

Investigating soil carbon dynamics along land use change gradients in subtropical and tropical grazing lands

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Statement of Authorship

This thesis has been prepared as a set of papers intended for submission to peer-reviewed journals. The chapters are presented in the format of the papers intended for submission to journals. Each paper's reference list is found in a combined reference list at the end of the thesis.

Chapter 1 provides a general introduction of the research area and the aims, objectives and key hypotheses of the thesis. It is not intended for publication.

Chapter 2 is intended for publication

Durcan, R., Ostle, N., Quinton, J. N., Rufino, M. C. (2022). RD and MCR designed the research. RD carried out the data collection and analysis. RD prepared the manuscript with input from MCR, JQN and NO.

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Chapter 5 comprises a general discussion and conclusions and is not intended for publication

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Abstract

Tropical and subtropical forests play an important role in the carbon cycle. Being situated in warmer climates, these forests have the potential to sequester large amounts of soil carbon and mitigate against climate change. Despite this, due to increased demand for livestock and other agricultural products, deforestation followed by grazing in these climates have led to the emission of large amounts of greenhouse gas emissions, and degradation of soils. In order to understand how soil carbon sequestration can be maximized in these environments in the future, it is imperative to understand the impacts of land use change on soil carbon dynamics and the complex interactions between soil characteristics and vegetation, something which is understudied in particular for the tropics and sub-tropics. This thesis aimed to investigate the impacts of land use conversion for grazing on soil carbon dynamics and soil quality through the analysis of existing data, and further detailed research into a subtropical dry forest ecosystem. A meta-analysis gathered existing data to investigate the impacts of grazing and land management practices on soil organic carbon and other soil properties in subtropical and tropical climates, identifying that low intensity grazing (<1 animals per ha) can increase soil organic carbon stocks by 14%, whilst high intensity grazing decreases soil carbon by 18% in tropical grasslands. However, in the subtropics grazing practices decrease carbon stocks by 18 – 28% under all grazing intensities. Changes in soil carbon under grazing were also affected by soil nutrients (N, P), vegetation type, dominant photosynthesis pathway, mean annual precipitation and temperature. More studies were undertaken in the tropics (n = 40) than the subtropics (n = 14 highlighting that further work is needed in order to understand drivers of soil carbon dynamics in this climate. Further investigation into the conversion of subtropical forests into grazing lands through a chronosequence design over a land use transition in the Chaco, Argentina (Forest – Young Pasture (0-5 years) – Intermediate Pasture (10-15 years) – Old Pasture (>20 years), showed that deforestation decreases soil carbon stocks (0-50 cm) in the topsoil by 16 – 53%, alongside decreases in soil nitrogen (5 – 55%), total phosphorus (6 – 68%), and root biomass (68 – 82%). Whilst age of pasture did not impact soil C or N, total P was observed to increase by 46% between intermediate and old pastures. Deforestation in this region has increased pH and decreased electrical conductivity (by 8 – 13%, and 28 – 69% respectively), and increased the salinity and sodicity of these soils, with changes in the dominance of cations in sites with the loss of native vegetation. The analysis of phospholipid-derived fatty acids (PLFAs) in soil samples – a proxy of microbial community composition showed that intermediate pastures had decreased total fungal and total gram-negative bacteria than forest soils in the topsoil, whereas in the subsoil total fungal PLFAs decreased in comparison to young pastures.

A manipulation study using intact soil cores, which incubated soils to mimic current seasonal climate conditions (hot moist summers, and cold dry winters) and potential future climate changes (hot dry and cold wet) revealed that soil greenhouse gas emissions (CO_2 , N_2O and CH_4) were the greatest under hot wet (35°C and 80% Water Holding Capacity (WHC)) conditions, followed by hot dry (35°C and 20% WHC) and lowest under cold wet and cold dry (10°C and 80% WHC and 10°C and 20% WHC respectively) conditions. The findings of this study show that whilst management practices in the tropics exist which can promote soil carbon sequestration, in the subtropics land use change causes large changes in soil carbon and soil quality, particularly in dry forest ecosystems. This thesis demonstrates that it is not only the direct loss of carbon from soils which is detrimental to the environment, but the impacts of deforestation and grazing on soil salinity, nutrient availability and vegetation also impact soil carbon dynamics, something which will likely be exacerbated by climate change.

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1 Introduction

1.1 Grazing lands and land use change

The conversion of native vegetation to grazing systems has been widespread over the last century, particularly over recent decades as the human population increases and with it demand for animal-based products such as meat and dairy (Godde *et al.* 2018; Smith *et al.* 2010). In fact, up to one half of the earth's terrestrial surface is now allocated to grazing systems (Byrne *et al.* 2018), and with projected growth in the population, this area will likely continue to increase. Such a demand for livestock production, combined with globalization, improved market access and international development has resulted in high rates of deforestation, with the conversion of approximately 4.5 million km² of forest to make way for grazing from between 2001 and 2015 alone (Chini *et al.* 2021). The removal of native vegetation has resulted in the emission of vast quantities of greenhouse gasses (GHG), which have directly contributed to climate change. Indeed, land use contributes 25% of global anthropogenic GHG emissions, 12 - 17% of which is from land use change (LUC), including deforestation (Paustian *et al.* 2016).

Extensive land use change and subsequent poor management of agricultural land can result in detrimental environmental impacts, such as loss of biodiversity, changes to climate through GHG emissions, and increased water stress (Powers & Jetz, 2019; Marhaento *et al.* 2018; Ramankutty *et al.* 2018). One increasing issue arising from the conversion of forest ecosystems and establishment of livestock grazing is that of soil degradation (Veldkamp *et al.* 2020). The degradation of soils is a global problem, with around 30% of land affected by varying degrees of soil degradation which could be physical (erosion, compaction, crusting), biological (soil organic carbon (SOC) loss, greenhouse gas emissions, decreased soil biodiversity), chemical (salinization, nutrient depletion), or ecological (disruptions to nutrient cycling, hydrological impacts) degradation (Lal, 2015).

1.2 Soil carbon

The soil biosphere is an important carbon reservoir, which holds more carbon than both the atmosphere and carbon stored in aboveground biomass (Bossio *et al.* 2020; Smith *et al.* 2015; Post & Kwon, 2000), and as such, soils play a vital role in the provision of ecosystem services, and the global C cycle. Through photosynthesis and the decomposition of dead above and belowground biomass, atmospheric carbon dioxide (CO₂) can be taken up and sequestered

belowground as soil organic matter (Smith *et al.* 2015; Janzen, 2006). In addition, changes in soil carbon can feedback to the earth's climate system both through the release of CO₂ and CH₄, and uptake of carbon (Smith *et al.* 2015; Bardgett *et al.* 2008).

Soil carbon sequestration has been identified as a potential climate change mitigation option (Minasny *et al.* 2017; Paustian *et al.* 2016). Atmospheric CO₂ can be taken up and sequestered belowground as soil organic matter through plant growth, the decay of litter and biota living within the soil profile (Janzen, 2006). Improved soil management which can increase SOC through increasing soil organic matter content, can not only reduce atmospheric CO₂, but also increase soil fertility through supplying more nutrients, increasing soil biodiversity and can help to protect grasslands from the impacts of climate change (Amelung *et al.* 2020; Paustian *et al.* 2016). Contrastingly, where land and soil management practices are poor, soils will move from a sink for CO₂ to a source, and lose carbon to the atmosphere (Lal, 2015). Heavy grazing practices have reduced soil fertility, with nutrient loss through increased organic matter breakdown, wind and water soil erosion, run off and nutrient leaching (Centeri, 2022; Byrnes *et al.* 2018; Aubault *et al.* 2015; Schuman *et al.* 2002; Steele *et al.* 1984). Furthermore, processes such as acidification, alkalinisation, compaction and changes to hydrology can limit plant productivity, reducing nutrient inputs into the soil, and in turn carbon sequestration from vegetation growth (Lai & Kumar, 2020; Goulding, 2016; Savadogo *et al.* 2007).

The degradation of soils and loss of carbon and other nutrients ultimately decreases agricultural productivity and thus threatens food security (Bindraban *et al.* 2012). In livestock systems, the net primary productivity of pasture grasses is decreased in degraded soils and the consequent low vegetation cover and increased bare soil area leads to loss of livestock carrying capacity, production and economic decline (Kwon *et al.* 2016; Tesfa & Mekuriaw, 2014; Zika & Erb, 2009). However, due to the substantial loss of carbon from deforestation, grazing systems and other agricultural practices, the capacity for soils to sequester carbon in grazing soils is high with the correct interventions and management strategies. This potential was highlighted in the "4 Per Thousand" initiative recommended in the Paris Climate Agreement which recommended the sequestration of soil carbon at a rate of 0.4% annually (Lal, 2018; UNFCC, 2015). But in order to identify management strategies which can promote soil carbon sequestration, it is necessary to understand how land use practices such as deforestation and grazing systems impact soil carbon dynamics, and other soil health indicators. Further to this, it is worth noting that the impacts of land use change on soil quality, and subsequent strategies for increasing sequestration can be regionally, or event site specific, with many interacting factors such as climate, geomorphology, soil type, and hydrology impacting soil responses to land use change (LUC).

1.3 Soil carbon and land use change in the tropics and subtropics

Whilst deforestation in temperate regions has slowed down greatly in modern times, in the tropics and subtropics it has increased, and forest systems in these climates are typically the biomes most subjected to land use change (Burrows, 2021; Macchi *et al.* 2013). Due to increased demand from a growing population, improved markets for livestock products, and slow technological development which favour extensive systems, 50% of the world's grazing systems can be found within the tropics and subtropics, and 19% are in Brazil, Australia and Argentina alone (Burrows, 2021; FAO, 2017; Asner *et al.* 2014). Understanding the impacts of land use change and land and grazing management on soils in these climates is incredibly important, due to their high SOC content (32% of global SOC is stored in tropical soils alone), and their potential to be large sinks for CO₂ (Lal, 2004).

The degradation of soil due to land use change and grazing on soil carbon dynamics and other soil characteristics has been extensively studied in the tropics. The soils of the tropics tend to be highly weathered and nutrient limited and are more vulnerable to degradation than soils in more temperate regions (Basak *et al.* 2022; Frankl *et al.* 2015; Daroub *et al.* 2003; Gijsman *et al.* 1996). Multiple studies have demonstrated that land use change from forest to pasture has resulted in the loss of carbon from soils (Dalal *et al.* 2019; Aryal *et al.* 2018; Johnson & Wedin, 1997). However the overall impacts of grazing on soil health in the tropics are not clear, with multiple studies demonstrating contrasting results and observing that under certain conditions grazing practices have promoted soil carbon sequestration. Extensive grazing practices which involve livestock at low stocking rates over a large area can promote carbon sequestration over time (Costa de Oliveira *et al.* 2022; Chen *et al.* 2015; Conant *et al.* 2003. A study by Navarette *et al.* (2017), identified using a chronosequence design that following deforestation soil carbon increased by 41% over 20 years under low intensity grazing, whereas it decreased by 20% under higher stocking rates.

Increasing soil carbon with improved grazing management has been found in a number of other studies, all of which found that low intensity grazing can stimulate root growth in turn improving soil structure and promoting the accumulation of plant carbon belowground (Garnett *et al.* 2017; Chen *et al.* 2015; Schumann *et al.* 2002). Furthermore, light intensity grazing has been shown to breakdown old and dead plant tissues and increase the photosynthesis of living vegetation (Alhamad & Alrababah, 2008; Loeser *et al.* 2004). Beyond grazing management, it has also become common practice to increase pasture productivity with the sowing of

“improved” pasture grasses, which tend to be more productive and can promote carbon sequestration when managed correctly. (Navarette *et al.* 2017; Amezcuita *et al.* 2004).

Improved pastures are implemented as a land management practice, particularly in the tropics and subtropics, to increase foraging and subsequently productivity. They typically are achieved through the sowing of introduced grasses such as *Brachiaria decumbens*, *Panicum maximum* and *Chloris gayana* which use a C4 photosynthesis pathway over C3 photosynthesis. In warmer climates such as the tropics and subtropical summers, C3 vegetation tends to be less efficient due to higher rates of photorespiration by enzymes in the presence of oxygen (Sage *et al.* 2007; Ehleringer & Cerling, 2002). C4 photosynthesis however results in the concentration of CO₂ in plant cells due to the activity of an enzyme which is absent in C3 grasses, and which increases carbon fixation and reduces photorespiration (Gowik & Westhoff, 2011). As a result, such grasses tend to be more productive in warm climates than native C3 grasses, have increased root biomass and depth (Banegas *et al.* 2019; Saraiva *et al.* 2014; Gowik & Westhoff, 2011), and have repeatedly been associated with greater soil carbon accumulation in the tropics. However, in the subtropics this association is less well demonstrated, and whilst a number of studies have observed changes in soil respiration with photosynthetic pathway (Kjelgaard *et al.* 2008), the impacts on soil carbon sequestration are less well known, or how the impact of photosynthetic pathway on soil characteristics compares to that in the tropics.

Perhaps due to subtropical forest ecosystems receiving less attention than those in tropical regions, such as the Amazon or the Congo rainforests, it is difficult to draw definitive conclusions regarding the impacts of land use change and grazing on soil carbon and other soil quality indicators. Similar to the tropics, previous studies have found mixed results with some demonstrating that with increasing grazing pressure, soil carbon and nitrogen concentrations decrease, alongside the breakdown of soil aggregates and increase in bulk density (Talore *et al.* 2015; Kotze *et al.* 2013). The majority of studies in the subtropics utilize an enclosure experimental design, whereby grazing is restricted and soil conditions are compared between grazed and non-grazed areas. These studies have shown that the removal of grazing from an area can increase carbon sequestration rates (Banegas *et al.* 2019; Wilson *et al.* 2018; Raiesi & Riahi, 2014; Witt *et al.* 2011; Shrestha *et al.* 2004) through increased aboveground biomass production and thus inputs into the soil, improved soil structure, and nutrient cycling. However, such focus solely on grazing practices and do not take account of land use history, and the impacts of land use transitions on soil carbon stocks.

The tropics and subtropics have distinctly different climate types, and whilst often grouped together in studies such as meta-analyses and literature reviews (Abdalla *et al.* 2017; Mcsherry & Ritchie, 2013; Conant & Paustian, 2002), it is important that the impacts of land use change and grazing on soil characteristics are considered separately and compared, in order that conditions which facilitate soil carbon sequestration can be identified. The tropical climate is characterized by year-round high temperatures, and predominantly humid conditions with semi-arid and arid regions occupying a lesser area. Contrastingly, the subtropical climate is characterized by pronounced seasonality, with hot moist summers, and cold dry winters, and are dominated by semi-arid and arid precipitation patterns. The pronounced differences between the two climates will inevitably impact the soil response to land use change, with the year round hot and moist conditions of the humid tropics facilitating processes such as root turnover, fungal activity and increasing NPP, all of which contribute to carbon sequestration (Hayakawa *et al.* 2014; Raich *et al.* 2006). Contrastingly arid regions are water limited, with lack of precipitation limiting vegetation growth, microbial activity and thus restricting carbon turnover (Li *et al.* 2018; Sawada *et al.* 2016; Heisler-White *et al.* 2008)

1.4 Land use change and grazing in the dry Chaco, Argentina

The Gran Chaco is an ecological region in South America which spans over three countries; Argentina, Bolivia and Paraguay and has three separate climate zones, the humid Chaco, the transitional Chaco and the dry Chaco (Caldas *et al.* 2015). It is the second largest natural biome in South America, second only to the Amazon rainforest, and possesses the largest seasonally dry forest in the continent (Caldas *et al.* 2015; Bucher & Huszar, 1999). The dry Chaco in northwestern Argentina, which is the main focus chapters three and four of this thesis, encompasses 1.9 million km² in northwestern Argentina and is characterized by 500 – 800 mm of precipitation per year, falling predominantly between October and March (Banegas *et al.* 2019; Macchi *et al.* 2013). The ecosystem has a subtropical seasonal climate, characterized by high summer temperatures (mean of 25°C) and cold winters with a mean temperature of 13°C (Banegas *et al.* 2019). This ecosystem is dominated by xerophytic forest, including the species *Schinopsis balansae*, *Schinopsis quebracho-colorado* and *Aspidosperma quebracho-blanco*, and with a grassy understory and halophytic shrub layer (Bucher, 1982).

