.79	4.76	35
MC	Bodoquena	5.71-6.14	5.41	45
NH	Bodoquena	6.43-6.61	6.61	45
PV	Bodoquena	6.03-6.47	6.48	45
SA	Corguinho	4.8-5.28	5.05	35
SD	Corguinho	4.57-4.9	4.81	5
SP	Bodoquena	5.66-6.34	5.75	45
UF	Bodoquena	6.5-6.55	6.54	35

Table 4: Soil Characteristics (pH and Clay Content) of the sampled farms.

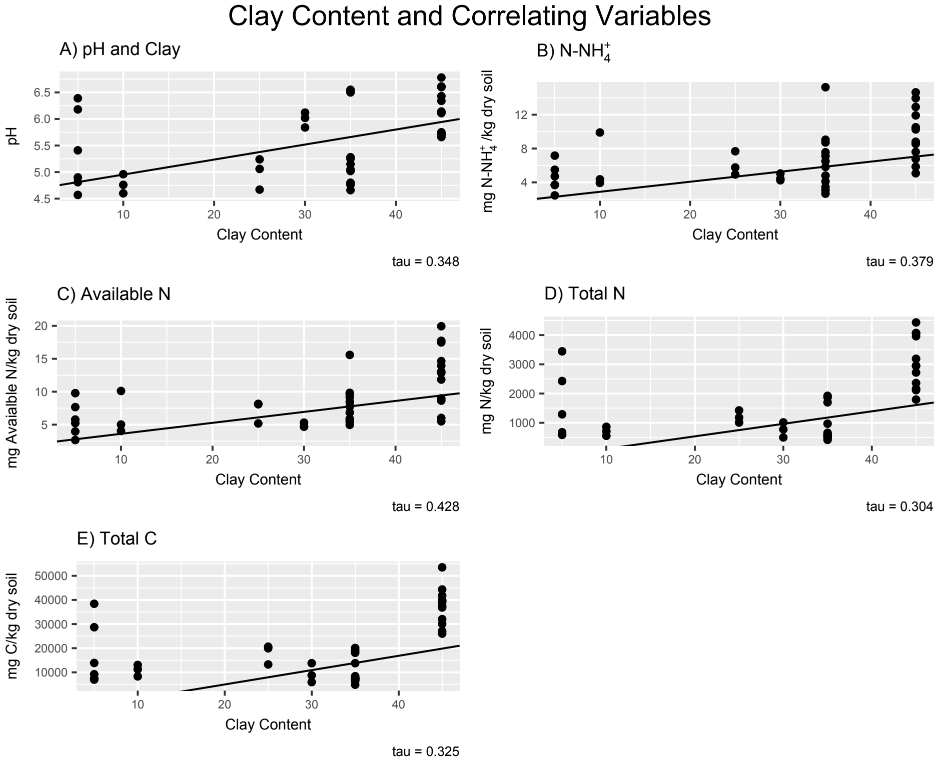

Figure 8: Clay content and significantly correlated soil characteristics and nutrient variables. A) Kendalls rank showed a positive correlation between A) clay and pH ($p = 0.001$; $\tau = 0.348$), B) clay and ammonium ($p = 0.0005$; $\tau = 0.379$), C) clay and available nitrogen ($p << 0.0001$; $\tau = 0.428$), D) clay and total soil nitrogen ($p = 0.005$; $\tau = 0.304$) and E) clay and total soil carbon ($p = 0.003$; $\tau = 0.325$).

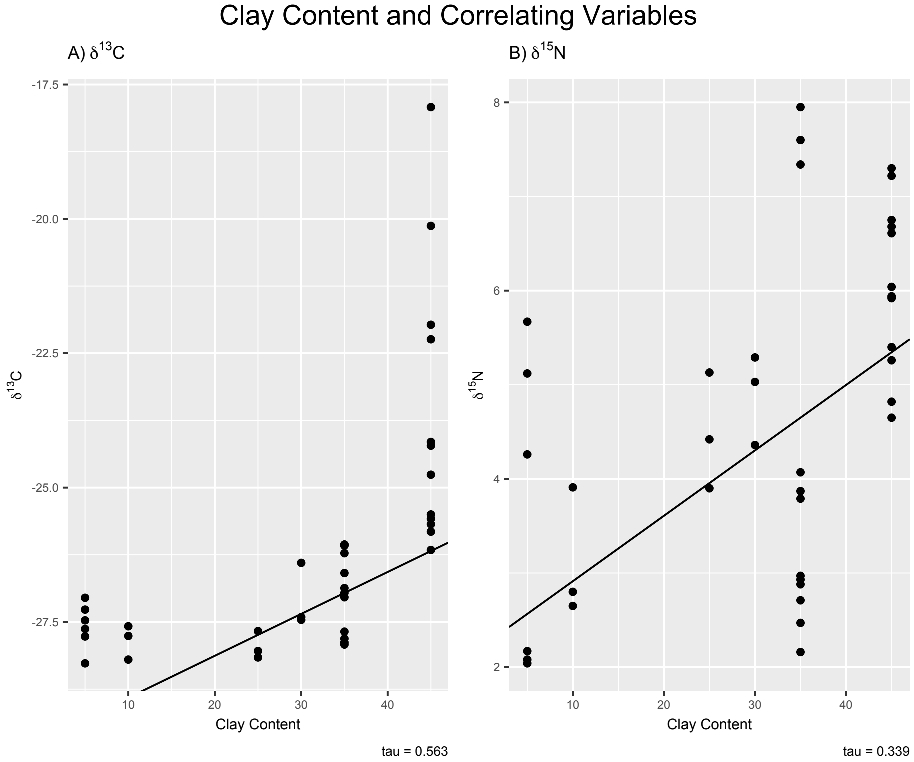

Figure 9: Clay content and significantly correlated soil isotope values. A) Kendalls rank showed a negative correlation between clay and δ^{13}C (p << 0.0001;; τ = 0.563) and B) a positive correlation between clay and δ^{15}N (p = 0.002; τ = 0.339).

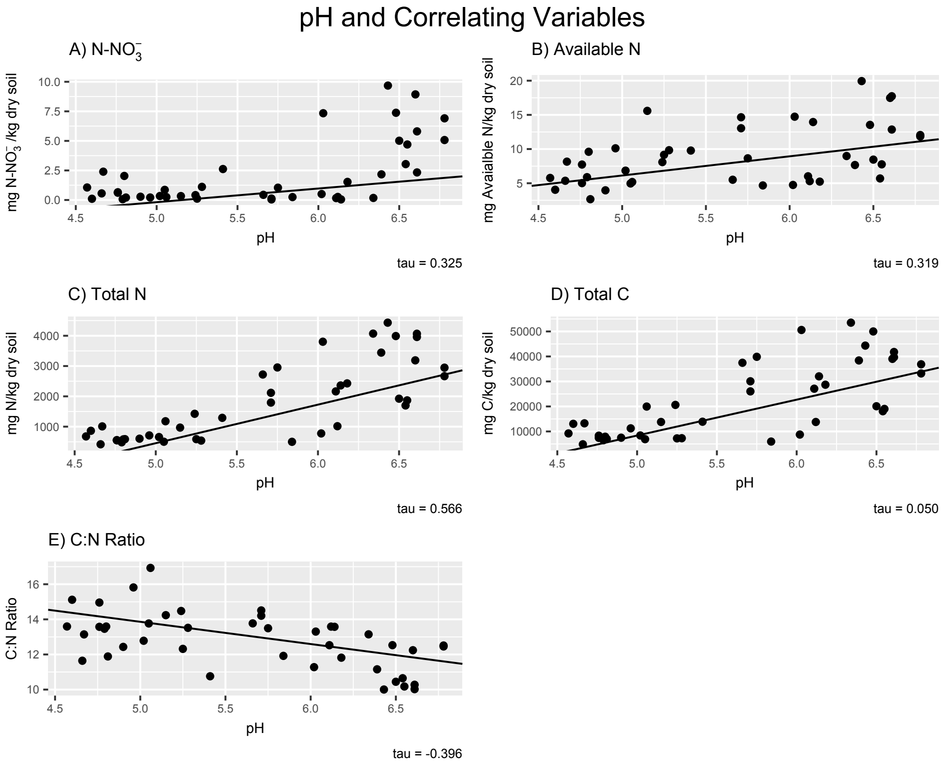

Figure 10: pH and significantly correlated soil nutrient variables. A) Kendall's rank showed a positive correlation between pH and soil nitrate (p = 0.002; τ = 0.325), B) pH and soil available nitrogen (p = 0.003; τ = 0.319), C) pH and total soil nitrogen ($p << 0.0001$; τ = 0.566), D) pH and total soil carbon ($p << 0.0001$; τ = 0.050), and a negative correlation between E) pH and soil C:N (p = 0.0002; τ = -0.396).