Cattle ranching is widespread across the Argentinian Chaco, producing 3 million tonnes of beef each year, 10% of which is exported (Piquer-Rodriguez *et al.* 2018). As a result of this and crop production, over 142,000 km² of native forest has been converted to grassland between 1985

and 2013 (Baumann *et al.* 2017). In this region, grazing systems are dominated by extensive ranches, with low stocking rates over large areas of pastures, which are typically sown with improved pasture grasses. Despite the levels of land use change in this region, the Gran Chaco has received little attention from science and policy and although research into this area has grown in recent years, there are still many unknowns surrounding its soils.

The removal of native vegetation for the establishment of such grazing systems has been found to impact soil carbon and factors which influence soil carbon dynamics. Multiple studies have found that deforestation and grazing in the Chaco has resulted in detrimental impacts on soil health, with decreased soil carbon stocks, increased carbon mineralization, compaction, reduced water availability and infiltration rate (Baldassini & Paruelo, 2020; Osinaga *et al.* 2017; Magliano *et al.* 2017; Abril & Bucher, 2001; Abril & Bucher, 1999). Contrastingly a study which investigated wider soil health indicators have observed increases in SOC and total nitrogen over a period of 3 years, alongside decreases in pH, electrical conductivity and soil respiration (Banegas *et al.* 2019). However, the soil characteristics of pastures were compared to those of a grazing enclosure, so conclusions cannot be made surrounding the impacts of land use change. To the best of our knowledge, no studies to date have considered LUC impacts on soil phosphorus or root biomass.

A number of studies have investigated greenhouse gas emissions from land use change and management in the Chaco, however, these studies are predominantly modelling experiments which do not use experimental data. In 2017, Baumann *et al.* predicted through remote sensing and carbon modelling that a total of 824 Tg of carbon was emitted between 1985 and 2013 as a direct response to deforestation for grazing systems. Another study, which modelled both historic emissions from soil, and predicted future emissions levels across a range of LUC scenarios, found that between 1996 and 2012 CO₂ emissions increased with time, and if rates of deforestation remained the same, CO₂ emissions would increase by 48% respective to 2012 (Villarino *et al.* 2018). There are few experimental studies which have investigated the impacts of land use change and grazing on soil GHG emissions in the Chaco. Two, which examined nitrogen dynamics, found that N₂O emissions were dependent on climate factors and the seasonality of the region, with higher emissions in the summer than winter (Oliva *et al.* 1993; Mazzarino *et al.* 1991). However, similarly to those investigating soil carbon dynamics, they did not take into account any land management variables. In 2001, Abril and Bucher, analysed soils for carbon mineralisation rates in degraded pastures, finding that mineralisation rate increased along a grazing gradient and CO₂ production decreased along the same gradient, indicating a reduced humification potential and increased soil carbon loss with increasing degradation.

Similar to Mazzarino *et al* they also observed that CO₂ production was dependent on climate factors, which highlights the need to better understand the potential impacts of climate changes on Chaco soils.

With climate change, weather patterns are predicted to change globally, with increased temperatures and increased probabilities of extreme weather events such as droughts, floods, and heatwaves (Stott, 2016). In the Chaco, there is already evidence that the removal of native vegetation is impacting regional weather patterns, with increased precipitation in the region (Magliano *et al.* 2016). If deforestation continues at current rates and GHG emissions from the soil increase further, it is likely that climate factors will continue to change which could in turn increase greenhouse gas emissions further. However, there has been no research to date which examines the impacts of potential climate change on soils from this region, thus it is difficult to predict the scale of impact which may occur.

1.5 Thesis aim, objectives and hypotheses

The main aim of this PhD research was to investigate the impacts of both deforestation and livestock grazing on soil carbon dynamics, and the key variables which impact soil carbon stocks in tropical and subtropical ecosystems. In order to address this question, the following key objectives were set:

1. To evaluate and compare through a meta-analysis the effects of grazing on soil organic carbon in subtropical and tropical grazing systems and to identify key biotic and abiotic variables which influence soil carbon dynamics (Chapter 2).
2. To quantify the impact of deforestation and grazing over time since removal of native vegetation on soil properties, and identify interactions between soil carbon stocks and other soil chemical and biological characteristics (Chapter 3).
3. To quantify soil greenhouse gas fluxes (CO₂, CH₄, N₂O) in forest and pastures of increasing time since deforestation under moisture and temperature incubation treatments, and identify the impact of land use change on key soil biological and chemical properties (Chapter 4).

The hypotheses which were tested in this research were as follows:

1. Grazing effects on soil organic carbon will be greater in the tropics due to higher mean temperatures, increasing soil respiration and carbon losses. The impacts of grazing on soil

organic carbon under pastures of different photosynthetic pathways will vary according to climate. The effects of grazing on SOC will be dependent not only on grazing intensity, but a range of interacting variables including vegetation type, soil texture, and soil chemical properties (Chapter 2).

2. Changes in soil carbon and other nutrients (nitrogen and phosphorus) will be impacted not only directly by land use change but also by indirect effects of deforestation on other physical, biological and chemical properties of soil such as pH, electrical conductivity (EC), and root biomass. Whilst soil carbon stocks will decrease following deforestation, recovery in carbon and other soil nutrients will be expected with grazing over time since deforestation (Chapter 3).
3. Soil fluxes and microbial community composition will be impacted by deforestation and time since land use change with soil emissions being greater in pasture soils than in those of native vegetation. Soil trace gas fluxes from native vegetation and grazed pastures will be influenced by incubation treatment, with greater emissions in warmer and wetter soils. Soil greenhouse gas fluxes will be significantly impacted by soil chemical characteristics (Chapter 4).

1.6 Thesis Structure

Chapter 1: An introduction to the PhD research which gives the background to the research topic and sets out the rationale for the research

Chapter 2: A meta-analysis which used existing data from primary literature to investigate the impacts of grazing and land management practices, and the soil chemical, biological and physical attributes of, on soil carbon stocks in subtropical and tropical climate systems, comparing drivers of carbon dynamics between the two and identifying areas for future research. This chapter addressed Objective 1.

Chapter 3: An experimental study of soil nutrient dynamics and other chemical, physical and biological properties over a chronosequence of sites from native forest to pastures of >20 years of extensive grazing in the dry Chaco, Argentina. The chapter investigated the effects of land use change and grazing over time on soil chemical, biological and physical attributes of soil. The study analysed destructive soil samples for soil carbon, nitrogen and phosphorus stocks, pH, EC, root biomass and texture, and employed a statistical path analysis to identify key drivers of soil carbon stock changes. This chapter addressed Objective 2.

Chapter 4: An experimental study which investigated the impacts of land use change and grazing in the dry Chaco, Argentina, on soil greenhouse gas emissions (CO_2 , CH_4 , and N_2O), microbial community composition, and soil salinity (electrical conductivity (EC), pH, cation concentrations and cation exchange capacity (CEC)). The study used intact cores in incubation experiments to investigate the impacts of climate factors (moisture and temperature) on soil trace gas fluxes in forest and pastures of increasing time since deforestation. It also analysed phospholipid fatty acids (PLFAs) and soil cations and investigates their interactions and impacts on soil fluxes. This chapter addressed Objective 3.

Chapter 5: A summary and discussion of the key results found in the experimental and analytical chapters of this thesis which reflects on the current research, discusses its wider impacts and makes recommendations for future research.

2 Unequal effects of livestock grazing on soil organic carbon in tropical and subtropical grasslands

2.1 Abstract

Tropical and subtropical grassland soils are recognised as important sinks for carbon, with high net primary productivity, due to warmer climates, increasing the potential of ecosystems to capture carbon and sequester it as organic matter. However, poor grazing management is limiting this potential, with high stocking densities and overgrazing resulting in land and soil degradation. Previous reviews have found that grazing can both increase and decrease soil carbon stocks depending on factors such as precipitation class, soil texture and vegetation type, but to date there are no meta-analyses which consider the tropics and subtropics exclusively, or how grazing effects on soil organic carbon (SOC) differ between these climate types. This study aimed to compare and contrast the effects of grazing and other factors on grassland soil carbon, nitrogen and phosphorus stocks in the tropical and subtropical climatic regions. We performed a statistical meta-analysis on data collected from 54 studies, with the effect of grazing calculated as a natural log response ratio. Multivariate models were used to test the hypotheses; 1) that the effects of grazing on soil organic carbon are amplified in the tropics due to higher temperatures and different photosynthetic pathways, and 2) that SOC changes depend not only on grazing pressure but also on soil chemical, physical and biological properties. Studies investigating the impacts of grazing on soil carbon were more common in tropical ($n = 40$) than subtropical systems ($n = 14$). Soil carbon was found to increase by 14% in the tropics under low intensity grazing but decrease by 18% under high intensity grazing, whereas in the subtropics carbon decreased under both high and low grazing intensities. Tropical grasslands with a dominance of C4 grasses had more soil carbon in the top 10 cm of the soil profile than those with a mix of C3/C4 grasses. A lack of data was found regarding grassland microbial biomass. Our results show that increases in soil organic carbon in tropical and subtropical grasslands are driven by multiple factors, with grazing intensity interacting with vegetation photosynthesis pathway (C3/C4) and vegetation type to affect soil carbon dynamics. This indicates that there are differences between climate types in how the soil responds to management practices, with vegetation playing a dominant role in driving soil carbon dynamics. Improved understanding of the interactions between these factors is needed to make meaningful predictions and plans for the sustainable grazing management of tropical and

subtropical grassland ecosystems. Furthermore, more research is needed into the impacts of grazing on carbon in subtropical ecosystems, as this area remains relatively under researched.

2.2 Introduction

Increasing pressure from a growing human population to provide sustenance has led to the expansion of livestock systems, and subsequently, much of the world's native and managed grasslands are intensively grazed and degraded (McSherry & Ritchie, 2013; ²Lal, 2004; Conant *et al.* 2001). Grasslands, which cover approximately 40% of the earth's surface, play an important role in the exchange of greenhouse gases (GHG) with the atmosphere, and grazing is not only hindering their potential to sequester and store carbon, but also turning them into carbon sources (Scurlock & Hall, 1998; Hirota *et al.* 2005). Overgrazing can decrease carbon stocks by reducing net primary productivity (NPP), increasing microbial activity and, altering plant nutrient availability in the soil (Piñeiro *et al.* 2010). Whilst poor livestock management has led to soil organic carbon (SOC) losses in many instances, improved management of pastures and grasslands such as low intensity grazing can prevent such losses, and in some cases increase soil carbon stocks (Conan & Paustian, 2002). Indeed, Schumann *et al.* (2001) predicted that through improved grazing practices and conservation of native grasslands, a loss of 43 Tg carbon yr⁻¹ could be prevented, whilst Henderson *et al.* (2015) estimated that the world's grazing lands have the potential to sequester 148.4 Tg CO₂yr⁻¹. However, although the mechanisms by which overgrazing is detrimental to soil carbon stocks are well known, those which can promote carbon sequestration are less well defined.

Previous reviews, which investigated the effects of grazing and land management practices on soil carbon, have found mixed results. Thus, it remains unclear what the driving forces of soil carbon dynamics in grazing systems really are. Grazing was found to decrease soil carbon stocks, with higher intensities of grazing resulting in greater losses of carbon in a number of reviews (Abdalla *et al.* 2017; Lu *et al.* 2017; Dlamini *et al.* 2016). Moreover, others indicated that whilst at medium to high grazing intensities soil carbon decreases, low grazing intensities promoted carbon sequestration (Zhou *et al.* 2017; Mcsherry & Ritchie, 2013). Similarly, Piñeiro *et al.* (2010) and Schipper *et al.* (2017) found both positive, negative and neutral effects of grazing on soil carbon depending on other influencing variables, such as soil type, topography, and vegetation type. However, management factors such as grazing intensity were not factored into the analysis. Management practices aside from grazing intensity can greatly influence soil carbon dynamics, with the use of fire to clear native vegetation, improved grass species which photosynthesise more efficiently, and rotational grazing all influencing soil carbon sequestration. Conant *et al.* (2017) looked further into grazing management practices such as fertilisation, silvopastoralism and fire, and their effects on carbon dynamics, discovering that

practices such as use of legumes, improved grass species, and fertilisation on top of improved grazing practices (reduced stocking rate, removal of livestock, rotational grazing) all led to increased soil carbon content.

Improved grass species can include those which utilise a C4 photosynthesis pathway over a C3 pathway, particularly in tropical and subtropical climates. C3 photosynthesis is catalysed by the enzyme rubisco, which is capable of catalysing a five carbon molecule – rubisco bisphosphate – into either phosphoglycerate (in the presence of CO₂) or phosphoglycerate and phosphoglycolate (in the presence of O₂) (Ehleringer & Cerling, 2002). The reaction in the presence of oxygen leads to photorespiration with decreased carbon fixation and CO₂ production and increases with increasing temperatures. As a result, C3 photosynthesis is more efficient and results in higher carbon sequestration in temperate climates (Sage & Kubien, 2007; Ehleringer & Cerling, 2002). Contrastingly C4 photosynthesis reduces the activity of rubisco due to a much more active enzyme – phosphoenolpyruvate carboxylase – fixing CO₂ into a four carbon acid which is then decarboxylated in close proximity to Rubisco which concentrates CO₂ in this region and limits photorespiration. This ultimately results in higher carbon fixation in higher temperatures where photorespiration is predominant in C3 plants (Gowik & Westhoff, 2011; Sage & Kubien, 2007; Ehleringer & Cerling, 2002).

Factors such as precipitation or temperature have been used in previous reviews to investigate the impacts of climate factors on soil carbon dynamics in grazing systems (Abdalla *et al.* 2017; McSherry & Ritchie, 2013; Conant & Paustian, 2002). However, few studies at present have studied the impact of land use variables and soil characteristics on soil carbon dynamics with respect to specific climate types. Soil carbon dynamics in grazing systems are particularly under researched in the tropics and subtropics. Grazed soils in the tropics and subtropics carry great potential for carbon sequestration, with approximately 30% of global SOC being sequestered in these climates (Dalal & Carter, 1999; Adewopo *et al.* 2014). With a large proportion of grazing land located in these climatic regions (i.e., 19% of global land under permanent pastures is in Brazil, Australia and Argentina) (FAO, 2017; Asner *et al.* 2004), it is imperative that grazer effects on soil carbon dynamics are better understood in order to identify management practices which maintain and increase soil carbon stocks.

Abdalla *et al.* (2017) investigated different climatic zones, climates such as tropical, subtropical, and temperate were grouped into zones such as “moist and warm” and “wet and cold”. Whilst informative, this does not allow the comparison of grazing effects and other management and soil variables in different climate types. Similarly, both McSherry and Ritchie (2013), and Conant

and Paustian (2002) evaluated the relationship between carbon sequestration and mean annual precipitation, rather than specific climates. These reviews found that the effect of grazing intensity on SOC is regulated by precipitation, with the amount of rainfall affecting microbial composition and activity, rooting depth and plant water availability (Abdalla *et al.* 2017; Conant and Paustian, 2002). Dlamini *et al.* (2016) investigated soil carbon changes due to grazing under different climates and soil textures, finding that soil carbon losses with degradation were greater in coarser soils (<20% clay), and in areas with decreased rainfall (<600mm yr⁻¹). Dlamini *et al.* (2016) investigated soil carbon changes due to grazing under different climates and soil textures, finding that soil carbon losses with degradation were greater in coarser soils (<20% clay), and in areas with decreased rainfall (<600 mm yr⁻¹). However, this research only investigated carbon stocks in relation to soil texture, it did not consider the influence of additional variables in carbon dynamics which must be considered (i.e., nutrient availability (CNP), soil chemical attributes, vegetation type, or management practices).

Aside from land and grazing management variables, soil biological, chemical and physical characteristics are important to consider in the investigation of the impacts of grazing on soil carbon dynamics across climate types. These variables such as soil nutrient availability (nitrogen and phosphorus), soil texture, bulk density, pH, and microbial biomass all impact soil carbon dynamics, and more importantly, climate specific factors will not only impact soil characteristics directly, but also impact how they influence soil carbon cycling. For example, critical contributory processes to carbon sequestration such as microbial respiration and root turnover are highly dependent on temperature and precipitation (Wu *et al.* 2011; Gill & Jackson, 2000). It is key that differences in the effects of grazing on soil carbon within different climate types are recognised in order to formulate the best climate change mitigation efforts in these areas. In the humid tropics, soil respiration is heightened in comparison to more temperate climates, with high temperatures and moisture providing humid environments for decomposers (Schlesinger & Andrews, 1999; Sombroek *et al.* 1993). Moreover, soils in tropical regions, particularly in areas of high rainfall, tend to be highly weathered and nutrient poor, rendering them more susceptible to degradation when poorly managed, leading to high rates of erosion and soil loss, loss of nutrients and carbon (Hossain *et al.* 2020; Veldkamp *et al.* 2020). Indeed, in the tropics alone, 75% of soil carbon stocks have been lost primarily due to the conversion of native ecosystems to agricultural land (Lal, 2004). In the subtropics, temperatures are more variable with pronounced seasons which affect microbial biomass and activity in degraded sites, and increased SOC losses in wet seasons (Abril & Bucher, 1999; Abril & Bucher, 2001). It is key

that differences in the effects of grazing on soil carbon within different climate types are recognised in order to formulate the best climate change mitigation efforts in these areas.