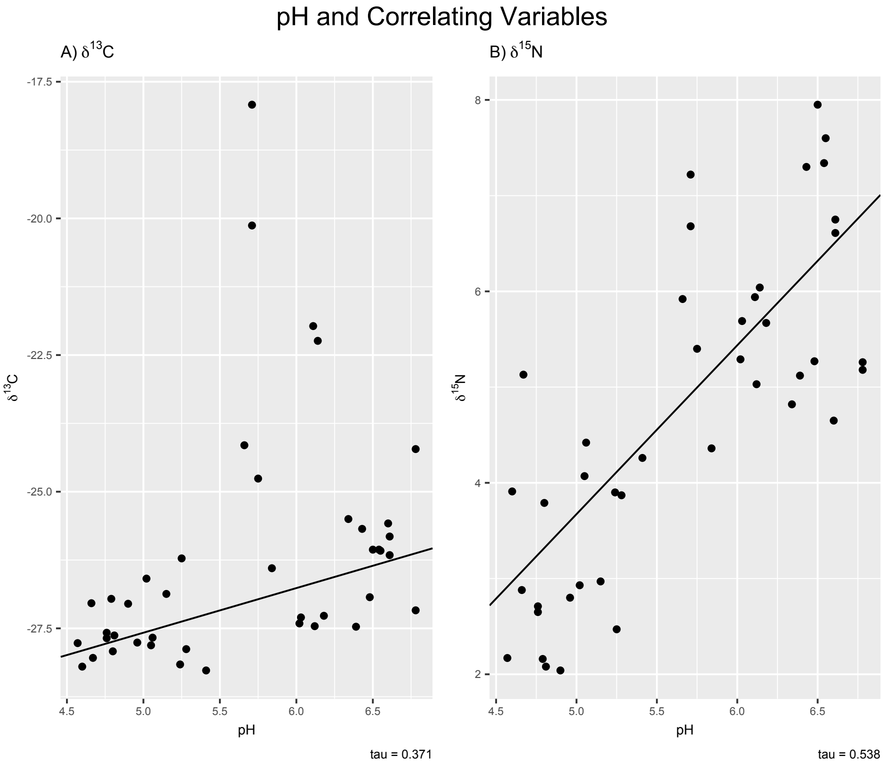

Figure 11: pH and significantly correlated soil isotope variables. A) Kendalls rank showed a positive correlation between pH and more positive soil δ^{15}N values (p $<<$ 0.0001; τ = 0.538). and B) pH and less negative soil δ^{13}C values (p = 0.0005; τ = 0.371).

Corguinho vs Bodoquena

An initial principal component analysis was used to investigate regional differences in both soil and nutrient characteristics. The results suggested there was significant variation between the two regions (Figure 12) and thus a Mann-Whitney U test for significant differences between the regions in all soil characteristics, soil nutrients, and soil isotopic variables.

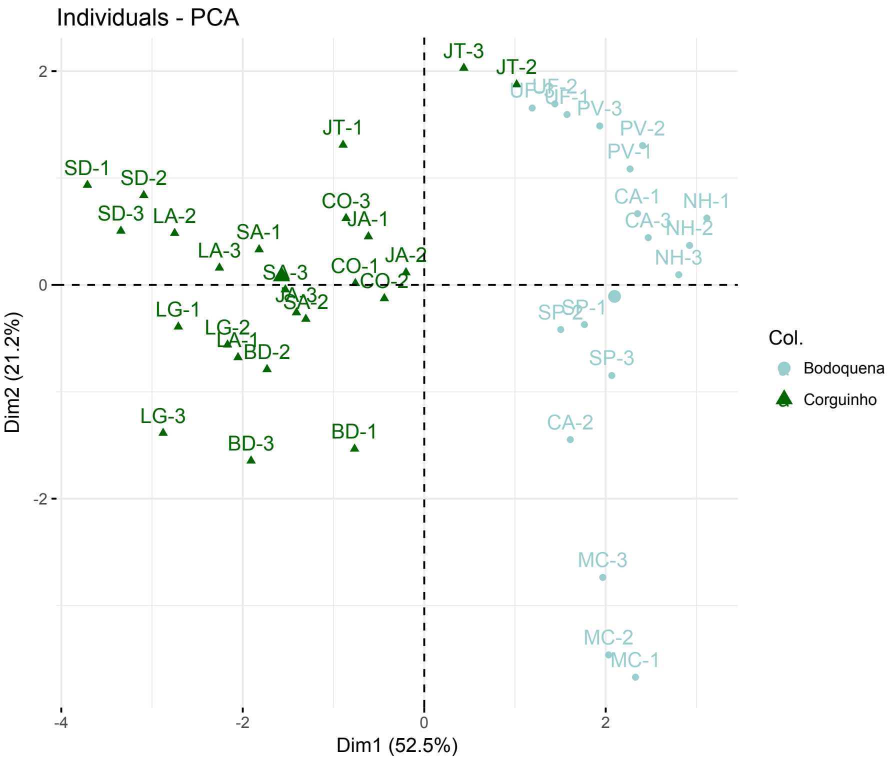

Figure 12: Principal Components Analysis using all measured nutrient and soil characteristics. All data was log transformed. Analysis shows location is the dominant discriminating factor.

Several soil characteristics were statistically different between the two study regions. Bodoquena had significantly higher clay content (Mann Whitney $p << 0.0001$) and pH (Mann Whitney $p << 0.0001$) (Figure 13) when compared to Corguinho. Most nutrient characteristics were different between the two regions as well. Forest patches in Bodoquena had higher nitrate concentrations (Mann Whitney $p= 0.03$), more available nitrogen (Mann Whitney $p = 0.0001$), more total soil nitrogen (Mann Whitney $p << 0.0001$) and more total soil carbon (Mann Whitney $p << 0.0001$). Ammonium concentrations were also marginally significantly higher in Bodoquena (Mann Whitney $p = 0.06$) but C:N ratios were not (Mann Whitney $p = 0.08164$) (Figure 14). Nitrogen isotopes were significantly more positive in Bodoquena (Mann Whitney $p << 0.0001$) while carbon isotopes were significantly less negative (Mann Whitney $p << 0.0001$) (Figure 15 and 16).

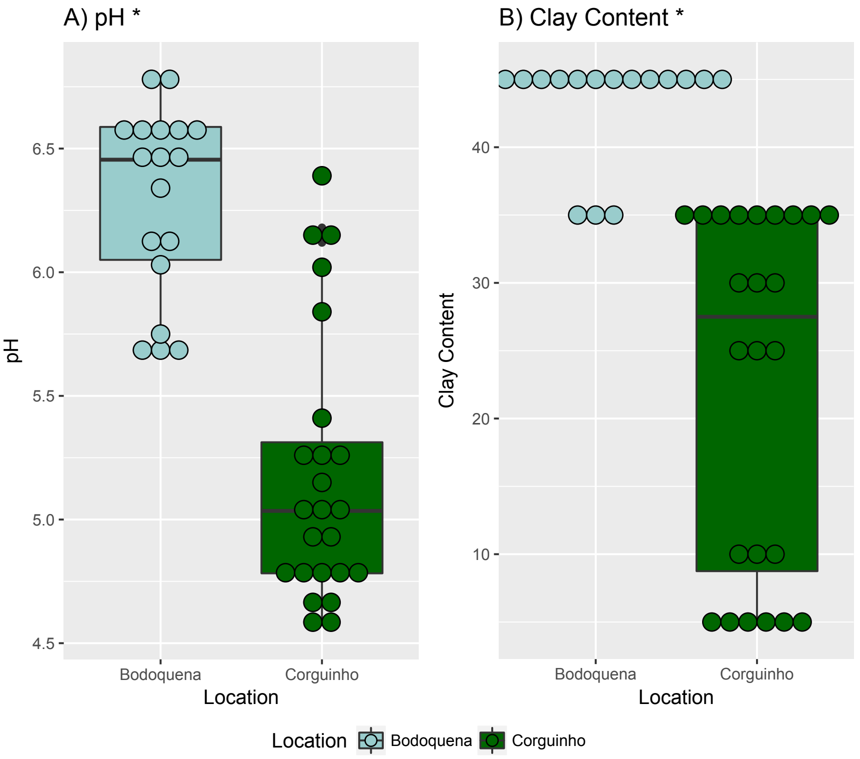

Figure 13: Box plot of soil characteristics between the regions with asterisks marking significant differences. A) pH values were statistically higher in the Bodoquena region compared to the Corguinho region (Mann Whitney $p << 0.0001$). B) Clay content was statistically higher in the Bodoquena region compared to the Corguinho region (Mann Whitney $p << 0.0001$).

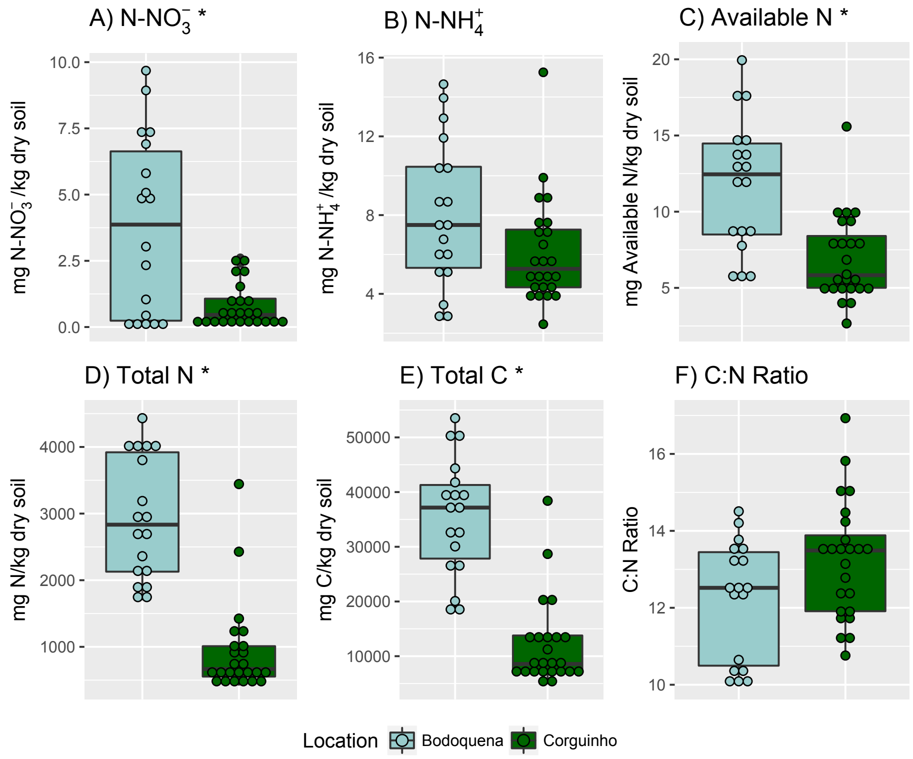

Figure 14: Box plot of nutrient characteristics between two regions with asterisks marking significant differences. Total soil nitrogen and total soil carbon were based on median values. A) Soil nitrate values were statistically higher in the Bodoquena region compared to the Corguinho region (Mann Whitney p = 0.03). B) Soil ammonium values were not quite statistically higher in the Bodoquena region compared to the Corguinho region (Mann Whitney p = 0.06). C) Total soil available nitrogen values were statistically higher in the Bodoquena region compared to the Corguinho region (Mann Whitney p = 0.0001). D) Total soil nitrogen values were statistically higher in the Bodoquena region compared to the Corguinho region (Mann Whitney $p << 0.0001$). E) Total soil carbon values were statistically higher in the Bodoquena region compared to the Corguinho region (Mann Whitney $p << 0.0001$). F) Soil C:N ratios were not significantly different between the two regions. (Mann Whitney p = 0.08164).

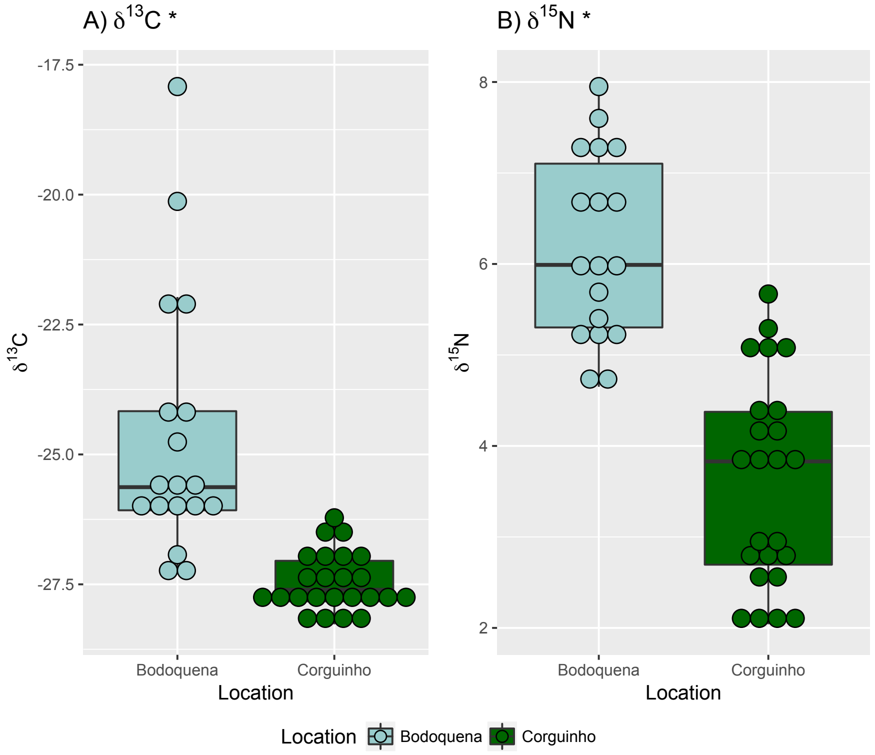

Figure 15: Box plot of isotope characteristics between two regions based on median values with asterisks marking significant differences. A) Soil nitrogen isotopes values were significantly more positive in the Bodoquena region compared to the Corguino region (Mann Whitney $p << 0.0001$). B) Soil carbon isotopes values were significantly less negative in the Bodoquena region compared to the Corguino region (Mann Whitney $p << 0.0001$.)

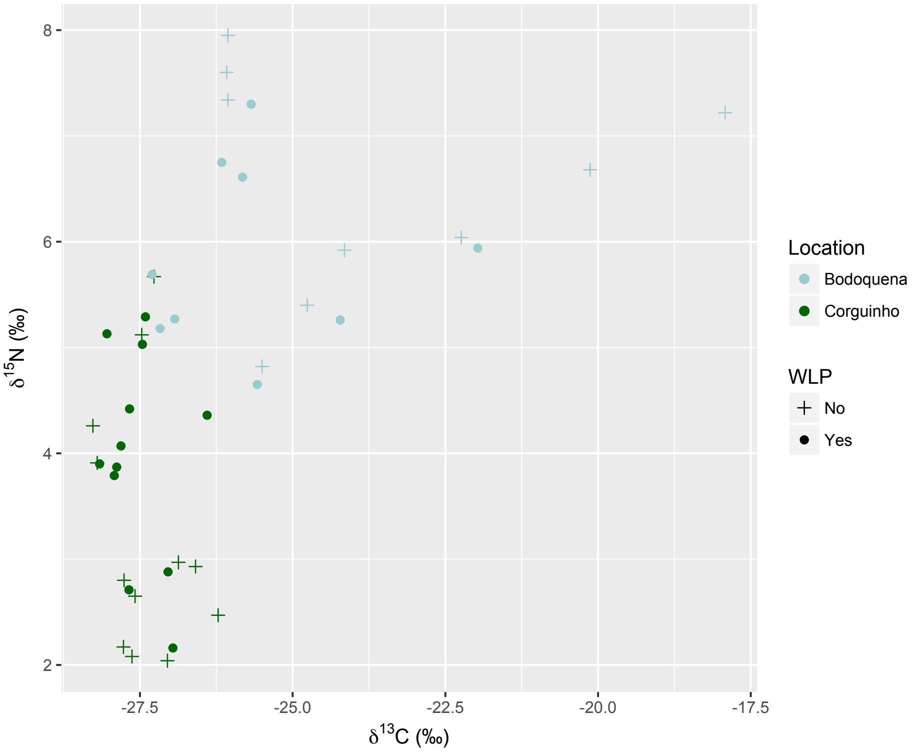

Figure 16: Isoscape of data split by region and white-lipped peccary presence. Triangles represent samples with white-lipped peccaries, circles represent samples without white-lipped peccaries. Green symbols represent Corguinho samples while blue symbols represent Bodoquena samples.