This review investigates the abiotic and biotic factors that affect soil carbon changes in subtropical and tropical grazing lands and identifies those which promote the sequestration of carbon. The key aim was to determine the influence of grazing and pasture management practices, soil biological, chemical and physical attributes, and climate variables on the impacts of grazing on soil carbon in tropical and subtropical climates through a statistical meta-analysis, ultimately allowing a comparison of the carbon dynamics between these systems. A meta-analysis was used as it permits the analysis of effects across multiple studies allowing a better assessment of significance (Arnqvist & Wooster, 1995; McSherry & Ritchie, 2013). The specific hypotheses to be tested in this research were as follows; 1) Grazing effects on soil organic carbon will be greater in the tropics due to higher mean temperatures increasing soil respiration and carbon losses; 2) The impacts of grazing on soil organic carbon will be dependent on the photosynthetic pathways of the pasture vegetation (C3/C4), and the effect of photosynthetic pathway will be different between tropical and subtropical pastures; 3) The effects of grazing on SOC will be dependent not only on grazing intensity, but a range of interacting variables including vegetation type, soil texture, and soil chemical properties.

2.3 Methods

2.3.1 Data Collection

Data were compiled from the literature regarding the influence of grazing on soil carbon stocks in tropical and subtropical grasslands. An extensive literature search was conducted using Scopus and Web of Science using the key words: soil carbon; tropical; subtropical; grassland; grazing; carbon sequestration; livestock; SOC and soil C storage. The searches yielded 3516 results. Firstly, papers were reviewed and selected based on title, then again with the abstracts being reviewed, which led to 213 being selected for review. Papers were rejected based on the following criteria; 1) the study was found outside of the subtropical and tropical latitudes (see below); 2) the study did not include soil carbon measurements (either stock or concentration/percentage); 3) the study did not include bulk density measurements to allow for the calculation of soil carbon stocks (if not already reported); 4) an ungrazed control (i.e., native

vegetation, exclusion area) to act as a comparison to grazed sites. Studies were classified as having sampled tropical soils if the sample location was found between the latitudes of the Tropic of Cancer (23°26'14"N) and the Tropic of Capricorn (23°26'14"S), and subtropical if found between the latitudes of either the Tropic of Cancer (23°26'14"N) and 35°N, or the Tropic of Capricorn (23°26'14"S) and 35°S.

Country specific searches were also conducted with the keywords soil carbon, grassland, grazing and the specific country. For example, "Soil carbon" AND "Grazing" AND "Colombia". All countries from tropical and subtropical regions were included in searches. This process resulted in 90 additional papers being added to the preliminary review process. Once the exclusion criteria were applied to the selected papers, the final number of studies used for data collection was 54. Reference lists of each paper sampled were also searched to ensure all possible studies were found. Data collected included: latitude/longitude; location; grazing intensity; grazing animal type; soil type; soil texture; sand, silt and clay contents; photosynthetic pathway of the vegetation type recorded; native vegetation type (grassland/forest); current vegetation type (grassland/shrubby grassland/woody grassland/woody shrubby grassland); bulk density (g cm^{-3}); carbon stock (kg m^{-2}); nitrogen stock (kg m^{-2}); C:N ratio; phosphorus stock (kg m^{-2}); microbial biomass (mg g^{-1}); pH; mean annual precipitation (MAP) and mean annual temperature (MAT); precipitation class (arid/semi-arid/humid/subhumid); Climate type (Koppen's Classification); Time since deforestation or implementation of treatment (i.e., of enclosure); and the depth of soil sample.

Data for soil carbon, nitrogen, and phosphorus which were presented as a percentage or concentration were converted to stocks with the units kg m^{-2} , using the reported bulk density value and sampled horizon depth. Due to the highly variable units of grazing intensity between studies (e.g., Livestock Unit (LSU), Animal Unit (AU), Heads of Forage-fed Livestock (HFFL)) each entry to the database had a grazing intensity of either high, low, or medium, and was characterised based on the authors description within the study following the criteria used by McSherry & Ritchie (2013). Vegetation was classified into one of four types: grassland (pastures with planted/native grasses but no shrubs); shrubby grassland (pastures with shrubs present); woody grassland (pastures with trees present); woody shrubby grassland (pastures with both shrubs and trees present) according to Abdalla *et al.* (2017). Photosynthetic pathway was recorded as either C3, C4 or C3&C4 for mixed vegetation. Finally, sampled depths were grouped into 3 classes; 0-10 cm, 11-40 cm and >40 cm, as described by McSherry & Ritchie (2013).

2.3.2 Data Analysis

The studies included in the review reported a multitude of different methods to calculate changes in soil carbon, and therefore we calculated the natural log response ratio (RR) of grazing to analyse the effect of different variables on soil carbon. A positive effect of grazing ($RR > 0$) indicates an increase in soil carbon, whereas a negative effect ($RR < 0$) shows a decrease. The standard log response ratio was calculated by taking the natural log of the ratio between the mean carbon stock of the grazing treatment and mean carbon stock of the ungrazed control. Response Ratio was calculated for each soil layer using the following equation:

$$RR = \ln \left(\frac{C_{grazed}}{C_{ungrazed}} \right)$$

Overall, of the 54 studies selected for data collection with a total of 569 independent contrasts, 40 were from tropical regions, and 14 were from the subtropics (Figure 1). Statistical analyses were carried out for each climate separately. Firstly, ANOVA analysis with type 3 sum of squares was carried out to identify any significant effects of specific variables on grazer effect size. Type 3 sum of squares was used due to inconsistent reporting of the selected variables from each study (i.e., not all studies reported all of the included variables). This was followed by univariate linear models to investigate the nature of statistically significant relationships. The reporting of soil texture and soil type was inconsistent across the selected studies, with many studies listing multiple types and texture. Thus, where possible, data was collected regarding the percentage of clay, sand, and silt particles in a soil sample. Similarly, grazing livestock type was not used in the statistical analysis, as multiple types of grazers were often used to graze, so it would not be possible to distinguish a relationship between individual animals and soil carbon stocks. Univariate analyses were carried out for all depths followed by each depth class separately (Table 2).

Multivariate linear models were then used to test for significant interactions between groups of explanatory variables such as management variables (grazing intensity/vegetation type/photosynthesis pathway), soil physical properties (texture/bulk density) and environmental variables (MAP/MAT/Climate). Stepwise regression allowed the selection of best fitting models using Akaike's Information Criterion, with the `step()` function in R identifying models with the lowest AIC value (table 1). Similarly, only three studies reported microbial

biomass, with 6 observations. Due to the small sample size, statistical analyses could not be carried out on this data, so no relationship between the effect of grazing on soil carbon and microbial biomass could be investigated.

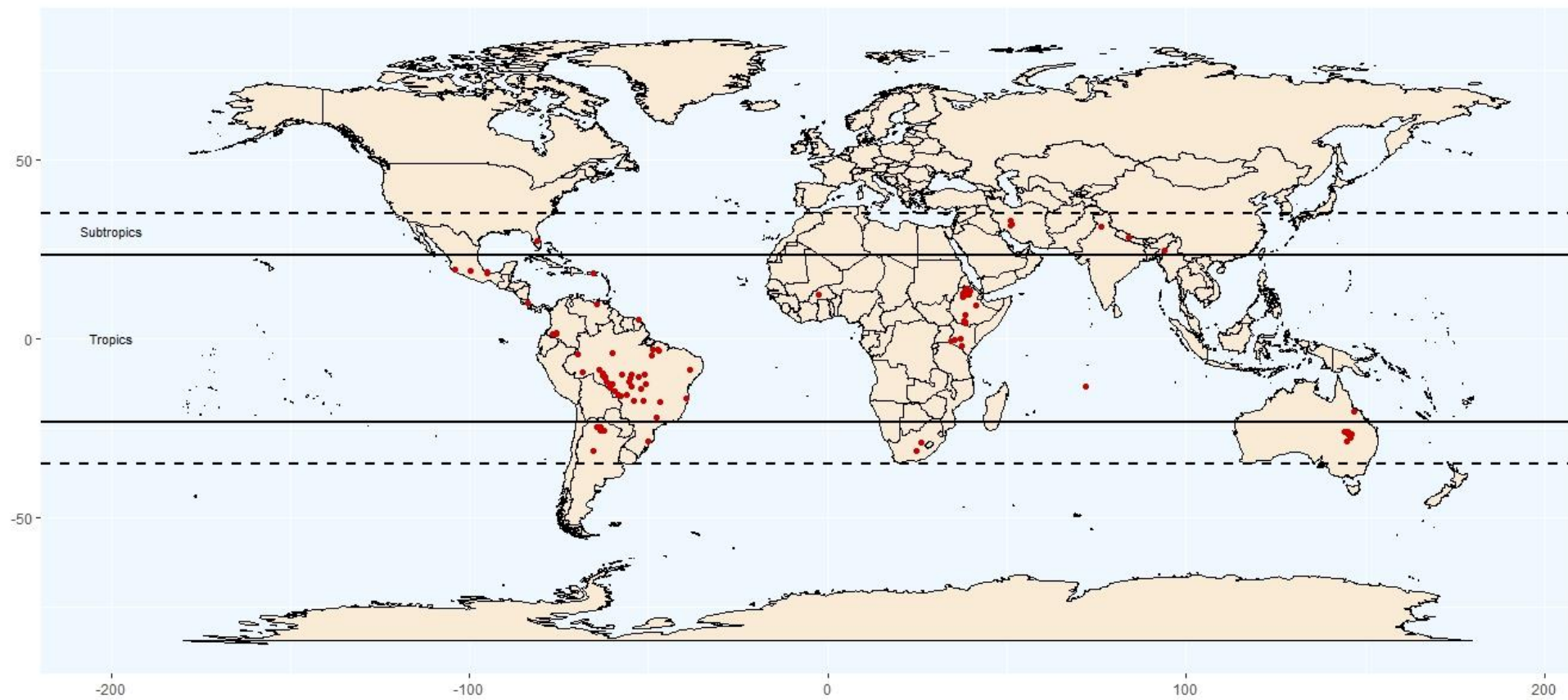


Figure 1. Locations of 120 sample sites in this meta-analysis.

2.4 Results

Carbon stocks reported in tropical grazed soils ranged from 0.05 to 11.90 kg C m⁻² with a mean of 1.99 (±0.3 s.e.) kg C m⁻² in the top 10 cm, 2.91±0.1 at 11-40 cm; and 2.79±0.3 below 40 cm, compared to 0.08 to 25.21 kg C m⁻² with a mean of 1.96 (±0.1s.e.), 3.48±0.2, and 2.59±0.26 kg m⁻² respectively in ungrazed controls (Table 1). This corresponded to a mean effect size of -0.04 (±0.2 s.e.) over all depths, (0-10 cm: 0.03±0.003; 11-40 cm: -0.10±0.02; >40 cm: -0.02±0.0 (Table 2) showing a small, but overall increase in soil carbon across all depths. In the subtropics, the mean effect size of grazing was -0.22(±0.03) over all depths (0-10 cm: -0.31±0.07; 11-40 cm: -0.14±0.04; >40 cm: -0.46±0.09) (Table 2), showing an overall decrease in soil carbon over all studies and depths, which was greater than that of the tropics. Carbon stocks in the subtropical climate ranged from 0.27 to 9.78 kg m⁻², with a mean of 1.6 (±0.2 s.e) kg m⁻² in the top 10 cm, 2.05±0.3 kg m⁻² at 11-40 cm and 2.03±0.2 kg m⁻² below 40 cm, under grazed pastures compared to a range of 0.67 to 13.74 kg m⁻² and mean of 2.18 (±0.3 s.e), 3.58±0.4, and 2.01±1.5 kg m⁻² respectively under ungrazed controls (Table 1).

Table 1. Climate and soil variables of subtropical and tropical sites recorded in the studies (n) included in the review. Table shows the soil carbon stocks (kg m^{-2}), bulk density (g cm^{-3}), pH, soil phosphorus stocks (kg m^{-2}), nitrogen stocks (kg m^{-2}) and microbial carbon of ungrazed control sites and grazed pastures in both the tropical and subtropical climates.

		Tropical			Subtropical	
	n	Mean \pm se		n	Mean \pm se	
MAT ($^{\circ}\text{C}$)	453	23.7 \pm 0.19		46	18.9 \pm 0.62	
MAP (mm yr^{-1})	481	2129.5 \pm 50		84	1123.31 \pm 124	
Texture (%)						
Clay	270	52.0 \pm 4.3		57	26.9 \pm 1.5	
Sand	292	39.0 \pm 1.6		30	56.7 \pm 2.6	
Silt	264	20.1 \pm 1.9		30	21.0 \pm 2.2	
		Ungrazed	Grazed		Ungrazed	Grazed
Soil carbon (kg m^{-2})						
0-10 cm	134	1.96 \pm 0.1	1.99 \pm 0.3	27	2.18 \pm 0.25	1.6 \pm 0.2
11-40 cm	282	3.48 \pm 0.2	2.91 \pm 0.1	50	3.58 \pm 0.43	3.05 \pm 0.3
>40 cm	69	2.59 \pm 0.26	2.79 \pm 0.3	7	3.01 \pm 1.52	2.03 \pm 1.0
Bulk density (g cm^{-3})	304	1.07 \pm 0.02	1.15 \pm 0.02	73	1.29 \pm 0.03	1.32 \pm 0.03
pH	314	4.89 \pm 0.05	5.39 \pm 0.05	47	6.38 \pm 0.12	6.50 \pm 0.14
Phosphorous (kg m^{-2})	88	0.036 \pm 0.01	0.027 \pm 0.008	36	0.004 \pm 0.001	0.003 \pm 0.0009
Total nitrogen (kg m^{-2})	238	0.20 \pm 0.01	0.20 \pm 0.01	64	0.24 \pm 0.03	0.2 \pm 0.02
Microbial biomass (mg m^{-1})	0	NA	NA	1	NA	NA

Mean annual precipitation (MAP) in tropical studies ranged from 391 to 4,900 mm with a mean of 2129.50 mm, and annual temperature (MAT) had a mean of 23.70 $^{\circ}\text{C}$ with a range of 1.50 to 28.50 $^{\circ}\text{C}$. The review of literature found that more studies were undertaken in the tropics (n = 40) than the subtropics (n = 14). Studies were located between the latitudes of -22.02 and 19.44, with the most commonly sampled country being Brazil (n = 14) followed by Ethiopia (n = 10) (Figure 1). In the subtropics, studies were located between -31.36 and 32.81, and the most sampled country was Argentina (n=3). MAP ranged from 255 to 4300 mm yr^{-1} with a mean of 1123.29 mm yr^{-1} , and MAT ranged from 6.70 to 24.0 $^{\circ}\text{C}$, with an average of 18.86 $^{\circ}\text{C}$ (Table 1).

Subtropical soils were reported to be of coarser textures than those in the tropics (Table 1) with higher percentages of sand particles (56.70 ± 2.6 and 38.97 ± 1.6 respectively) and lower percentages of clay particles (26.94 ± 1.5 and 52.20 ± 4.3 respectively). Bulk density measurements were also higher in the subtropics than the tropics. In both climates, grazing was found to decrease soil phosphorus content, with the subtropics seeing a decrease from 0.004 kg m^{-2} to 0.003 kg m^{-2} (Table 1), and in the tropics phosphorus stocks decreased from 0.036 kg m^{-2} to 0.027 kg m^{-2} . With regards to nitrogen stocks however, an overall decrease was seen with grazing only in the subtropics (0.24 to 0.20 kg m^{-2}), whereas in the tropics, nitrogen content remained the same (0.2 kg m^{-2}).

Table 2. Mean effect of grazing on soil chemical, physical and biological properties within each climate and depth class. NA: No data collected for these variables at the specified depths

Variables	Depth (cm)	Mean Response Ratio \pm S.E.				Confidence Interval (95%)	
		n	Tropical climate	95% Confidence Interval	n		Subtropical climate
Soil Organic Carbon (SOC)	0-10	134	0.03 \pm 0.03	-0.02 -m- 0.08	27	-0.31 \pm 0.07	-0.44 -m- -0.18
	11-40	282	-0.10 \pm 0.02	-0.15 -m- -0.05	50	-0.14 \pm 0.04	-0.24 -m- -0.06
	>40	69	-0.02 \pm 0.04	-0.25 -m- 0.22	7	-0.46 \pm 0.09	-0.67 -m- -0.25
Total Nitrogen (TN)	0-10	66	-0.16 \pm 0.02	-0.34 -m- 0.2	43	-0.28 \pm 0.05	-0.39 -m- -0.17
	11-40	137	-0.08 \pm 0.02	-0.14 -m- -0.02	40	-0.18 \pm 0.03	-0.25 -m- -0.11
	>40	25	-0.02 \pm 0.12	-0.26 -m- 0.22	2	-0.21 \pm 0.07	-0.49 -m- 0.7
Phosphorous (P)	0-10	19	-0.14 \pm 0.09	-0.31 -m- 0.07	19	0.09 \pm 0.1	-0.14 -m- 0.32
	11-40	9	-0.18 \pm 0.09	-0.31 -m- 0.03	16	-0.25 \pm 0.11	-0.49 -m- -0.01
	>40	0	NA	NA	1	NA	NA
Microbial Carbon (MC)	0-10	0	NA	NA	1	0.84 \pm 0.84	NA
	11-40	0	NA	NA	1	-0.13 \pm 0.13	NA
	>40	0	NA	NA	0	NA	NA
Bulk Density (BD)	0-10	129	0.11 \pm 0.01	0.10 -m- 0.12	39	0.05 \pm 0.02	0.035 -m- 0.065
	11-40	140	0.07 \pm 0.01	0.06 -m- 0.08	43	0.024 \pm 0.014	0.011 -m- 0.037
	>40	35	0.07 \pm 0.01	0.06 -m- 0.08	7	-0.005 \pm 0.0-5	0.01 -m- 0

Univariate linear models that included all data and management variables (grazing intensity/vegetation type/photosynthesis pathway/native vegetation/use of fire) showed that within the tropics, high intensity grazing was found to decrease soil carbon over all depths in comparison to native vegetation on average by -18%, which was significantly different to low intensity which observed an increase of 14% in soil carbon stocks ($p < 0.001$). This was also observed at 0-10 cm and 11-40 cm individually, with high intensity grazing decreasing soil carbon stocks ($p < 0.001$), and low intensity increasing soil carbon ($p < 0.05$) (Figure 2). The RR of medium intensity grazing was not significantly different to other grazing intensities. In the subtropics, grazing intensity was found to decrease soil carbon over all depths under both low (-36%) and high grazing intensities (-18%) with low intensity grazing resulting in a 50% greater decrease than high intensity grazing ($p < 0.001$). Similar to the tropics, this was also observed in each of the sample depths (Figure 2).

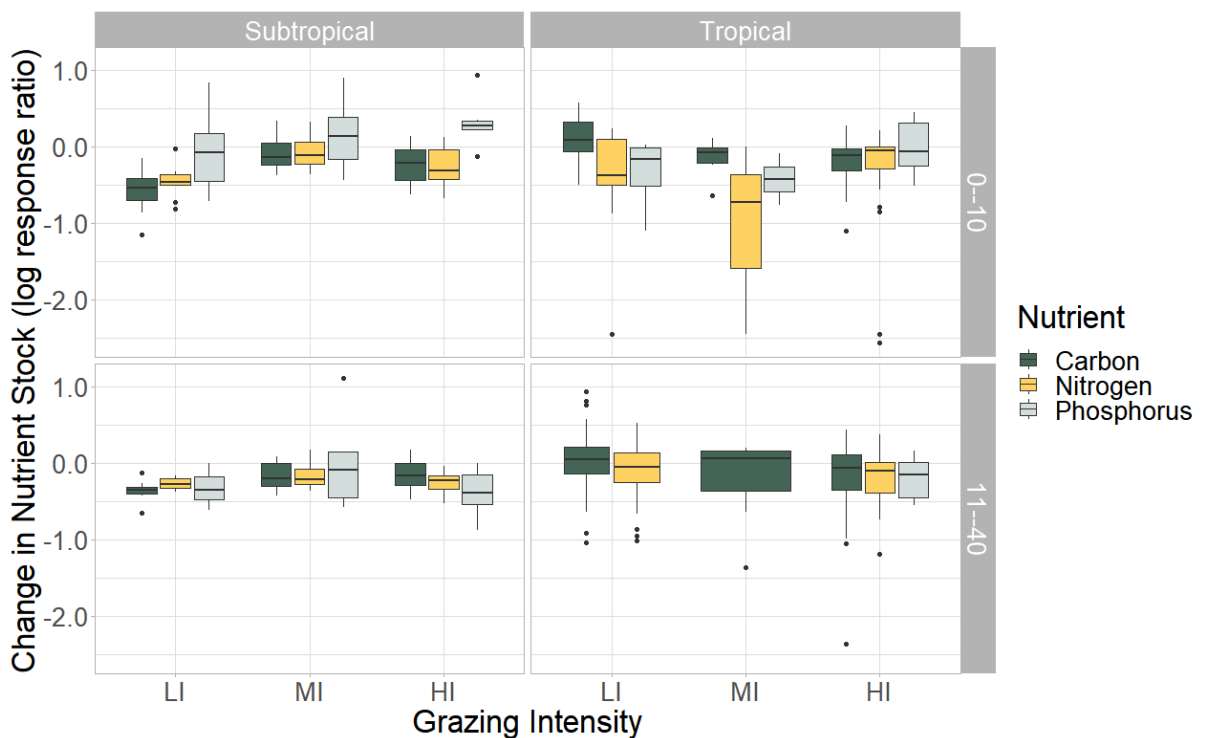


Figure 2. Response ratios of soil carbon, nitrogen and phosphorus under low medium and high grazing intensities at 0-10cm and 11-40cm within both the subtropical (left) and tropical (right) climate zones.

At 0-10 cm, photosynthetic pathway of pasture vegetation in the tropics was found to significantly affect the log response ratio, and pastures with C4 grasses had a significantly greater RR than those with C3/C4 vegetation ($p < 0.001$). However, negative RR's were observed

under all photosynthetic pathways, with a 0.6% increase in soil carbon in C4 pastures in comparison to the ungrazed control and a decrease of 13% in C3/C4 pastures. Similarly in the subtropics, pastures with C4 photosynthesis vegetation had a significantly higher RR at 11-40 cm than pastures with C3 grasses ($p < 0.05$), which demonstrates a 20% increase in soil carbon in pastures with C4 grasses in comparison to ungrazed controls, and a 15% decrease in soil carbon in pastures with C3 grasses. Analysis of the vegetation on grazed lands showed that over all depths, woody shrubby grassland had a significantly higher RR than pure grassland vegetation ($p < 0.05$) and resulted in a 7% increase in soil carbon stocks in comparison to ungrazed controls (Figure 3). Multivariate analyses found no significant interactions between management variables in either climate.

Univariate analyses were carried out using land use change variables which included the type of native vegetation, the time since land use change or implementation of exclusion treatments, and the use of fire to clear land (Figure 3). The RR of soils which had an absence of fire to clear land in tropical study sites was found to be significantly lower than those on which fire had been used ($p < 0.05$). This observation continued at each sample depth; 0-10 cm ($p < 0.001$) and 11-40 cm ($p < 0.001$), and indicated that soil carbon stocks decreased more in unburnt grazed lands in comparison to ungrazed controls, than those which had been burnt for clearance. Similarly, this effect was also observed in the subtropics, both over all depths ($p < 0.05$) and at 0-10 cm ($p < 0.05$). Native vegetation type in the tropics significantly affected the impact of grazing across all depths, 0-10 cm and >40 cm, with sites which were originally grasslands having a significantly lower RR and thus a greater decrease in soil carbon stocks with grazing than sites which were converted from forest ($p < 0.001$) (Figure 3). In the subtropics, in sites which were originally native grasslands, grazing was found have a lower RR than sites which were converted from forest ($p < 0.05$) and thus greater decreases in soil carbon in comparison to ungrazed control sites at 11-40 cm.

In the tropics, soil carbon over the soil profile was found to increase with time since land use change or implementation of exclusion over all classes (all native vegetation, native grasslands and native forest) with the strongest increase being found in those sites which were originally forest (coefficient: 0.006, $p < 0.001$) (Table 3). This relationship was also found at 0-10 cm and 11-40 cm (coefficient: 0.005, $p < 0.001$; coefficient: 0.005, $p < 0.05$), and in deep soil (>40 cm) there was also an increase with time since conversion from native forest (coefficient: 0.014, $p < 0.001$). Contrastingly, in subtropical climate, time since conversion from all native vegetation types (all native vegetation, native grasslands, and native forest) were found to decrease RR and thus lose soil carbon over time, with the strongest decrease across the soil profile being found

in sites which were converted from native ungrazed grasslands (coefficient: -0.003, $p < 0.05$). Sites which were converted from native grasslands were also found to result in decreased RR and thus decreased soil carbon stocks with increasing time since conversion in the 0-10 and 11-40 cm depth classes (coefficient: -0.008, $p < 0.05$; coefficient: -0.003, $p < 0.05$, respectively).

Table 3. Univariate linear models of carbon Response Ratio (RR) = $\ln(C_{\text{grazed}}/C_{\text{control}})$, against soil physical and chemical variables and land management variables across the whole soil profile, showing the R squared values, predicted P-values and number of observations (*k*)

Variable	R Sq		P Value		<i>k</i>	
	Tropical	Subtropical	Tropical	Subtropical	Tropical	Subtropical
Grazing intensity	0.1	0.3	<0.001	<0.001	328	63
Photosynthesis	0.03	0.04	<0.05	0.3	430	69
Depth	0.03	0.1	<0.05	<0.05	485	84
Native vegetation	0.03	0.04	<0.001	0.07	485	84
Vegetation Type	0.04	0.1	<0.001	<0.01	482	84
Use of Fire	0.04	-0.01	<0.001	0.9	485	84
Sand	-0.002	0.18	0.59	<0.05	292	30
Silt	-0.003	0.2585	0.69	<0.05	264	30
Clay	0.004	-0.02	0.16	0.81	270	57
Bulk density	-0.003	0.09	0.844	<0.05	304	73
Nitrogen	0.002	-0.02	0.229	0.972	238	64
Phosphorus	0.02	0.1	0.08	<0.05	88	36
MAP	0.2192	0.01	<0.05	0.162	481	84
MAT	0.03	-0.02	<0.05	0.7	453	46
Precipitation Class	0.1	0.07	<0.001	0.1	485	84
Duration (from Forest)	-0.01	0.37	<0.05	0.96	386	16
Duration (from Grassland)	0.06	-0.04	0.81	<0.05	65	25
Duration (from All)	0.05	0.09	<0.05	<0.05	451	41

In the subtropics, soil carbon stocks decreased in grazed sites with increasing bulk density ($p < 0.05$) across all depths, and again at 11-40 cm ($p < 0.05$). Furthermore, with increasing sand content was found soil carbon under grazing decreased over the whole subtropical dataset ($p < 0.05$) and at 11-40 cm ($p < 0.05$). Silt was also found to decrease soil carbon at 0-10 cm and 11-40 cm ($p < 0.05$). With regards to soil texture in tropical studies, RR and thus soil carbon decreased with increasing silt content in comparison to ungrazed control sites in overall depths and at 11-40 cm respectively. Multivariate analysis on soil physical variables in tropical studies found significant positive interactions between bulk density and clay, sand and clay, and bulk density, sand silt and clay ($p < 0.05$) and negative interactions between sand and silt, bulk density, sand and silt, and bulk density, sand, and clay ($p < 0.05$). In the subtropical dataset, weak positive interactions were found between sand and silt, sand and clay, and a negative interaction between sand, silt, and clay.

At 0-10 cm in the tropics, soil carbon significantly increased with increasing nitrogen stocks ($p < 0.05$) and over all depths decreased with increasing phosphorus stocks ($p < 0.05$). For the subtropical dataset, no significant relationships were found between RR and nitrogen. However, with increasing phosphorus over all data and at 11-40 cm soil carbon was found to increase ($p < 0.05$; $p < 0.001$) respectively. A significant negative relationship between carbon RR and pH was also found in the subtropics, with soil carbon stocks decreasing with increasing pH ($p < 0.05$). In both the tropical and subtropical multivariate model significant negative interactions were found between nitrogen and phosphorus ($p < 0.05$), and in the subtropical multivariate model there was also a negative interaction between soil phosphorus and pH ($p < 0.05$).

Across the data a weak positive relationship was found between MAP and grazer response ratio, with an increase in MAP increasing RR and thus changes soil carbon stocks between ungrazed controls and grazed sites across all depths ($p < 0.05$). With regards to MAT, significant positive relationships were found across all data and each of the depth classes, with increasing MAT increasing changes in soil carbon stocks over all data ($p < 0.05$), 5% ($p < 0.001$) at 0-10 cm ($p < 0.001$), and by at 11-40 cm ($p < 0.0001$). Similarly, the semi-arid precipitation class had a significantly lower RR than sites in both subhumid and humid sites ($p < 0.05$), which corresponds to greater losses of soil carbon in -semi-arid grazing lands. There were no impacts of climate factors on soil carbon change in the subtropics.

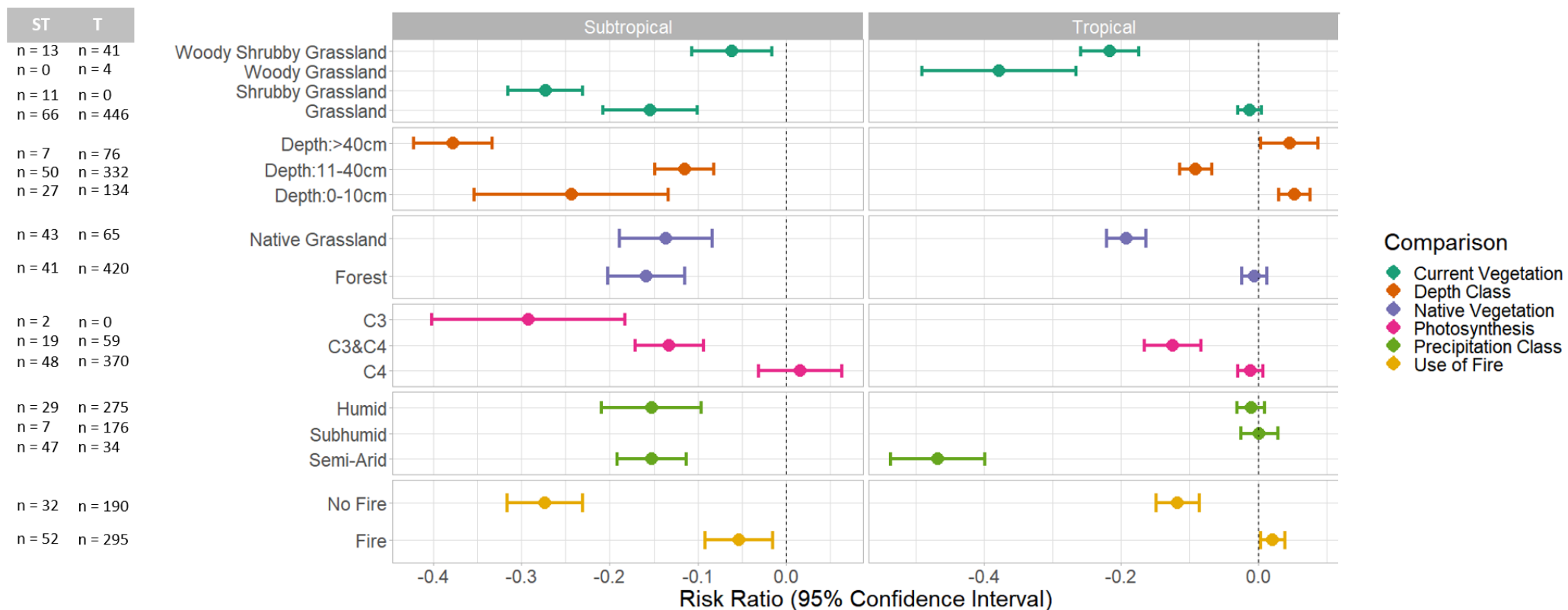


Figure 3. Forest plots showing the mean (+se) of carbon Response Ratio size under different management, climatic and sample depths in both subtropical and tropical climate types. Each plot shows a comparison between levels within the different management and climatic variables and sample depths (i.e., Photosynthesis pathway: C3, C4 or C3&C4 mixed) shown in the key on the right hand side. Sample size (n) is shown on the left-hand table. Values which are greater than 0, shown to the right of the dashed line denote a positive change in carbon in comparison to the control plots (native vegetation).