White-Lipped Peccary

Forest patches with white-lipped peccary presence did not have significantly different pH values (Mann Whitney p = 0.399) or clay content (Mann Whitney p = 0.195) when compared to forest patches without white-lipped peccaries (Figure 17). Nitrate was significantly higher in forest patches with white-lipped peccaries (Mann Whitney p = 0.04), however no other soil characteristic was significantly different between forest patches of white-lipped peccary presence or absence, including ammonium (Mann Whitney p = 0.533), total soil available nitrogen (Mann Whitney p = 0.410), total soil nitrogen (Mann Whitney p = 0.901), total soil carbon (Mann Whitney p = 0.980) or soil C:N ratio (Mann Whitney p = 0.533) (Figure 18). The isotopic nitrogen (Mann Whitney p = 0.881) and carbon (Mann Whitney p = 0.291) signals were also not statistically different (Figure 19). Data was then averaged for each forest patch and a linear discriminant analysis using pH and nitrate was able to predict white-lipped peccary presence with a 21.4% error (Figure 20).

Given the different regional results, the impact of white-lipped peccaries within each region was also evaluated. Results showed no significant difference in any nutrient characteristics in the Corguinho forest patches. Clay content was significantly higher in areas with white-lipped peccaries (Mann Whitney p = 0.008), though this seemed to have no impact on any other nutrient or soil characteristic. In the Bodoquena region, however, forest patches with white-lipped peccaries had higher pH values (Mann Whitney p = 0.034). Further, forest patches with white-lipped peccaries had significantly higher nitrate (Mann Whitney p = 0.006), available nitrogen (Mann Whitney p = 0.050) and total soil nitrogen (Mann Whitney p = 0.019) (Figure 21). No other soil or nutrient characteristic was different between forest patches with and without white-lipped peccaries in Bodoquena. While a significant positive correlation was found between pH and nitrate when only considering the Bodoquena region, the correlation was only accounted for almost 40% of the variance (Kendalls rank p = 0.033; $\tau = 0.373$) (Figure 22). Therefore, a linear discriminant analysis was used within the Bodoquena region using pH and nitrate. The results show that given these two variables, the model is able to predict white-lipped peccary present with 100% accuracy (Figure 23).

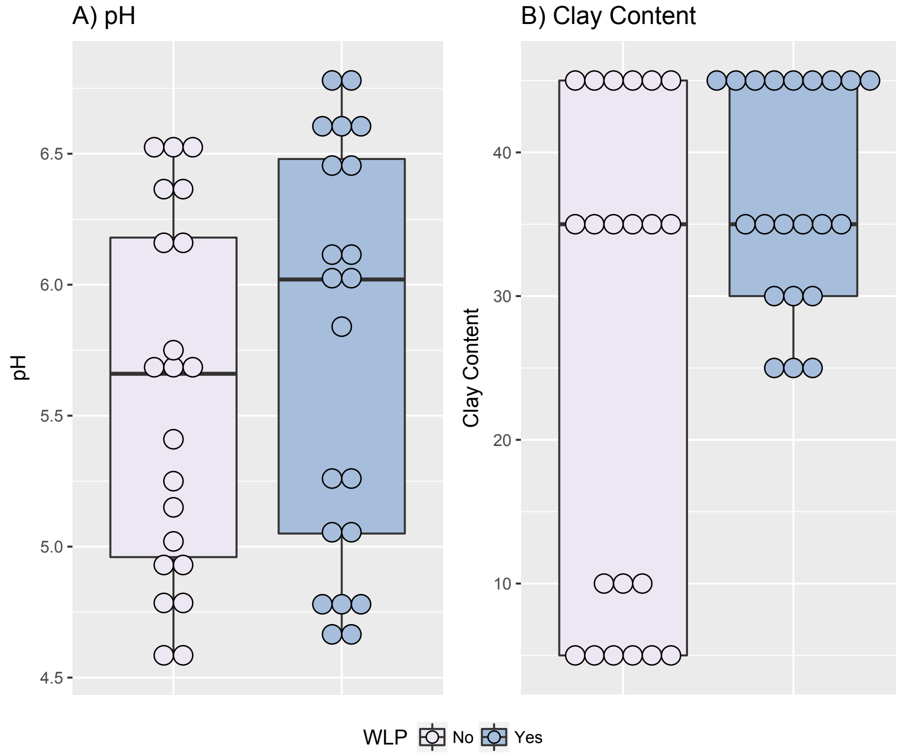

Figure 17: Box plot of soil characteristics in areas with white-lipped peccaries presence and absence with asterisks marking significant differences. A) pH values were not statistically different in areas with white-lipped peccaries or without white-lipped peccaries (Mann Whitney p = 0.399). B) Clay content was not statistically different in areas with white-lipped peccaries or without white-lipped peccaries (Mann Whitney p = 0.195).

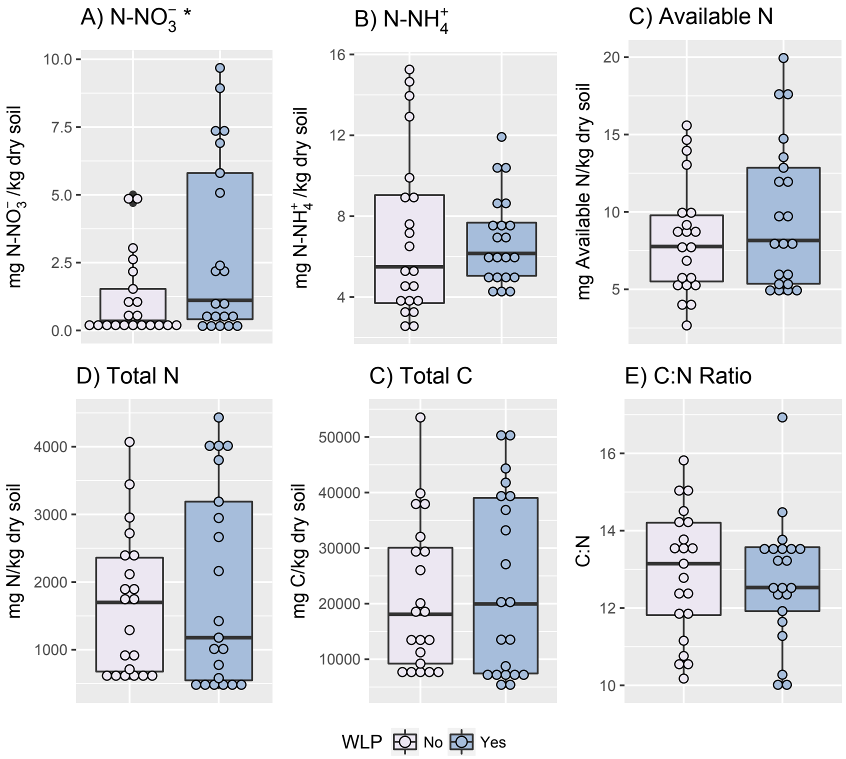

Figure 18: Box plot of nutrient characteristics in areas of white-lipped peccaries presence and absence with asterisks marking significant differences. A) Soil nitrate concentrations were significantly higher in samples with white-lipped peccaries compared to areas without white-lipped peccaries (Mann Whitney p = 0.04). All other nutrient characteristics, including B) ammonium (Mann Whitney p = 0.533), C) total soil nitrogen concentrations (Mann Whitney p = 0.901), D) total soil carbon concentration (Mann Whitney p = 0.980) and E) soil C:N ratios (Mann Whitney p = 0.5333) were not significantly different in samples with white-lipped peccaries compared to areas without white-lipped peccaries.