2.4 Discussion

Our analyses show that as our hypotheses predicted, grazing intensity impacted soil carbon stocks in both climate zones and that this hypothesis can be accepted. In the tropics low intensity grazing causes a small increase in soil carbon stocks. This result is consistent with previous studies in which pastures with low livestock stocking rates (for example 0.1 heads of foraged livestock per hectare (HFFL), or 1 Livestock Unit (LSU per hectare) have the potential to act as carbon sinks, continuously accumulating carbon (Navarrete *et al.* 2010; Stahl *et al.* 2017). Grazing can stimulate plant growth, increasing carbon which is stored both above and belowground as plant material (Schumann *et al.* 2002). Furthermore, defoliation can break down standing dead biomass and remove old plant tissues, increasing light interception and photosynthesis of living vegetation (Garnett *et al.* 2017). However, establishing such grazing intensities which favour soil carbon sequestration can be a challenge, with variations in climate, soil type and vegetation also playing a role in carbon dynamics. Unlike in the tropics, subtropical soils exhibited an overall decrease in soil carbon under low grazing intensities, both over the whole dataset and within each depth class, suggesting that grazing decreases soil carbon stocks regardless of management practice. High intensity grazing was found to have a negative effect on carbon stocks in both climates, an effect of grazing which has long been accepted and is well documented globally (Tessema *et al.* 2011; Enriquez *et al.* 2015; He *et al.* 2011).

We predicted that the impacts of grazing on soil carbon would be dependent on the photosynthetic pathway of pasture vegetation, and that the impacts of photosynthetic pathway would be different between tropical and subtropical climates. This hypothesis was found to be true, with soil carbon in C3/C4 pastures showing a greater decrease in soil carbon in the topsoil than in those which had solely C4 grasses. C4 photosynthesis is highly efficient for fixing CO₂ in environments with higher temperatures and dominates primary productivity in warmer climates (Ehleringer & Cerling, 2002). Thus, in the tropics, pastures using the C4 photosynthetic pathway will naturally have greater soil carbon inputs than those using C3 photosynthesis which has higher efficiency in cooler regions (Ehleringer *et al.* 1997). As a result, pastures which have both C3 and C4 vegetation in the tropics tend to be those which have a mixture of both shrubs and grasses, or pastures which are sown with legumes (Valentim & Andrade 2004; Thomas *et al.* 1994). Pastures which have vegetation with mixed photosynthetic pathways (C3 and C4) yet are not sown with legumes are likely to be degraded, with unpalatable C3 shrubs encroaching into managed pastures and competing with improved grasses (Fonte *et al.* 2014; Moleele *et al.* 2002; Martinez & Zinck, 2002).

In the subtropics, woody shrubby grassland was associated with an increase in soil organic carbon, whilst the dominance of C3 grasses decreased soil carbon stocks with grazing. Previous studies have found that the invasion of woody plants into grasslands can increase both carbon and nitrogen stocks through higher rates of organic matter production, and greater resistance of woody plant material to decomposition (Liao *et al.* 2006; Liao & Bouton, 2008). Furthermore, the wider temperature amplitude between seasons in the subtropics, such as South Africa which in parts has a mean winter temperature of 7.9°C, but summer temperatures of 20.9 (Talore *et al.* 2014) indicates that unlike in the tropics, C3 photosynthesis is most efficient during winter months and shrubby vegetation will have the greatest NPP. However, whilst C3 photosynthesis is more efficient during these months, it is likely that overgrazing and degradation of pastures increases the rate of carbon loss over that of photosynthesis, and more carbon will be lost than sequestered belowground.

In both tropical and subtropical climates which converted to managed pastures from native grasslands or savannahs were observed to have decreased grazing-carbon response ratios and thus greater decreases in soil carbon with grazing in comparison to those converted from forest. This could be due to more carbon being stored belowground in forests prior to land use change, particularly in deep soil horizons. These forest carbon stores are more likely to be recalcitrant and less susceptible to change and subsequent loss through microbial activity. Sites which were not exposed to fire to clear woody vegetation prior to establishment of pastures were found to have a greater decrease in soil carbon stocks than those which were burnt. This could be due to the formation of black carbon or charcoal, which is formed from partially charred plant material (González-Pérez *et al.* 2004; Kuhlbusch, 1998). However, it should be noted that this effect could also be related to the impacts of grassland composition that were previously discussed, with a decrease in soil carbon found with wood shrubby vegetation.

Aside from grazing intensity, it was expected that soil carbon would be affected by other interacting physical and chemical variables. This analyses has shown that physical characteristics such as bulk density, and soil texture, and chemical characteristics such as nutrient availability and pH are all observed to interact with soil carbon stocks, and as a result we can accept this hypothesis. With increasing bulk density, carbon RR was found to decrease in the subtropics, indicating a greater decrease with grazing in soil carbon stocks. Livestock trampling is well known to cause compaction and to increase soil bulk density (Steffens *et al.* 2008; Stavi *et al.* 2008; Van Haveren, 1983), which leads to a multitude of detrimental effects on soil carbon storage including reduced soil water

filtration and availability for plant growth, disruption of aggregates; reduced root growth and reduced carbon inputs to the soil (Brevik *et al.* 2002; Jimenez & Lal, 2006; Martinez & Zeck, 2002; Pineiro *et al.* 2010). However, it should be noted that this could be an indirect effect of varying soil textures, with finer textures soils having lower bulk densities (Chaudhari *et al.* 2013). Contrastingly, data from the tropics exhibited a positive relationship with RR, indicating an increase in carbon stocks with grazing in sites with increasing bulk density. As there were no significant effects of soil textural fractions in the tropics, these results would have to be verified, but this could be due to a number of studies which had a high proportion of sand (>50% sand).

Whilst no significant interactions were found between proportion of sand, silt, and clay in the tropics, increasing proportions of sand and silt particles were found to be negatively correlated grazer RR in the subtropics. Silt particles are similar to clay in that it can bind to and protect carbon from microbial attack (Jimenez & Lal, 2002), however this is to a much lesser extent than clay. It is likely that with increasing silt particles, clay particles decrease and the soil texture becomes coarser with a high proportion of coarse silt, which is less able to bind to organic matter (Balesdent *et al.* 1998). With regards to the effects of sand content, larger particles are less effective stabilising and sequestering carbon due to the absence of charged surfaces such as on clay or silt minerals which prevents the binding of organic matter (Augustin & Cihacek, 2016; Hobley *et al.* 2013). Thus, an increase in sand particles in these areas would decrease the ability of the soil to stabilise organic matter in aggregates.

Contrasting relationships were found between nutrients in the two climates, with RR increasing with soil nitrogen, and decreasing with soil phosphorus in the tropics, whilst in the subtropics there was no effect of nitrogen, and effect size increased with phosphorus stocks. The increase found between nitrogen stocks and grazer RR in the tropics is expected due to the close linking of the carbon and nitrogen cycles (Pineiro *et al.* 2010). Other studies (Frank *et al.* 1995; Hiernaux *et al.* 1998) have found that carbon sequestration is highly dependent on nitrogen availability, with an increased availability of nitrogen in the soil potentially increasing NPP, transferring from soil to plants, and potentially decreasing soil respiration, reducing loss of carbon from the soil (Pineiro *et al.* 2010). Increasing levels of phosphorus can increase soil carbon as seen in the subtropics, which is potentially due to increased livestock inputs directly, or microbial activity releasing available phosphorus, which in turn can be utilised in plant and root growth, enhancing organic matter inputs into the soil (Touhami *et al.* 2020; Sato *et al.* 2019). Within the tropics however, this relationship

was contrasting, with increasing phosphorus decreasing carbon RR and thus soil carbon stocks. This pattern has previously been observed by Townsend *et al.* (2002), who hypothesised that this could be occurring through the decoupling of organic phosphorus and organic matter pools, with livestock consuming plant matter and returning it to the soil as manure which has a much lower C:P ratio.

However, whilst it was predicted that effects of grazing on SOC will be dependent on a range of interacting variables including vegetation type, soil texture, and soil chemical properties, multivariate interactions were only found between soil chemical properties. In both the tropics and subtropics significant interactions were found between soil nitrogen and phosphorus, and their combined influence on grazer RR. The stoichiometric ratios of C, N and P in the soil are recognised to be important maintaining the stability of an ecosystem, and thus concentrations and the cycling of these elements in soils are closely linked (Fazhu *et al.* 2015; ²Li *et al.* 2018). The negative effect of an interaction between nitrogen and phosphorus on grazer response ratio may well be due to increase inputs of N and P to the soil through animal excreta. Such increases in nutrient deposition can increase microbial activity and biomass which ultimately will lead to reduced organic matter content. The lack of other interactions in the collected data could be due to the nature of the study, with data collected from a range of studies in different climates, soil types and spatial locations. The spatial nature of soils will inevitably result in high variability in the database, and as a result multivariate interactions between variables may not be observed. Despite this, it is clear that soil carbon is impacted by multiple different variables of both biotic and abiotic nature, and the conditions which facilitate carbon sequestration are complex.

In this analysis, we hypothesised that the effects of grazing on soil carbon would be greater in the tropics due to higher mean temperatures increasing soil carbon respiration and carbon losses. The results of this study however, indicate that in the tropics with both increasing precipitation and temperature soil carbon stocks under grazing sites increase, so we must reject this hypothesis. In the tropics, these relationships are likely explained due to high humidity and temperatures facilitating processes such as humification and root turnover which can increase carbon sequestration into the soil (Zech *et al.* 1997). As shown in the present study, the tropical climate has consistently high temperature with very little seasonal variation, and therefore can favour such processes which promote soil carbon sequestration. This contrasts with the subtropics, where no significant effects of the climate variables (precipitation and temperature) were found on soil carbon response ratio. Temperatures in these climates have a much wider range, subsequently

producing varying effects on carbon sequestration, with low rates of sequestration at lower temperatures due to low microbial activity and NPP, to higher rates as temperature increases (Davidson & Janssens, 2006; Mellilo *et al.* 1993). Such effects are highly dependent on precipitation. For example, NPP may increase or decrease at higher temperatures depending on water availability, with low rainfall/increased temperature resulting in decreased soil moisture (Mellilo *et al.* 1993). Thus, the increase of RR and corresponding increase in soil carbon in response to increasing precipitation and temperature is to be expected, as increasing rainfall means that there is higher water availability for vegetation growth and microbial respiration and higher temperatures increase biological activity. This result is consistent with the findings of previous reviews which examine grazer effects on soil carbon (Abdalla *et al.* 2017; Conant and Paustian, 2002; Dlamini *et al.* 2016; McSherry & Ritchie, 2013) and is further highlighted in the analysis of precipitation class and effect size.

In the tropics, response ratios were decreased in semi-arid conditions, suggesting that areas with low rainfall have increased soil carbon loss under grazing. This has been widely reported in previous studies, with increasing aridity bringing with it a host of issues which can exacerbate the loss of carbon from soils, including increased vulnerability to erosion, reduced aggregate stability, abundance of fungal bacteria, and vegetation productivity (Berdugo *et al.* 2020). Furthermore, semi-arid agricultural lands have a higher tendency to become saline and sodic, particularly where irrigation is used or groundwater tables are shallow, which can affect soil carbon through plant toxicity and detrimental effects on soil structure (Vecchio *et al.* 2018; Wong *et al.* 2010). On the other hand, in the tropics, soil carbon RR was reported to be higher in humid and subhumid conditions, which as mentioned previously can increase soil carbon.

2.4.1 Gaps in the Literature

The present study includes no data regarding of the effects of grazing on soil microbial biomass carbon, and subsequent effects on carbon stocks. Previous studies which focus on more temperate climates have found mixed effects of grazing, with some showing an increase in microbial biomass at low grazing intensity, but a decrease at high intensity (Liu *et al.* 2012; Bardgett *et al.* 2001), while others show an overall decrease in microbial biomass (Fu *et al.* 2012; Wang *et al.* 2006), and some found no effect of grazing on microbial biomass (Tracy & Frank, 1998; Kieft, 1994;). The uncertainty

in the effects of grazing on microbial biomass and activity could be due to the wide range of environmental, physical and chemical factors, such as temperature, soil texture, and nitrogen availability upon which soil microbes rely on (Ramirez *et al.* 2012; Kirschbaum, 2004; Waldrop *et al.* 2004; Wang *et al.* 2003). The distinct lack of studies reporting microbial biomass and activity in the tropics, highlights a key and important gap in the literature. Microbial activity and biomass both control carbon dynamics in the soil profile through producing binding agents in soil aggregates, transforming organic matter into humic compounds, and providing nutrients to plants (Jimenez & Lal, 2006; Zech *et al.* 1997). As a result, soil microbes are key in the process of carbon sequestration. In tropical regions, which see year-long high temperatures and very little seasonal variation, the role of microbes is particularly important as rates of microbial respiration can be consistently high. Understanding these patterns and how they are further impacted by grazing management is key in understanding where carbon sequestration can be achieved under grazing lands, thus more research would be beneficial in this area.

Whilst the review did not use root biomass as a search term, vegetation type and photosynthetic pathway were considered, and it was noticed that few studies considered root biomass when reporting carbon stocks (most removed roots from samples prior to analysis). Roots make up a large proportion of soil organic matter, with over 80% of plant biomass being found belowground (Jackson *et al.* 1996; Caldwell & Richards, 1986) and the rhizosphere exerts a huge influence within the soil profile over the activity of soil fauna (De Deyn *et al.* 2008). The lack of studies in the tropics and subtropics which consider the effect of on both soil carbon stocks and root biomass is an important knowledge gap, with root growth and turnover being highly dependent on climate and other environmental variables. Root turnover in grasslands has been found to positively correlate with mean annual temperature (Gill & Jackson, 2000), thus the warmer conditions typically found in the tropics no doubt impact belowground biomass. A review by Schenk & Jackson (2004), found that deep rooting structures are mostly found in arid and humid tropical regions with either coarse or fine textured soils due to their capacities to store plant available water.

In subtropical savannas, many of which are arid or semi-arid, it was found that root biomass decreased with depth to 50 cm yet increased in deeper soil layers, likely due to the ability of woody patches to acquire more nutrients in this type of ecosystem (Zhou *et al.* 2018). Furthermore, in the tropics and subtropics, introduced African C4 grasses are widely used which exhibit deep rooting structures, ultimately sequestering significant amounts of carbon within deeper soil layers (Fisher

et al. 1994). Similarly, Nepstad *et al.* (1994) reported rooting structures of grasses within a managed pasture of *Brachiaria humidicola* to a depth of ~8 m in the Brazilian Amazon. Such structures can contribute to carbon sequestration through increased root turnover and subsequent humification of exudates in deeper soil layers.

2.4.2 Limitations of the present study

A factor which was not considered in the present study yet has previously been shown to effect soil carbon dynamics, is altitude. High altitudes can decrease the carbon sequestration potential of a grassland, due to lower temperatures which limits NPP and decreases organic matter and root turnover (Dlamini *et al.* 2016; Shi *et al.* 2009; Garcia-Pausas *et al.* 2007). In the tropics however, such lower temperatures could favour carbon sequestration, with a decreased respiration in favour of photosynthesis. With a number of studies sampling regions with high altitudes – Ethiopia, China – and many more regions in the tropics and subtropics not included in this study – Ecuador, Peru, India – it would be useful to further understand the dynamics of soil carbon in these environments, particularly in these climate zones. Also worth taking into account, would be the effect of landscape position on soil carbon dynamics. Previous studies have found that topography can have significant effects of the distribution of soil carbon in an ecosystem, with upland areas having less carbon than low lying areas or depressions where there is soil deposition and waterlogging (Ritchie *et al.* 2007). Further investigation in this area would allow a clearer view of soil carbon dynamics in the tropical and subtropical climate zones.