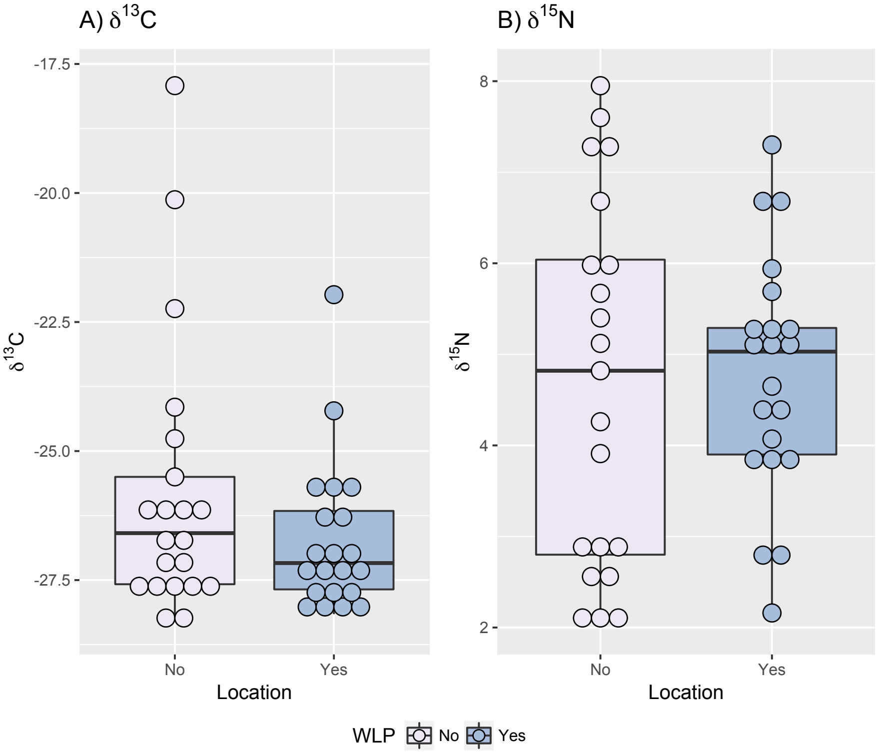

Figure 19: Box plot of isotope characteristics in areas of white-lipped peccaries presence and absence with asterisks marking significant differences. Neither A) soil isotopic nitrogen values (Mann Whitney p = 0.881) nor soil isotopic carbon values t (Mann Whitney p = 0.291) were significantly different in areas with white-lipped peccaries compared to areas without white-lipped peccaries.

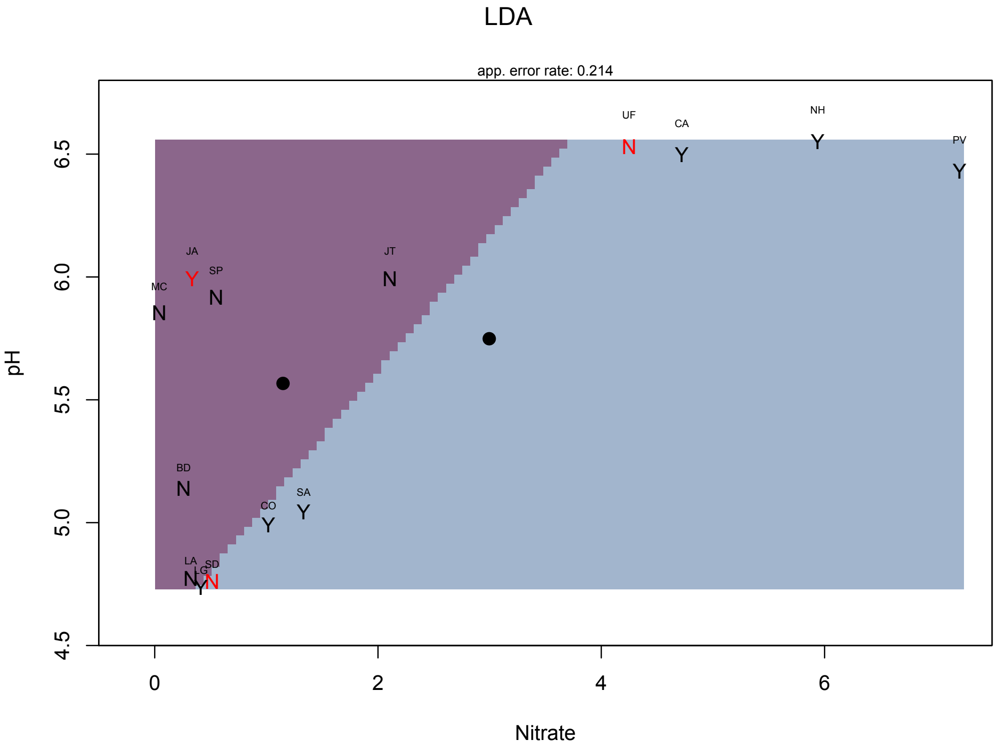

Figure 20: Linear discriminant analysis using nitrate and pH in the forest patches to determine white-lipped peccary presence and absence. Results show a 78.6% discrimination success rate when considering white-lipped peccary presence and absence based on these two characteristics.

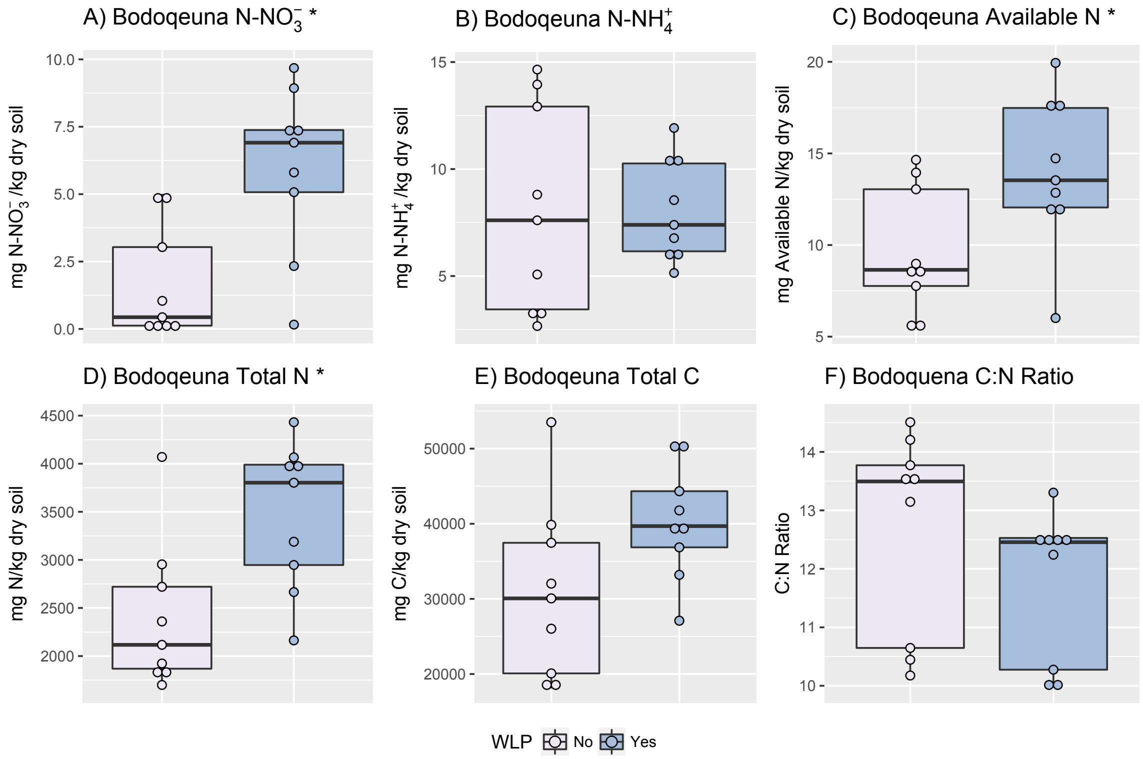

Figure 21: Box plot of nutrient characteristics in Bodoquena with white-lipped peccaries presence and absence with asterisks marking significant differences. A) Soil nitrate concentrations were significantly higher in samples with white-lipped peccaries in Bodoquena compared to areas without white-lipped peccaries (Mann Whitney p = 0.006). B) Soil ammonium concentrations were not significantly different (Mann Whitney p = 0.863) C) Total available nitrogen was significantly higher in samples with white-lipped peccaries in Bodoquena compared to areas without white-lipped peccaries (Mann Whitney p = 0.050). D) Total nitrogen was significantly higher in samples with white-lipped peccaries in Bodoquena compared to areas without white-lipped peccaries (Mann Whitney p = 0.019). E) Total soil carbon was not significantly different (Mann Whitney p = 0.063). F) The C:N Ration was not significantly different (Mann Whitney p = 0.077).

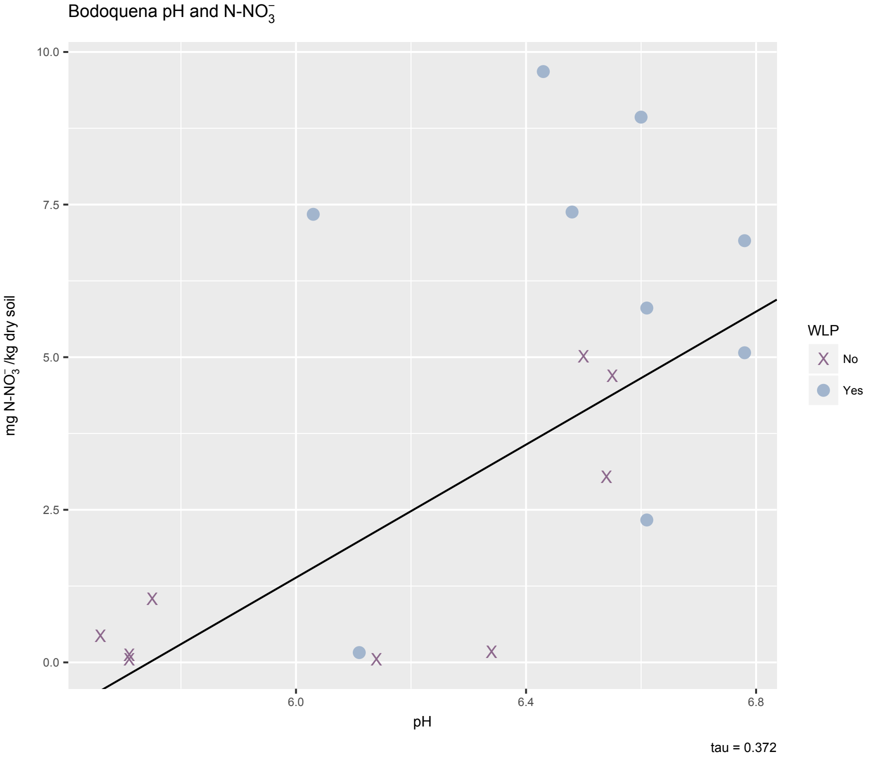

Figure 22: A Kendall’s rank showed a positive correlation between pH and nitrate within the Bodoquena region and accounted for approximately 37% of the variation (Kendall’s rank p = 0.033; $\tau = 0.372$). Patches with white-lipped peccary presence and absence are shown.

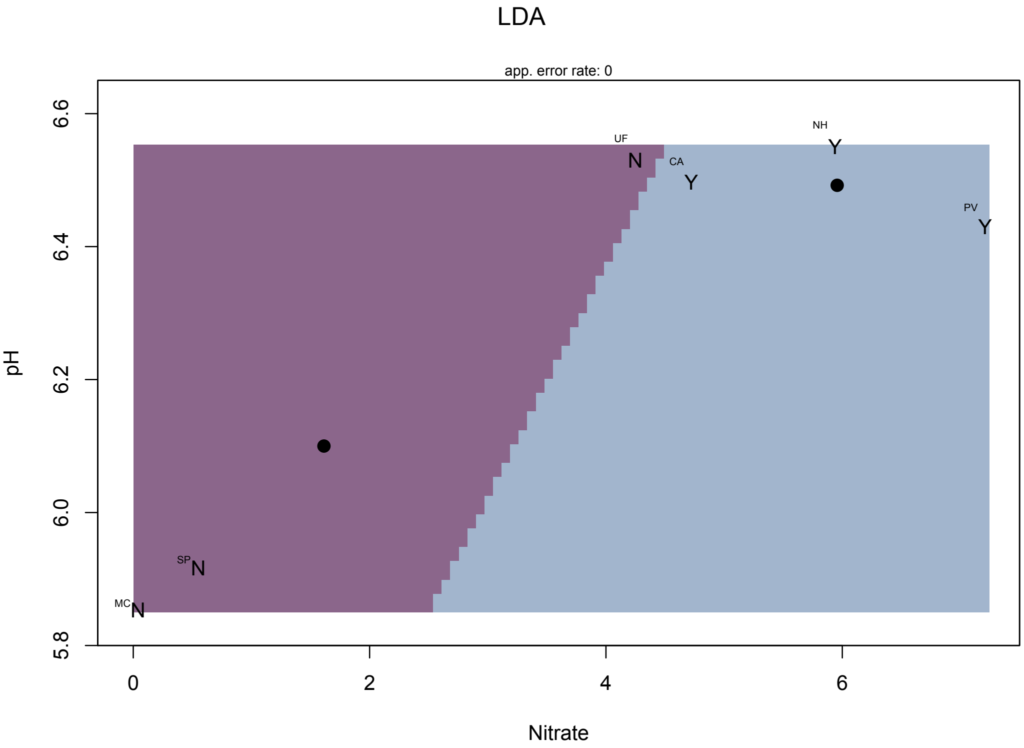

Figure 23: Linear discriminant analysis of the nitrate and pH in the forest patches of Bodoquena. Results show a 100% discrimination success rate when considering white-lipped peccary presence and absence based on these two characteristics.

Discussion

General Characteristics of the Forest Patches

Overall, forest patches were of comparable plant compositions. While the DCA may suggest that the farms Lagoa and Bidi have different plant communities (Figure 8), this signal is likely due to the presence of *Siparuna guianensis* within the sampling scheme. This plant, however, is typical of a dense savannah woodland system (cerradão) (Duca Santos, pers. comm.) and thus does not indicate these two farms are drastically different in vegetation type.

The δ^{13}C signal is however, likely dominated by slight vegetative differences. The forest patches with less negative δ^{13}C values (Mato do Celso, Cachoeira, and St. Peters Farm) have slightly more open vegetation schemes, and are possibly still in the final stages of the transition between cerrado denso and dense savannah woodland (cerrado). There is, however, no significant difference in the δ^{13}C regarding plant composition or peccary use and the composition of plants within is not significantly different.

Clay content was significantly positively related to pH, which translates into a decrease in acidity correlating with an increase in clay content. Frank and Groffman (1998) also found this to be the case in Yellowstone grasslands. Clay acts as a buffer in soils given its inherent negative charge, which attracts positive H^+ ions within the soil and absorbs them (Tan, 2010). Thus, in this case, where the soil is already quite acidic, clay is acting as a buffer and reducing the acidity of the soil.

Clay content was also significantly positively correlated with available ammonium and overall available nitrogen. As mentioned, clay has the ability to fix ammonium due to its inherent negative charge (Osborne, 1976). While fixed ammonium is not specifically measured in this study, the correlation with available ammonium is likely due to the adsorption of ammonium from the clays which occurs when nitrogen is the limiting element to growth. Thus clay acts as a storehouse of ammonium to replenish supplies as needed, resulting in higher ammonium and higher total available nitrogen concentrations. Clay content was also positively correlated with total soil nitrogen and total soil carbon. This may be a result of the increased available nitrogen, which stimulates growth. Further, the presence of fixed ammonium may decrease the loss of nitrogen in the system as fixed ammonium is less likely to be converted into nitrate, which is susceptible to denitrification (Nider et al., 2011). Finally, clay was significantly positively correlated with more positive δ^{15}N values. Soil ammonium is relatively enriched in δ^{15}N , especially compared to nitrate (Craine et al., 2009b), and therefore this relationship may be due to increased ammonium in the system.

Soil pH values fell within the range of mesotrophic facies (Ratter et al., 1977; Furley and Ratter, 1988). The results show that soil pH was significantly positively correlated with nitrate, total available nitrogen, total soil nitrogen, and total soil carbon and significantly negatively correlated with C:N ratios. As mentioned, pH is positively related to nitrification, the biological processes of transforming ammonium into nitrate, in tropical soils (Sahrawat, 1982). The results here suggest soil conditions with a pH below 6 may limit nitrification to some extent, however once this threshold is met, nitrate is significantly more abundant. Again, increased levels of available nitrogen stimulate growth, and thus the relationship between pH and nitrate may account for the correlation between pH and total soil nitrogen and total soil carbon and the negative correlation with C:N ratios. Soil pH was also positively correlated with δ^{15}N values. With more nitrate in the system, the more denitrification occurs. The processes of both nitrification and denitrification leaves the substrate enriched in δ^{15}N (Nadelhoffer and Fry 1994; Frank and Evans, 1997) and therefore increased pH may result in a faster nutrient cycling.