2.5 Conclusions

The present study shows that increases in soil carbon under grazed lands are driven by multiple interacting factors in both the subtropical and tropical climates. Overall, all grazing intensities decrease soil carbon in comparison to ungrazed control sites in the subtropics, yet in the tropics, low intensity grazing was shown to increase soil carbon. Furthermore, the effects of grazing intensity interact with vegetation type and photosynthetic pathway. The interlinked nature of the drivers of soil carbon sequestration highlights that several variables and multiple processes should be considered at a site-specific level when formulating management plans to increase rates of

carbon storage. This is particularly important in the subtropics and tropics, where climate has a strong effect on soil carbon dynamics, and agricultural expansion is so prevalent that grazed lands are already significantly degraded.

With changing climates, increasing precipitation and temperatures, driving variables within sites or whole ecosystems may change, in turn influencing soil carbon sequestration. For instance, with warmer temperatures, there may be an increase in the dominance of C4 species, and decrease in productivity of C3 vegetation, decreasing sequestration rates in C3 pastures. Moreover, higher temperatures will also increase soil microbial activity, increasing respiration within soils which will increase carbon outputs from the soil. Thus, it is critical to understand not only interactions between soil and land management, but also how these may be affected by changes in climate.

The present study identified that there has been substantially more research into the impacts of land use change and grazing on soil carbon dynamics in tropical ecosystem, likely due to ecosystems such as the Amazon rainforest receiving significantly more public and policy attention. In order to maximise the potential of subtropical soils to sequester soil carbon in grazing systems, it is imperative that more research is undertaken in this climate. Further key areas for future research which have been recognised in the present study are the effects of grazing on microbial and root biomass in the tropical and subtropical climates. In order to fully understand where carbon sequestration can be achieved, it is important to investigate multiple factors which affect carbon dynamics. Both microbial biomass and respiration, and root dynamics directly affect soil carbon, with microbes decomposing organic matter inputs, and roots making up a large proportion of inputs to soil organic matter. Further investigations into these areas and their interactions with one another and grazing management could provide important insight into potential soil carbon sequestration in tropical and subtropical grazing lands.

3 Land use change effects on soil carbon dynamics and soil quality in the dry Chaco

3.1 Abstract

In the north-west region of the Chaco ecosystem, Argentina there has been widespread conversion of forests to grazing systems. This clearance of native vegetation and establishment of extensive grazing practices has been identified previously to decrease soil carbon stocks. However, whilst this negative impact of deforestation and grazing on soil carbon in the Chaco has been observed, it remains unclear if changes in soil carbon stocks are directly due to land use change or whether carbon stock changes are associated with changes in soil quality. This lack of understanding has the potential to reduce the value of soil for agriculture now and in the future, and will ultimately lead to further soil degradation. The present study aimed to quantify the impact of deforestation and time since establishment of grazed pastures on soil carbon and investigate whether there are other factors besides that of land use change which influence soil carbon dynamics and soil quality. Using a chronosequence design, soil samples were taken from forest patches and pastures of increasing time since conversion across two sites in the Tucuman, Catamarca and Santiago del Estero provinces in north-western Argentina. The samples were analysed for total carbon, nitrogen and phosphorus stocks, soil texture, pH, electrical conductivity and root biomass. We found that soil carbon and nitrogen stocks significantly decreased by 16-53% and 12-55% respectively in both sites with deforestation in the top 10 cm of the soil profile. Carbon stocks in Los Puestos also decreased by 17-22%, and in Lavalle nitrogen stocks were observed to increase post deforestation by 27-28% over 0-50 cm. Soil phosphorus stock decreased by 6-67% in Los Puestos with deforestation over all sampled depths, whilst in Lavalle soil P at 10-30 and 30-50 cm decreased in young and intermediate pastures then increased by 46-52% in old pastures. Root biomass was observed to be 44-82% lower pastures of both sites in comparison to forest, over all depths and significantly positively correlated to both carbon and nitrogen stocks at 0-10 cm ($p < 0.05$). Mediation path analysis showed that in Los Puestos, soil carbon is directly affected by nitrogen stocks and pH, but indirectly affected by the root biomass, pH and age class. In Lavalle, soil carbon stocks were directly affected by root biomass and nitrogen stocks, and indirectly by root biomass, depth, pH and age class. The results show that soil carbon, nitrogen and phosphorus stocks decrease in the topsoil of both sites with both the removal

of forest with no recovery over time, but that over 0-50 cm there was no impact. This indicates that there is a loss of organic matter from the biologically active horizon of the soil profile, which in turn may impact soil productivity in the future. Such impacts on soil health will be driven both by land use change, and also the detrimental impacts of conversion on other soil characteristics such as nitrogen availability, root biomass, and pH.

3.5 Introduction

Due to increased availability of livestock products and associated high consumption of animal-sourced products, there has been substantial intensification and expansion of grazing systems globally (Godde *et al.* 2018; Havlik *et al.* 2014). As a result, the global livestock sector is one of the leading causes of land use change, with extensive areas of forested lands being converted to grazing systems each year (Curtis *et al.* 2018). Such expansion of grazing lands into forest ecosystems releases vast amounts of soil carbon into the atmosphere, with accelerating soil erosion due to deforestation, disruption of soil microbial communities, changes to soil moisture and physical properties such as aggregate stability detrimentally impacting soil carbon dynamics (Wienkenkamp *et al.* 2020; Borrelli *et al.* 2017; Crowther *et al.* 2014). In addition, grazing practices can amplify soil carbon losses through decreased vegetation diversity and biomass, and compaction impeding root growth and hindering organic matter formation in the soil (Pineiro *et al.* 2010; Han *et al.* 2008; Pei *et al.* 2008). Soil carbon is reported to be the largest terrestrial pool of carbon (Deng *et al.* 2016; Jobbagy & Jackson, 2000), so the increasing rate at which forests are being cleared and grazing systems expanded is causing the huge loss of sequestered carbon to the atmosphere (Kim *et al.* 2015). As a result, it is imperative that the impacts of these practices on soil carbon dynamics are quantified and understood.

One region which has been subjected to greatly increased and widespread deforestation over the recent decades is the Gran Chaco, a mostly forested ecosystem in the subtropics which spans across Argentina, Paraguay, and Bolivia and 81.5% of which is in Argentina (Fernandez *et al.* 2019; Fenlenberg *et al.* 2017 Gasparri *et al.* 2008). Predominantly to make way for grazing systems and crop production, 142,000 km² (14.2 million ha) of dry forest was removed between 1985 and 2013 (Baumann *et al.* 2017). With rates of deforestation in this area accelerating rapidly between 2000 and 2010 due to increased demands on agricultural products, it is estimated that 824 Tg of carbon emissions resulted from forest to grazing land conversion over a 29-year period (Baumann *et al.* 2017; Vallejos *et al.* 2015). Despite the high rates of deforestation (estimated at 1% per year) which occur here, and the associated detrimental ecological effects on soil condition and the ecosystem, this area of South America receives little attention from science and policy, particularly in comparison to the neighbouring Amazon rainforest (Gasparri & Grau, 2009). In the dry Chaco, which is characterised by seasonally dry forest vegetation (Grau *et al.* 2005), the impacts on soil carbon stocks of land use change from forest to grazing systems remains under researched.

Previous studies have been found that grazing and fire, which is traditionally used to clear forest, decreases soil carbon concentrations with increasing degrees of site degradation (Bonino, 2005; Abril & Bucher, 2001; Abril & Bucher, 1999; Abril *et al.* 1995). More recently, Osinaga *et al.* (2018), observed that newly established pastures showed a decrease in soil carbon compared with forest soils over 10 years since deforestation, mainly due to a decline in net primary productivity compared to the forests. Furthermore, deforestation followed by grazing in this ecosystem has been found to negatively impact physical soil properties, with higher bulk density and penetration resistance, lower infiltration rates and reduced water content in grazed compared to forested soils (Magliano *et al.* 2017; Osinaga *et al.* 2017). Such detrimental impacts affect soil carbon stocks are likely due to reduced root growth and limiting plant water availability as shown in a recent review of the literature (Veldkamp *et al.* 2021). More recently, a combination of experimental data and simulation modelling showed that the removal of forest followed by grazing over time had the potential to both decrease and increase soil organic carbon with respect to native vegetation, depending on management practices (Baldassini & Paruelo, 2020). The study found that soil carbon stocks were only observed to increase when grazing intensity was reduced by 50-75%, burning of pastures for invasive vegetation control was not used, or high levels of nitrogen were applied prior to sowing (Baldassini & Paruelo, 2020).

Despite these studies, it remains unclear what causes decreases in soil carbon stock, whether it be direct impacts of land use change, or indirect impacts due to changes in other soil or physical and chemical properties. Particularly with most previous studies either selecting pastures which were established for some time, or those with varying degrees of degradation (shrubby encroachment and bare soil), there is no clear evidence of how the soil carbon stock is impacted immediately post deforestation, and how these impacts change over time since the establishment of pastures. The use of chronosequence studies is highly valuable in this respect, as they allow the investigation of the direction of soil carbon changes and other soil properties over time, which can be translated an overview of long-term carbon dynamics (Hugget, 1998). This study quantified soil carbon and nutrient stocks over a chronosequence using sites which show a land use gradient from native forest to grazed pastures with increasing time since deforestation in the dry Gran Chaco.

The objectives of this study were to investigate how deforestation and time since the establishment of pastures impacts soil carbon and nutrient dynamics. Furthermore, the study aimed to identify soil characteristics which influence soil carbon stock changes, such as soil nutrients, root biomass

and other soil physical and chemical properties. The working hypotheses were that; 1) changes in soil carbon and other nutrients (nitrogen and phosphorus) will be impacted not only by land use change but also by indirect effects of deforestation on other soil physical, biological and chemical properties such as pH, electrical conductivity (EC), and root biomass; 2) Whilst soil carbon stocks would decrease following deforestation, recovery in carbon and other soil nutrients were expected with time since the removal of native vegetation.

3.5 Methods

3.3.1 Study area and site selection

The study was carried out in dry Chaco region of Argentina, in the Tucuman, Santiago del Estero and Catamarca provinces (Figure 4). The area is characterised by a mean annual rainfall of 500 – 880mm, 75% of which falls between October and March, with high summer temperatures (mean: 25°C) and cold (mean: 13°C) dry winters (Banegas *et al.* 2019; ²Banegas *et al.* 2019). The natural forest of the dry Chaco is a priority area for conservation, and has historically been exploited for logging, crop production and grazing systems (Gasparri & Grau, 2009). It is a subtropical seasonal forest which is dominated by dry, xerophytic open woodland, with an upper story characterised by species such as *Schinopsis lorentzii*, *Aspidosperma quebracho-blanco* and *Schinopsis balansae*, a mid layer of shrubs, such as *Schinus mole*, *Capparis atamisque* and *Celtis ehrenbergiana* and a grassy understory with species such as *Trichloris crinita*, *Trichloris pluriflora* and *Papophorum caespitosum* (Ledesma *et al.* 2022). Over the last 40 years, exploitation of the natural forest has increased, and more recently the ecosystem is dominated by very small patches of preserved forest which are typically maintained as parts of farms and exist in a mosaic of shrubland and areas of agricultural production. Where the Chaco has been cleared for livestock production, pastures are dominated by improved C4 grasses, in particular *Chloris gayana*, *Urochloa brizantha* and *Leucaena leucocephala* (Banegas *et al.* 2019). It is common for pastures in the area to be degraded and display the encroachment of shrub species mentioned previously.

The study followed a chronosequence design replicated six times, which consisted of a site of native vegetation (Figure 5) followed by pastures of increasing time since deforestation. Age classes of the plots selected were: Forest (Native Vegetation); (Young) 0-5 years; (Intermediate) 10-15 years; and

(Old) >20 years. The study sites were selected from a number of farms used for research by the *Instituto de investigación Animal del Chaco Semiárido* (INTA-IIACS), the *Instituto de Ecología* (IER) *Regional* in Tucuman, Argentina, and the University of San Luis. From this selection, two farms were chosen based on their comparability of soil types, land management practices (i.e. both used substantial areas for extensive livestock systems), and proximity in location which ensured similar weather patterns such as rainfall over the wet season. The two farms selected were in the settlements of Los Puestos (27°17'02"S 65°01'02"W) and Lavalle (28°12'18"S 65°05'49"W) which are found in the provinces of Tucuman, Santiago del Estero and Catamarca (Figure 4). An additional farm was selected at INTA-IIACS (27°11'27"S 65°14'39"W), which was neighbouring to Los Puestos, and added to the experimental design upon the flooding of the Los Puestos site. Each of the selected farms were mixed land use, with both livestock grazing and soybean crop production, and possessed an accessible patch of forest nearby. Typically, much of the forest in this area is privately owned, so it was necessary to select farms which had forest patches in their boundaries.

The specific plots were selected using Google Earth Engine in which a shape file of land use history provided by the University of San Luis, was overlaid onto Landsat imagery. The shape file outlined the boundaries of the farms which the University of San Luis, INTA-IIACS and IER use for research. Within each farm boundary was also the boundaries of individual plots of pasture, forest or cropland. The individual plots contained information on year of deforestation, previous land use and current land use which was used to identify potential sampling plots. Pastures and forest patches were selected based on year of deforestation (young; 0-5 years prior to 2019; intermediate; 10-15 years prior; old; >20 years prior) and excluded if they had a prior land use besides that of forest. To check the reliability of the site data, a freely available global time lapse of landsat imagery from 1984 – 2016 was used which allowed for the visual identification of deforestation year and establishment of pastures.

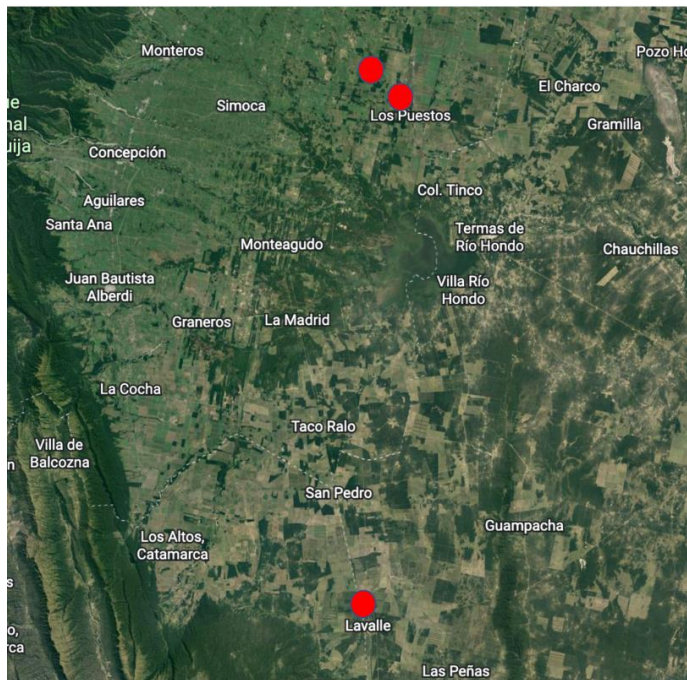
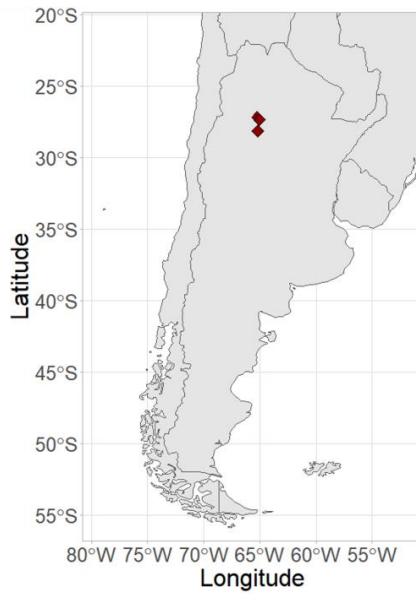


Figure 4. Location of sample sites in the Chaco, in Los Puestos and INTA-IIACS, Tucuman, and Lavalle, Catamarca and Santiago del Estero.

Three repeats of the chronosequence were selected at Lavalle (1 patch of forest, 9 pastures) and three at Los Puestos (1 patch of forest, 9 pastures). Due to difficulties in locating intact forest patches, 1 forest site was selected for each farm and was pseudoreplicated in laboratory analyses.

Within each age class, a 1m x 1m soil pit was dug in order to inspect the soil profile (Figure 5), which identified that there were no noticeable differences in soil horizons between each age class (including forest sites). The sites were found to have the same soil textural classification (Table 4) and were typically a silt loam. Whilst both sites were generally flat in topography, Lavalle was found to be at a higher altitude than Los Puestos, which was located in a flat depression. Both sites were situated close to the foothills of the Sierra del Aconquija mountain ridge of the Andean mountain range.