It should be noted that while the study measured available nitrogen in a single point in time at each plot, a better approach would be to use ion exchange membranes (Feeley et al., 2005; Lajtha, 1988). Variations in soil moisture or slope could inhibit single measurements to provide clear information on nitrogen availability (Binkley and Hart, 1989). Using an ion membrane, which acts as a sink for the available nutrients over a set amount of time, allows for a better picture of the total availability of nutrients in the soil (Feeley, 2005; Feeley and Terborgh, 2005; Lajtha, 1988).

Regional Difference

The Bodoquena region has significant higher nitrate, total soil nitrogen, and total soil carbon concentrations compared to Corguinho, indicating Bodoquena may be relatively enriched in nitrogen and have an accelerated nitrogen cycle. While the elevated nitrate values cannot identify which processes within the cycle are accelerated, they can help infer that the processes of returning available nitrogen to the system may be increased. Bodoquena is also δ^{15}N enriched compared to Corguinho. This further indicates a faster rate of transformation of nitrogen or a higher nitrogen availability, as proposed by previous studies (Cotesee et al., 2011; Craine et al., 2015), and faster nitrogen cycle, given the precipitation and temperatures are generally the same for the areas. It should be noted, the C:N ratio was not significantly higher in Bodoquena, which is a further indicator of accelerated decomposition and nutrient turnover (Frank and Groffman, 1998; Seagle et al., 2002; Singer and Schoenecker, 2003; Pastor et al., 1993). However, increased values of carbon may indicate more growth due to more nitrogen availability.

A faster nutrient cycling could be a function of certain soil characteristics. In general, the region sampled is primarily characterized by the karstic formations of the Parana Basin. The basin, however, contains a mosaic of underlying geologic formations, which are likely linked to the soil conditions described. The Bodoquena region is apart of the Serra da Bodoquena karst (Filho and Karmann, 2007), a formation characterized by elevated fertility (though nitrogen has not been examined before this study), high base saturation, and high activity of clay (Silva et al., 2017). The soils from this area are likely a product of nearby limestone parent material (Silva et al., 2017). The Corguinho region is apart of the

Aquidauana Formation, a Permo-Carboniferous sedimentary unit composed of sandstones and diamictites (Souza et al., 2014). The parent rock composition likely accounts for the lower clay content in the farms sampled.

This study shows that clay is positively correlated with ammonium. As mentioned previously, clay can both absorb and adsorb ammonium ions (Osborne, 1976), and the positive correlation is likely a product of the clays fixing ammonium when it is deposited and adsorbing it when it becomes limiting in the system (Marschner, 1995; Saha et al., 1986; Nieder et al., 2011). It may also explain the almost significant positive correlation of higher clay concentration within the Bodoquena region and elevated concentration of ammonium (Mann Whitney $p = 0.06$) compared to Corguinho. Further, Bodoquena had significantly higher pH concentrations, and even within the region, areas with higher pH have higher δ^{15}N signals. As mentioned, pH is linearly related to nitrification, the biological process of oxidizing ammonium to nitrate (Sahrawat, 1982) which is also demonstrated in the results. Therefore, it is possible that the rate of nitrification is faster within Bodoquena, which is supported by the higher concentration of nitrate and the more positive δ^{15}N signal.

Carbon isotopes were also significantly different between the two regions, though this is likely due to the fact that all three of the farms with more open vegetation (Mato do Celso, Cachoeira, and St. Peters Farm) are found within Bodoquena. It should be noted, these farms are still considered dense cerrado woodland (cerradão), yet are likely in the final stages of growth. This transitional environment is not necessarily characteristic of the forest types within the Bodoquena region, but simply a random coincidence of opportunistic sampling.

White-Lipped Peccary Effect on Soil Nutrients

Forest patches with the white-lipped peccary had no significant difference in soil characteristics or plant characteristics compared to forest patches without the white-lipped peccary. This lack of significant difference supports the claim that these patches can be used to compare the effect of white-lippedpeccary presence and absence on soil nutrients.

The forest patches with the white-lipped peccary did, however, have significantly higher nitrate values. Frank and Groffman (1998) also saw an increase in available nitrogen as a result of grazing ungulates, though it should be noted that the results do not show a significant difference in overall total available nitrogen, but only nitrate. In fact, nitrate was the only significantly different nutrient characteristic, with ammonium, total available nitrogen, total nitrogen and total carbon values showing no significant difference.

The isotopic results also showed no significant difference. While some studies have shown that ungulate presence increases δ^{15}N through the deposition of urine and fecal matter (Frank and Groffman, 1998), no such results were found in this study. This difference is likely because Frank and Groffman, (1998) examined soil specifically under urine patches, while our sampling was representative of the forest at large rather than directly below excretion points. Overall, while these results do not indicate if the increase nitrate values in areas of white-lipped peccary presence are due do to a direct deposition through excretion or through an acceleration of the cycle, they do support the claim that non-grazing ungulates can impact the nitrogen cycle within their system (Hobbs, 1996).

White-lipped peccaries impact on nitrate levels was driven by results from the Bodoquena region (Figure 20 - Corguinho: Mann Whitney p = 0.854, Bodoquena: Mann Whitney p = 0.006). Our results suggest that this is a consequence of the fact that in Corguinho, nitrate and nitrogen levels are, in general, highly depleted, thus any nutrients deposited via fecal matter are quickly assimilated by plants. On the other hand, in Bodoquena, which has an overall higher nitrogen composition, the nitrate added to the system by the white-lipped peccaries may not be immediately used. It should be noted that the farms with white-lipped peccaries in Bodoquena do have higher available nitrogen values (Mann Whitney p = 0.050) and total nitrogen values (Mann Whitney p = 0.019) but no other significant difference in nutrient composition. Bodoquena's overall higher nitrogen in the soil may be due in part to a significantly higher pH in the region (Mann Whitney p = 0.034), which, is positively correlated with higher rates of nitrification. Nevertheless, the lack of significant difference in the δ^{15}N signal suggests that the nitrogen cycle, and nitrification in particular, is not necessarily occurring at a faster rate. Further, a Kendall's rank correlation showed that the positive correlation between pH and nitrate only accounted for approximately 37% of the variance in the data (Kendall's rank p = 0.033; τ = 0.373) (Figure 22). Through another angle, a linear discriminant analysis (LDA) suggests that pH and nitrate can predict white-lipped peccary use in both regions with a 78% success rate (Figure 20). Two of the three outliers, (Jacobina and Santa Adelaide) are within the Corguinho region, and a LDA performed only with Bodoquena shows a 100% accurate prediction of white-lipped peccary presence given nitrate and pH values (Figure 23). It should be noted, the one farm with higher nitrate and pH values, yet to white-lipped peccary presence, (Unnamed Farm) had abnormally high evidence of tapir, which may be contributing nitrate in this system. Further, there were only six sites analyzed within the Bodoquena region, providing a small sample size. Regardless, these results support the suggestion that white-lipped peccaries are, at least in part, a source of the additional nitrogen in these systems.

Conclusion

The results presented in this study show a distinct difference in regional soil characteristics and soil nutrient content. This is likely partially due to underlying geological differences that alter the clay content and pH of the soil. Further, as noted in Furley and Ratter, (1988), rainfall and temperatures are rarely limiting to plant growth throughout the region. The results here suggest nitrogen may be acting as a limiting element to plant growth, as seen by increased total soil carbon in areas with increased available nitrogen.

While the results show that the white-lipped peccary may be impacting the nutrient cycle locally, it is the soil characteristics that are the prevalent control on the nitrogen cycle. As mentioned, the role of ungulates in nutrient cycling is highly ecosystem specific. In Yellowstone grasslands, net mineralization of soil nitrogen was higher outside of ungulate exclosure plots and in fact were about double that of the ungrazed plots. However no effect of ungulates was found in Rocky Mountain National Park (Binkley and Hart, 2003). In the boreal forests of Isle Royale National Park, Pastor et al. (1993) found that moose browsing over long periods (40 years) reduces soil nutrient activity and microbial activity. The study also found lower total soil carbon and total soil nitrogen, lower nitrogen mineralization rates and lower microbial respiration rates outside exclosure plots. The results presented here indicate white-lipped peccaries presence may be increasing nitrate values in some of the systems they inhabit, either through direct deposition from fecal matter or through increased rates of nitrification. Further investigations should measure the rate of nitrogen cycle process as well as ion exchange membranes, which capture nitrate and ammonium values more accurately (Feeley 2005; Feeley et al., 2005; Lajtha, 1988) to better untangle the exact role of the white-lipped peccary.