Figure 5. Newly deforested area in Lavalle (left) and soil profile to 1m in Los Puestos (right).

Pastures, which were established following deforestation using slash and burn techniques. Of the pasture plots used in this study, all were sown with the improved C4 grass – *Chloris gayana* – a salt tolerant grass which is able to withstand arid conditions, salinity and light frost (Taleisnik *et al.* 1997), so is well adapted to be productive in the subtropical seasonal climate of the region. In Lavalle, all plots of each age category (young, intermediate and old) were productive and in moderate, to very good condition. Particularly the young and old pastures, were dominated by *C. gayana* and showed little to no shrub encroachment. The intermediate pastures selected for sampling were in moderately good condition, but displayed some evidence of bare patches of soil

and shrub encroachment. Shrubs which encroached on these pastures were sparse, and were typically of the species *C. atamisque*. In Los Puestos, the selected pastures appeared to be more degraded, with young and intermediate plots displaying a greater encroachment of shrubby vegetation. Encroaching shrubs included the species *C. atamisque* and *C. ehrenbergiana*. Old pastures did not show shrub encroachment, but had moderately high occurrences of bare soil patches between areas of *C.gayana*. The forest plot of Los Puestos was in good condition, with a thick grassy understory with few bare soil patches, a moderate mid layer of shrubs and high tree coverage in the canopy layer. Contrastingly the forest plot of Lavalley was degraded, with a sparse grassy understory, a thick shrubby mid-layer, and a moderately thick canopy layer of trees.

3.3.2 Soil Sampling and processing

Within each plot soils were sampled using a soil auger at five points within each plot, from three depths; 0-10 cm, 10-30 cm and 30-50 cm. In addition, bulk density samples were taken using a 5cm x 5cm bulk density ring from three points within each plot and analysed as described under Section 3.3.3. Soil samples were sampled to 2mm and roots separated from the soil for the measurement of root biomass. After sieving and homogenising, samples were analysed for pH and electrical conductivity. Samples for total carbon, total nitrogen, total phosphorus, and texture were air dried for shipping back to Lancaster, UK.

Table 4. Site and soil characteristics at Los Puestos and Lavalle at 0-10 cm

Site	Los Puestos	Lavalle
Coordinates	27 11'34" – 27 20'29"S 65 14'46" – 65 01'27"W	28 11'07"S, 65 06'28"W
Mean Annual Precipitation (MAP, mm yr ⁻¹)	984.9	527.8
Mean Annual Temperature (MAT, °C)	13-25	13-25
Climate (Koppen)	Humid subtropical	Hot semi-arid
Elevation	300-325m a.s.l	450-550m a.s.l
Soil Type	Eutric Fluvisol	Eutric Fluvisol
Clay (%)	12.2±0.6	13.3±0.8
Silt (%)	64.7±1.2	60.8±0.9
Sand (%)	23.2±1.1	25.9±0.5
OM (%)	6.5±0.3	4.9±0.1
pH	5.9-9.1	6.3-8.2
Electrical Conductivity (dS/m)	0.09-3.6	0.03-0.36
Pasture Vegetation	<i>Chloris gayana</i> , <i>Acacia spp</i>	
	Trees: <i>Aspidosperma quebracho-blanco</i> , <i>Schinopsis quebracho-colorado</i> , <i>Chorisia speciosa</i> Legumes: <i>Caesalpinea paraguariensis</i> , and Shrub: <i>Prosopis spp</i>	

3.3.3 Laboratory Analysis

3.3.3.1 Physical and Chemical Properties

Immediately after sampling soils were measured for pH and electrical conductivity (EC), through the measurement of the soil water interface with a 1:2.5 soil water ratio (Allan, 1989) (10g soil sample). Soil samples were then left to air dry for transportation back to the UK for further analyses.

Soils were analysed for total carbon and nitrogen by dry combustion, using a Vario El Cube organic elemental analyser. The samples were ground using a ball mill and 30mg was wrapped into tin cups

for analysis. Carbon and nitrogen percentages recorded in the analysis were converted to carbon stocks using bulk density measurements for statistical analyses.

Total phosphorus was extracted with the acid digest method, which used a sulphuric peroxide acid mixture added to 0.2g of air-dried soil and boiled at 400 degrees (Allan *et al.* 1985). The resultant solution was further diluted 5 times and filtered through Whatmann grade 42 filter papers, after which it was diluted further and analysed using colometric determination on a SEAL Autoanalyser 3.

Samples were analysed for particle size distribution by boiling 5g of soil with 20% w/v hydrogen peroxide in order to digest soil organic matter (Gale & Hoare, 1991). Calgon was then added to the soil to disperse aggregates and the sample was passed through a particle size analyser three times. Percentages of sand, silt and clay were then used to designate a textural class to each sample.

Cores taken for the measurement of bulk density were placed into the oven for 105°C for 24 hours. After drying, the dry cores were weighed, and bulk density was calculated using the following equation:

$$BD = dW/dV$$

Where BD = Bulk Density, dW = dry weight of the core, and dV = dry volume of the core

The soils samples did not contain a significant number of stones so these were not taken into account.

3.5.1.1 Root Biomass

During sieving, roots were separated from the soil, with no distinction being made between live and dead roots. The separated roots were then washed using deionized water and placed in the oven at 105°C for 24 hours. Dry root weight was then weighed, and root biomass was calculated using the volume of the soil auger used during sampling.

3.5.1.2 Statistical analyses

Statistical analyses were carried out using a mixture of linear and non-parametric GLM models in R. Post hoc comparison of means were carried out on categorical explanatory variables in order to ascertain differences between treatment levels. Multivariate analyses were carried out in the form of mediation pathway analysis, using the SEM package in R. A mediation path analysis utilizes linear

regression models to identify direct effects of an independent variable on a dependant variable, and indirect effects of the same independent variable through an intermediate variable (i.e., Nitrogen stocks may affect soil carbon stocks both directly, and indirectly through interactions with root biomass) (Albert *et al.* 2019). This method of statistical analysis allows the investigation of the complex interactions between soil chemical and biological variables which can lead to changes in soil carbon stocks. Due to differences between sites, analyses were carried out on each site separately and the two were compared.

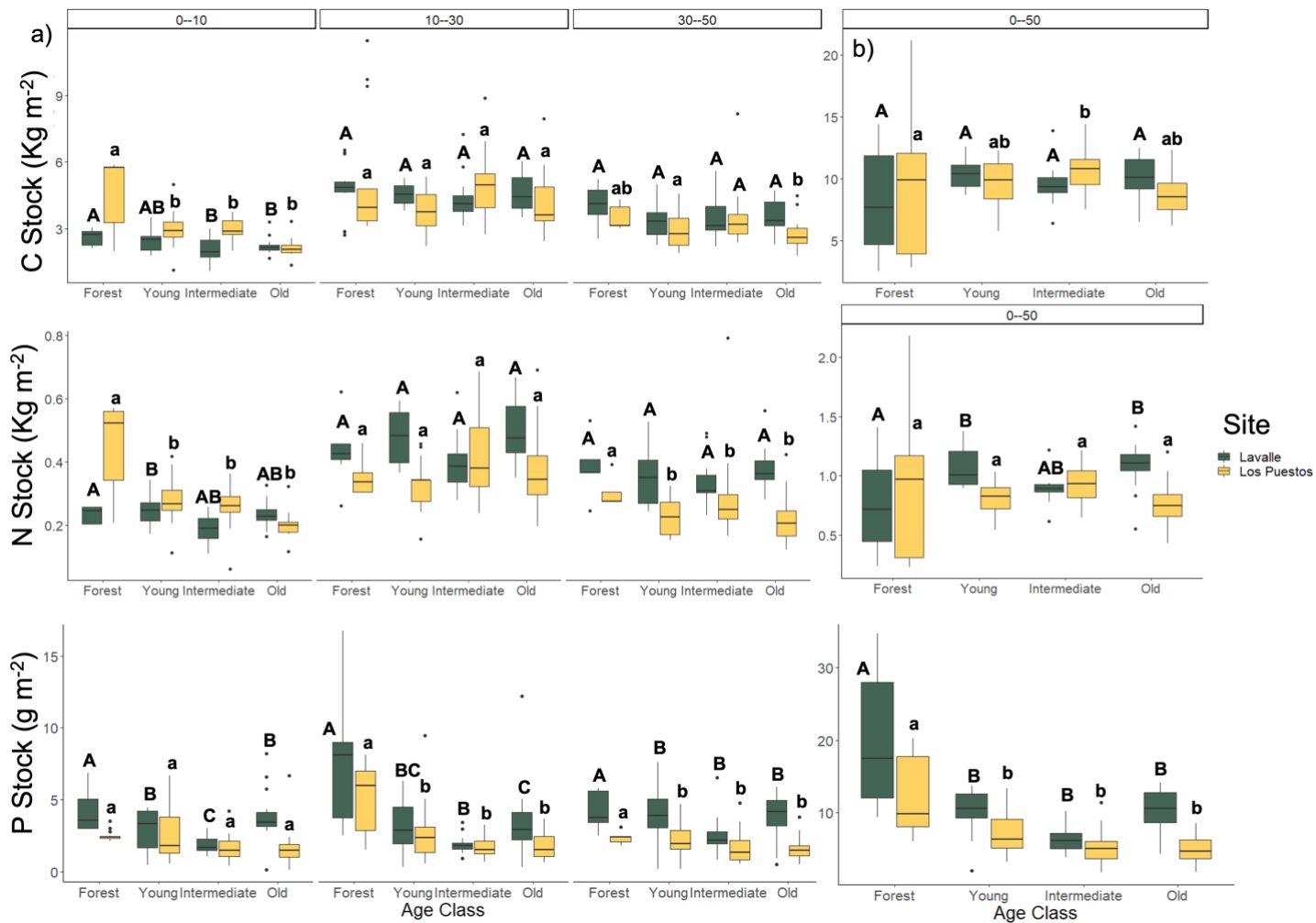


Figure 6. Soil carbon (C), nitrogen (N) (kg m^{-2}) and phosphorus (P) stocks (g m^{-2}) at both Los Puestos/INTA and Lavalle sites at 0-10, 10-30 and 30-50cm (left hand side) and at 0-50cm (right hand side). Letters denote significant ($p < 0.05$) differences in each observed chemical variable between age classes at each sample depth within individual sites. Age classes with labelled with the same letter are not significantly different (ABC = Lavalle, abc = Los Puestos).

3.6 Results

3.6.1 *Effect of deforestation and time since establishment of pastures on soil carbon, nitrogen and phosphorus stocks and nutrient interactions*

Total soil carbon stocks at 0-10 cm were 17% higher in Los Puestos than in Lavalle ($p < 0.001$), whilst at 30 – 50 cm in Lavalle soil carbon was 13% higher ($p < 0.05$). Over the soil profile (0-50 cm), soil carbon significantly increased by 12.5% between forest and intermediate sites in Los Puestos ($p < 0.05$) (Table 5), however contrast of means found no significant difference between pasture age classes. At 0-10 cm, soil carbon significantly decreased in all three age classes (Figure 6), with old pastures having 53% less carbon than forest sites ($p < 0.001$), intermediate pastures 36% less ($p < 0.001$) and young pastures 35% ($p < 0.001$). This was also observed at 30-50 cm, with the greatest decrease in soil carbon was found in old pastures (-22%, $p < 0.05$), followed by young pastures (-17%, $p < 0.05$). At 0-10 cm in Lavalle, intermediate and old age classes were found to have 21 and 16% lower carbon stocks than forest sites respectively ($p < 0.05$), but there were no significant differences between pasture age classes (Figure 6). At both sites, sample depth was found to have a significant effect on soil carbon stocks, with soil carbon being significantly greater at 10-30 cm than 0-10 cm ($p < 0.001$) in Los Puestos, and significantly greater at both 10-30 and 30-50 cm than 0-10 cm ($p < 0.001$) in Lavalle.

Table 5. Soil nutrient stocks and root biomass at 0-50 cm from both the Los Puestos and Lavalle sites. Letters denote significant ($p < 0.05$) differences in each observed variable between age classes within individual sites. Age classes with labelled with the same letter are not significantly different (ABC = Lavalle (LV), abc = Los Puestos (LP)).

Time since Deforestation (years)	Carbon Stocks (kg m ²)		Nitrogen Stocks (kg m ²)		Total Phosphorus Stocks (g m ²)		Root Biomass (g cm ³)	
	LP	LV	LP	LV	LP	LV	LP	LV
Native Vegetation	9.3±1.0 ^a	8.5±0.8 ^A	0.9±0.1 ^a	0.8±0.08 ^A	12.6±1.0 ^a	19.8±1.8 ^A	948.0±1490 ^a	648.3±139.0 ^{AB}
0-5 (young)	9.7±0.5 ^{ab}	10.3±0.4 ^A	0.8±0.04 ^a	1.1±0.05 ^B	7.0±0.7 ^b	10.0±1.2 ^B	362.6±85.8 ^b	458.5±58.7 ^A
10-15 (intermediate)	10.6±0.5 ^b	9.5±0.4 ^A	0.9±0.05 ^a	0.88±0.3 ^{AB}	5.1±0.6 ^b	6.3±0.7 ^B	169.2±22.2 ^b	455.0±56.3 ^A
>20 (old)	8.8±0.5 ^{ab}	10.1±0.5 ^A	0.8±0.2 ^a	1.1±0.05 ^B	5.0±0.5 ^b	10.3±0.7 ^B	242.3±52.4 ^b	817.8±116.3 ^A

Nitrogen stocks were higher at 10-30 than 0-10 cm ($p < 0.001$) in Los Puestos, and subsequently decreased at 30-50 cm ($p < 0.05$). Similarly, in Lavalle, nitrogen stocks increased with depth, with the greatest stocks being at 10-30 cm ($p < 0.001$), followed by 30-50 cm ($p < 0.001$). Total P significantly increased with depth in Los Puestos, with stocks being higher at 10-30 ($p < 0.05$) and 30-50 cm ($p < 0.05$) than in the top 10 cm. In Lavalle, phosphorus stocks at 30-50 cm were significantly greater than the top 10 cm ($p < 0.01$), but there was no significant difference between 0-10 and 10-30 cm.

Nitrogen stocks in the topsoil of Los Puestos significantly decreased with the clearance of forest (Figure 6), with 36 – 55% decreased soil nitrogen in young, intermediate and old pastures ($p < 0.001$). This was also observed at 30-50 cm which saw decreases of 4 – 30% in pasture age classes ($p < 0.05$). Contrastingly there was no significant effect of age class on total phosphorus stocks in the top 10 cm. At 10-30 and 30-50 cm however, total phosphorus significantly decreased in all age classes in comparison to forest sites, with the decreases of 46 – 67% at 10-30 cm ($p < 0.001$) and 6 – 29% at 30-50 cm ($p < 0.001$). However, there was no significant difference between pasture age at any depth. Similarly, over the soil profile (0-50 cm), all age classes had significantly lower phosphorus stocks than the forest plot (Table 5), with 44 – 60% less total phosphorus stock in young, intermediate and old pastures ($p < 0.001$). However, comparison of means found no significant differences between the pastures.

In Lavalle, deforestation significantly increased soil nitrogen stocks over 0-50 cm, with both the intermediate and old pastures having 28% and 27% higher nitrogen than the forest, respectively ($p < 0.05$). Contrastingly, at 0-10 cm, the intermediate age class was observed to be 12% lower in soil nitrogen than the forest site ($p < 0.05$). Total phosphorus stocks at 0-10 cm in Lavalle was 29% lower in young ($p < 0.05$) and 55% lower in intermediate pastures than in forest soils ($p < 0.001$). At 10-30 cm, all age classes were significantly lower in P than the forest sites, with 5–4 – 75% decreases in young ($p < 0.001$), intermediate ($p < 0.001$) and old pastures ($p < 0.001$). This pattern was also observed at 30-50 cm, with 8 – 39% decreases in total phosphorus. Post hoc tests found that at 0-10 cm, both young and old pastures had significantly higher (36% and 52% respectively) soil phosphorus than intermediate pastures ($p < 0.01$). Old pastures also had 46% higher soil phosphorus than intermediate plots at 10-30 cm ($p < 0.05$). Over 0-50 cm, deforestation was found to significantly decrease soil phosphorus by 48 – 68% ($p < 0.001$), however there was no difference between the pasture plots.