Further, to better understand the role of ungulates on the nutrient cycle in the forests of the Brazilian Cerrado, proper exclosure plots should be constructed. This will allow for a direct comparison of nutrient cycling when white-lipped peccaries are excluded and will allow for a precise knowledge of how long the animal has been removed from the system. In addition, this will allow for the exclusion of the tapir, whose scat may also act as a natural fertilizer to the system.

Our results do however, provide a basis for such further investigation. Understanding the role of large mammals will not only help in the conservation of the species investigated, but also help determine how to best develop land without losing important ecosystem services in tropical forests.

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APENDIX

Sample Name	Location	WLP	pH	Clay Content	NO3-N/g dry soil	NH-N/g dry soil
BD-1	Corguinho	No	5.15	35	0.331087064	15.2540294
BD-2	Corguinho	No	5.02	35	0.335960911	6.507153824
BD-3	Corguinho	No	5.25	35	0.112096552	9.044531042
CA-1	Bodoquena	Yes	6.6	45	8.932720779	8.548212896
CA-2	Bodoquena	Yes	6.11	45	0.160617386	5.855805019
CA-3	Bodoquena	Yes	6.78	45	5.072443247	6.771131572
CO-1	Corguinho	Yes	5.06	25	0.250077303	4.930416293
CO-2	Corguinho	Yes	5.24	25	0.413044359	7.677704542
CO-3	Corguinho	Yes	4.67	25	2.394487772	5.760919117
JA-1	Corguinho	Yes	6.02	30	0.504669519	4.244005831
JA-2	Corguinho	Yes	6.12	30	0.258444556	5.048143158
JA-3	Corguinho	Yes	5.84	30	0.246089503	4.432718779
JT-1	Corguinho	No	5.41	5	2.619046373	7.162653765
JT-2	Corguinho	No	6.39	5	2.170725468	5.494179419
JT-3	Corguinho	No	6.18	5	1.531197254	3.701824836
LA-1	Corguinho	No	4.96	10	0.210854442	9.900152538
LA-2	Corguinho	No	4.76	10	0.6556315	4.361071341
LA-3	Corguinho	No	4.6	10	0.103529461	3.939317224
LG-1	Corguinho	Yes	4.66	35	0.560139563	4.797772735
LG-2	Corguinho	Yes	4.76	35	0.615737544	7.116906011
LG-3	Corguinho	Yes	4.79	35	0.066752288	5.82111697
MC-1	Bodoquena	No	5.71	45	0.123239647	12.92167914
MC-2	Bodoquena	No	5.71	45	0.05	14.65030202
MC-3	Bodoquena	No	6.14	45	0.05	13.95173503
NH-1	Bodoquena	Yes	6.43	45	9.679067635	10.26190097
NH-2	Bodoquena	Yes	6.61	45	5.805094391	11.91914905
NH-3	Bodoquena	Yes	6.61	45	2.332821757	10.52086584
PV-1	Bodoquena	Yes	6.03	45	7.340409576	7.391184917
PV-2	Bodoquena	Yes	6.48	45	7.378187277	6.155912848
PV-3	Bodoquena	Yes	6.78	45	6.908641359	5.142383534
SA-1	Corguinho	Yes	5.05	35	0.85822981	4.108620288
SA-2	Corguinho	Yes	5.28	35	1.110660194	8.719398449
SA-3	Corguinho	Yes	4.8	35	2.034914672	7.564869929
SD-1	Corguinho	No	4.81	5	0.208321418	2.457493005
SD-2	Corguinho	No	4.57	5	1.058899362	4.715597624
SD-3	Corguinho	No	4.9	5	0.27691396	3.687652089
SP-1	Bodoquena	No	5.75	45	1.041739177	7.605162179
SP-2	Bodoquena	No	5.66	45	0.4356652	5.072934384
SP-3	Bodoquena	No	6.34	45	0.173707553	8.805400602
UF-1	Bodoquena	No	6.5	35	5.013761677	3.441230549
UF-2	Bodoquena	No	6.55	35	4.698006374	3.068095609
UF-3	Bodoquena	No	6.54	35	3.036248653	2.661483347

Total Avialable N	Median Total N	Median δ15N	Medina Total C	Median δ13C
15.58511646	968.505	2.97	13789.368	-26.87
6.843114735	657.389	2.93	8401.703	-26.59
9.156627593	586.941	2.47	7230.277	-26.22
17.48093368	3188.632	4.65	39032.896	-25.58
6.016422404	2162.911	5.94	27097.301	-21.97
11.84357482	2946.723	5.26	36862.871	-24.22
5.180493596	1178.504	4.42	19950.765	-27.67
8.0907489	1424.047	3.9	20614.976	-28.16
8.155406889	1008.547	5.13	13254.902	-28.04
4.748675351	775.837	5.29	8749.383	-27.41
5.306587714	1014.572	5.03	13785.064	-27.46
4.678808281	499.215	4.36	5950.373	-26.4
9.781700138	1290.557	4.26	13885.146	-28.27
7.664904887	3443.301	5.12	38407.462	-27.47
5.23302209	2427.882	5.67	28690.073	-27.27
10.11100698	710.665	2.8	11241.337	-27.76
5.016702841	555.776	2.65	8312.859	-27.58
4.042846685	863.34	3.91	13049.471	-28.2
5.357912298	417.793	2.88	4863.949	-27.04
7.732643555	548.217	2.71	7441.312	-27.68
5.887869258	483.428	2.16	6508.512	-26.96
13.04491879	1794.076	7.22	26027.769	-17.92
14.65030202	2116.54	6.68	30066.775	-20.13
13.95173503	2360.073	6.04	32035.765	-22.24
19.94096861	4431.841	7.3	44334.764	-25.68
17.72424344	3959.354	6.75	39689.422	-26.16
12.85368759	4066.284	6.61	41780.218	-25.82
14.73159449	3802.656	5.69	50586.221	-27.3
13.53410013	3990.13	5.27	49997.258	-26.93
12.05102489	2665.673	5.18	33202.186	-27.17
4.966850098	501.756	4.07	6907.175	-27.81
9.830058643	539.415	3.87	7290.867	-27.88
9.599784601	580.465	3.79	7889.302	-27.92
2.665814423	587.501	2.08	6983.467	-27.63
5.774496986	677.725	2.17	9212.862	-27.77
3.964566048	602.81	2.04	7493.296	-27.05
8.646901356	2953.459	5.4	39855.596	-24.76
5.508599584	2720.588	5.92	37466.493	-24.15
8.979108155	4070.399	4.82	53511.523	-25.5
8.454992226	1922.664	7.95	20082.023	-26.06
7.766101984	1868.292	7.6	19010.369	-26.08
5.697732	1699.413	7.34	18092.971	-26.06

Median C:N Ratio	Longitude	Latitude
14.2377871	7816358.57	694192.5632
12.78041312	7816258.82	694161.177
12.31857546	7816219.81	694043.0257
12.2412671	7711480.12	534525.1946
12.52816274	7711566.77	534473.1976
12.50978494	7711657.67	534509.8324
16.92889036	7822703.32	677773.2852
14.47633119	7822802.15	677797.7594
13.14257243	7822895	677823.4324
11.27734692	7790270.79	689225.394
13.58707317	7790198.19	689150.502
11.91945955	7790130.02	689075.8676
10.7590335	7814491.2	682347.2074
11.15425634	7814540.59	682269.9428
11.81691408	7814649.44	682224.7323
15.81805351	7804146.02	680988.7188
14.95721118	7804121.56	681085.4681
15.11510066	7804087.04	681180.7521
11.64200693	7815441.69	697958.2218
13.57366152	7815514.82	697883.1405
13.46324996	7815561.68	697801.0569
14.50761785	7719013.77	530366.5795
14.20562569	7718962.49	530266.9906
13.57405682	7718917.27	530177.4149
10.00369011	7685717.91	536925.7422
10.02421658	7685780.61	537001.2669
10.27479094	7685830.63	537100.2638
13.30286542	7737907.97	510188.4276
12.53023285	7737943	510286.282
12.45546097	7737933.2	510383.3795
13.76600379	7824703.68	707244.1296
13.51624816	7824641.76	707160.9678
13.59134832	7824578.74	707085.8703
11.88673211	7829648.41	692755.5376
13.59380575	7829556.48	692727.9003
12.43060998	7829452.03	692702.0167
13.4945486	7688618.26	543403.7899
13.77146889	7688724.45	543422.2658
13.14650554	7688801.05	543418.305
10.44489469	7657092.39	559615.4683
10.1752665	7657020.33	559709.565
10.64660033	7656910.61	559719.2358