At both Los Puestos and Lavalle, carbon stocks were positively correlated to nitrogen stocks ($p < 0.001$). This interaction was observed over 0-50 cm and at each individual sampling depth. No significant relationship between soil carbon and total phosphorus was found at Los Puestos. In Lavalle, total P was positively correlated to carbon stocks at 0-10 cm ($p < 0.05$).

3.6.2 Effect deforestation and grazing over time on root biomass

Root biomass of the forest understory was 11% higher in Los Puestos than Lavalle in the top 10 cm ($p < 0.05$) (Figure 7), and 54% higher at 10-30 cm ($p < 0.001$) and 70% at 30-50 cm ($p < 0.001$). Over the soil profile 0-50 cm, Los Puestos also had on average higher root biomass than Lavalle ($p < 0.001$) (Table 5). In pasture soils however, Lavalle had a higher root content than Los Puestos in all three age classes at 0-10 cm and 10-30 cm ($p < 0.05$).

Over the soil profile (0-50 cm) in Los Puestos, root biomass was significantly lower in the pastures than the forest understory ($p < 0.001$) with decreases of 68 – 82% in young, intermediate and old pastures (Figure 7). However, there was no significant difference between pasture sites. Similarly, at 0-10 cm, root biomass significantly decreased in all three age classes by 70 – 81% ($p < 0.001$), with no differences between pasture age classes. This pattern was also observed at 10-30 cm, and at 30-50 cm (Figure 7). In Lavalle, at 0-50 cm young and intermediate pastures had 44% and 45% lower

root biomass than old pastures respectively ($p < 0.05$). At 0-10 cm, the intermediate age class was found to have 62% lower root biomass than the forest understory ($p < 0.05$), whilst at 10-30 cm, old pastures were found to have 48% higher root biomass than forest understory ($p < 0.05$). In both sites, depth was found to significantly decrease root biomass ($p < 0.001$).

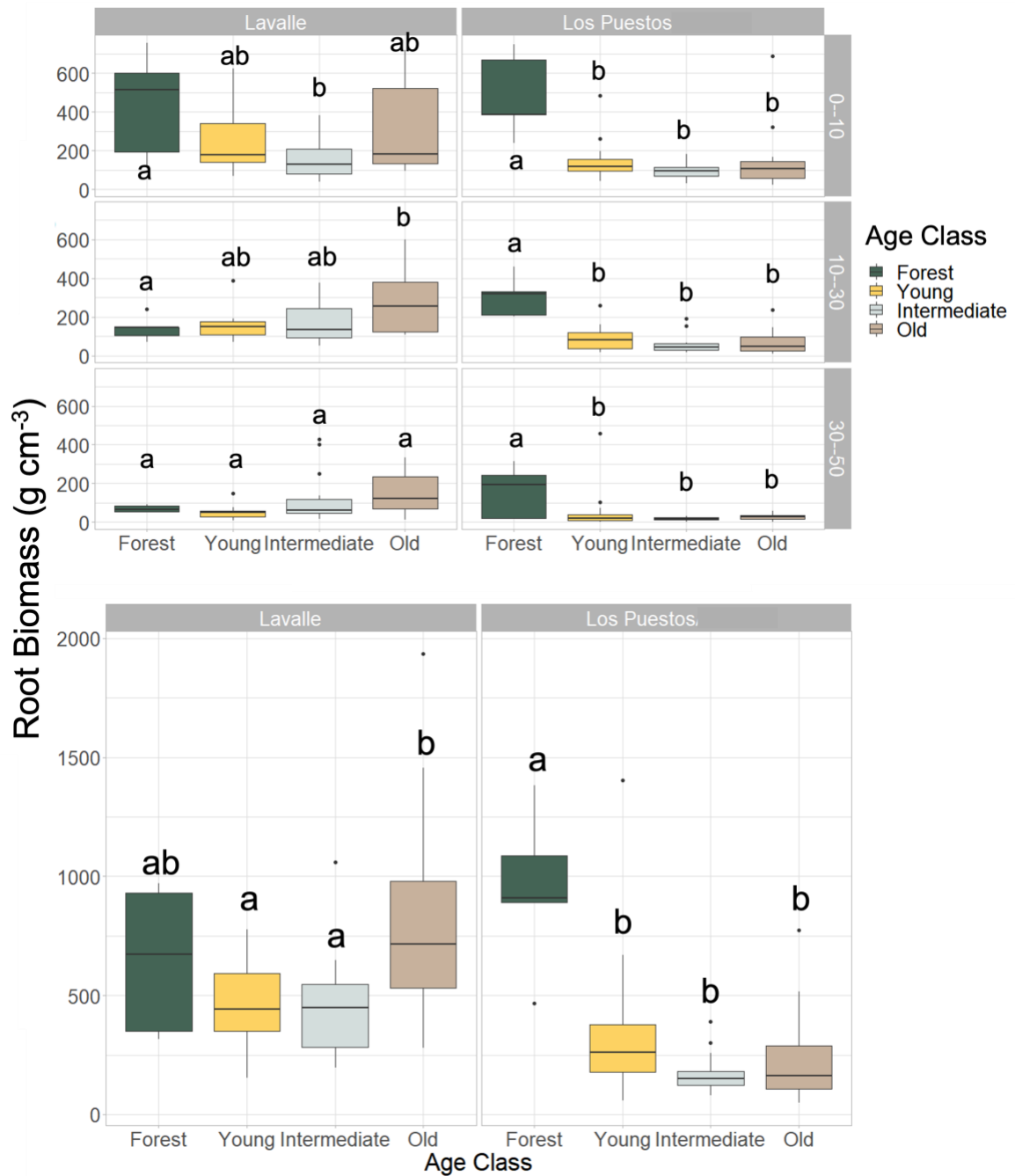


Figure 7. Root biomass of forest understory and pastures (g cm^{-3}) across land use and age categories at Los Puestos and Lavalle, at 0-10, 10-30 and 30-50 cm sample depths, and across all sample depths (0-50 cm). Letters denote significant ($p < 0.05$) differences in each observed chemical variable between age classes at each sample depth within individual sites. Age classes with labelled with the same letter are not significantly different.

3.6.3 *Root – Nutrient interactions*

In both Los Puestos and Lavalle, carbon and nitrogen stocks were weakly positively correlated with root biomass ($p < 0.05$). Increases in root biomass increased soil carbon and nitrogen stocks in the top 10 cm of the soil (Figure 8). However, R-squared values for the statistical models were low, likely due to high variation in the data which is not accounted for in the model. At 10-30 cm in Lavalle, root biomass was also positively correlated to total phosphorus stocks ($p < 0.05$). Multivariate analysis with carbon, nitrogen and phosphorus and age class were not significant.

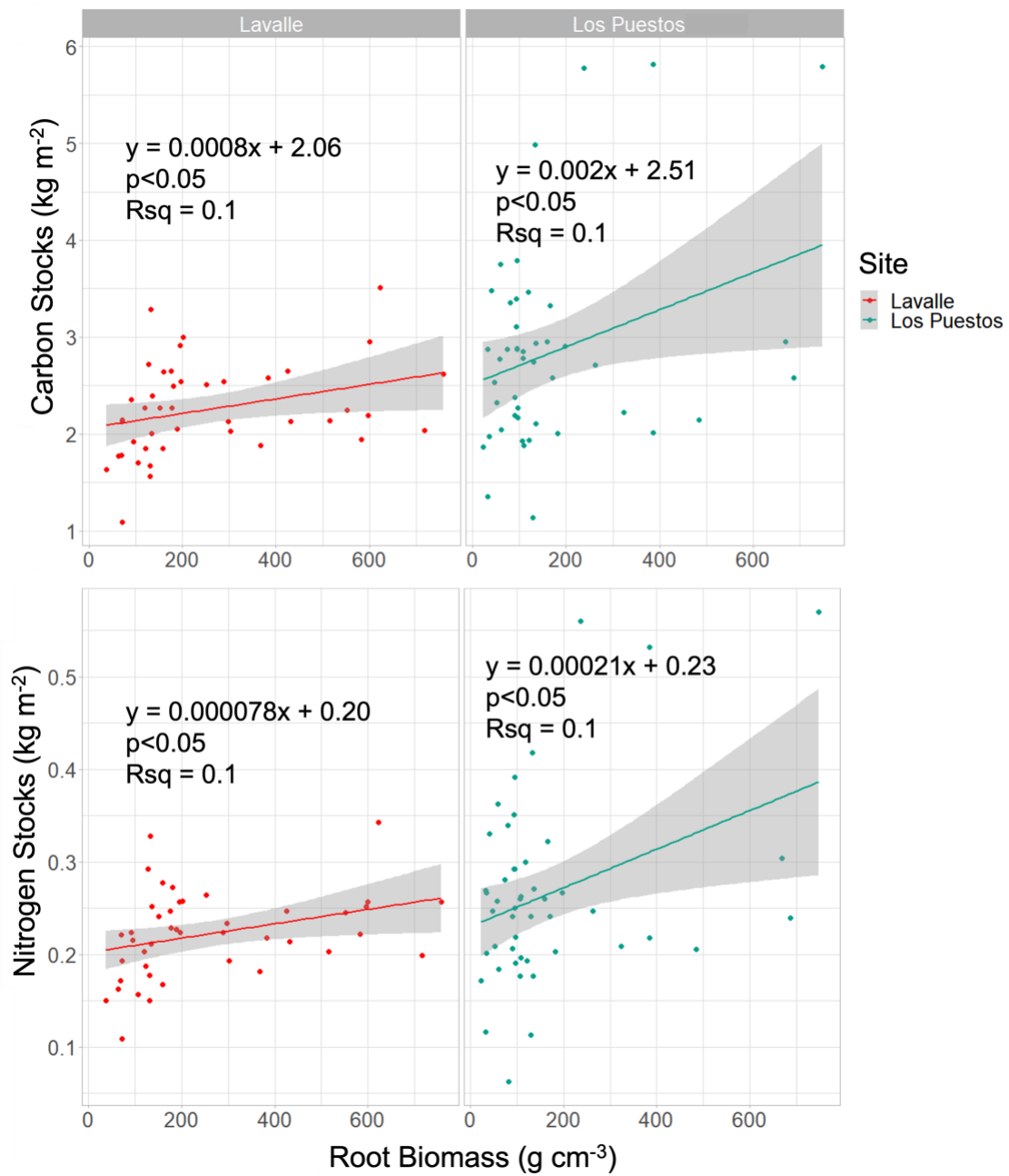


Figure 8. Relationship between root biomass (g cm⁻³) and carbon and nitrogen stocks (kg m⁻²) at 0-10 cm at both Los Puestos (blue) and Lavalle (red) sites.

3.6.4 pH and Electrical Conductivity

Soil pH and electrical conductivity (EC) were 7% and 93% higher in the Los Puestos site respectively ($p < 0.001$), with an average pH of 8.0 and EC of 1.5 dS/m at 0-50 cm, compared to 7.5 and 0.1 dS/m in Lavalle (Table 6). In both sites, EC was observed to be spatially variable within each plot, with a range of values across each pasture and forest plot (Figures 10 – 17, Supplementary Material)

No significant effect of deforestation of pH was found either over the soil profile or within specific sample depths in Los Puestos. Electrical conductivity significantly decreased by 28 – 51% with deforestation at 0-50 cm ($p < 0.05$). At 10-30 and 30-50 cm, this interaction was also observed, with EC decreases of 35 – 68% and 54 – 69% respectively in pasture sites in comparison to the forest ($p < 0.05$). In Lavalle, pH significantly increased by 8 – 13% in the pastures in comparison to the forest plot at 0-50 cm ($p < 0.05$). This pattern was also observed at 0-10 cm ($p < 0.05$), 10-30 cm ($p < 0.05$) and 30-50 cm ($p < 0.001$). Comparison of means found that at 30-50 cm pH was 7% higher in both old and young pastures respectively than in intermediate pastures ($p < 0.05$). Electrical conductivity was 47% lower in young pastures than the forest at 10-30 cm ($p < 0.05$) and 34% higher in old pastures than in young pastures ($p < 0.05$). At 30-50 cm old pastures had 45% higher EC than intermediate pastures at 30-50 cm ($p < 0.05$). There was no significant effect of land use change on EC at 0-50 cm. pH was significantly affected by depth, with values increasing with increasing depth in both sites ($p < 0.001$).

In Los Puestos, no significant interactions were found between carbon and pH either over the soil profile or within each of the sampling depths. Furthermore, there was no significant effect of electrical conductivity on soil carbon stocks. However, a significant positive correlation was observed between electrical conductivity and total phosphorus stocks at 0-50 cm ($p < 0.05$). In Lavalle, pH was not found to significantly affect soil carbon. Like in Los Puestos, soil total phosphorus was significantly positively correlated to EC in the top 50 cm of the soil profile ($p < 0.05$)

Table 6. Soil pH and Electrical conductivity (dS/m) within each sampling depth at Los Puestos and Lavalle. Letters denote significant ($p < 0.05$) differences in each observed variable between age classes within individual sites. Age classes labelled with the same letter are not significantly different (ABC = Lavalle (LV), abc = Los Puestos (LP)).

Age	Depth	Lavalle		Los Puestos	
		pH	EC	pH	EC
Forest	0-10	6.5±0.1 ^a	0.18±0.03 ^a	7.0±0.2 ^a	1.83±0.48 ^a
Young		7.2±0.1 ^b	0.13±0.01 ^{ab}	7.5±0.2 ^a	1.02±0.2 ^a
Intermediate		7.1±0.1 ^b	0.12±0.01 ^b	6.9±0.2 ^a	0.71±0.17 ^a
Old		7.0±0.1 ^b	0.16±0.01 ^{ab}	7.2±0.2 ^a	0.81±0.17 ^a
Forest	10-30	7.0±0.1 ^a	0.14±0.01 ^a	7.7±0.2 ^a	3.21±0.32 ^a
Young		7.6±0.1 ^{bc}	0.09±0.01 ^b	8.3±0.2 ^a	1.53±0.32 ^b
Intermediate		7.4±0.1 ^{ab}	0.11±0.01 ^{abc}	7.4±0.2 ^a	0.87±0.25 ^b
Old		7.6±0.1 ^{bc}	0.14±0.01 ^{ac}	7.9±0.2 ^a	0.82±0.21 ^b
Forest	30-50	7.0±0.1 ^a	0.11±0.01 ^{ab}	8.1±0.2 ^a	4.01±0.49 ^a
Young		8.2±0.2 ^{bc}	0.14±0.01 ^{ab}	8.9±0.2 ^a	1.35±0.35 ^b
Intermediate		7.7±0.1 ^b	0.08±0.001 ^a	8.1±0.3 ^a	0.97±0.28 ^b
Old		8.3±0.2 ^c	0.15±0.02 ^b	8.6±0.2 ^a	1.05±0.29 ^b

3.6.5 Physical Variables

Soil textural class was found to be the same in all pastures and forest plots and was identified as Silt Loam. Due to this it was excluded from statistical analyses.

3.6.6 Path Analysis

Mediation path analyses were carried out on each of the sites and constructed with the statistical results from univariate glm and lm models (Table 7). The model outputs found that in Los Puestos,

soil carbon is directly affected by nitrogen stocks ($p < 0.05$) and pH ($p < 0.05$) (Figure 9). Indirectly, carbon stocks are driven by the effects of root biomass and pH on soil nitrogen stocks ($p < 0.05$) and the effects of age class on root biomass ($p < 0.001$). Contrastingly in Laval, root biomass and nitrogen stocks affect soil carbon stocks directly ($p < 0.05$). Furthermore, carbon is indirectly affected in the soil by the effects of root biomass, depth, pH and age class on soil nitrogen ($p < 0.05$), and the effects of age class on root biomass ($p < 0.05$) (Figure 9).

Table 7. Mediation path analysis models used for the Los Puestos and Laval sites

	Los Puestos	Lavalle
Model	“Carbon Stocks ~ Nitrogen Stocks + Root Biomass + Bulk Density + pH + Age Class Nitrogen Stocks ~ Root Biomass + pH + Age Class pH ~ Age Class”	“Carbon Stocks ~ Nitrogen stocks + total Phosphorus + Root Biomass + Sample Depth + Electrical Conductivity + Age Class Nitrogen Stocks ~ Age Class + pH + Root Biomass + Depth Root Biomass ~ Age Class + Depth + Bulk Density”
Number of Observations	153	147
Test Statistic	28.3	16.486
Degrees of Freedom	2	7
P Value	<0.001	<0.05

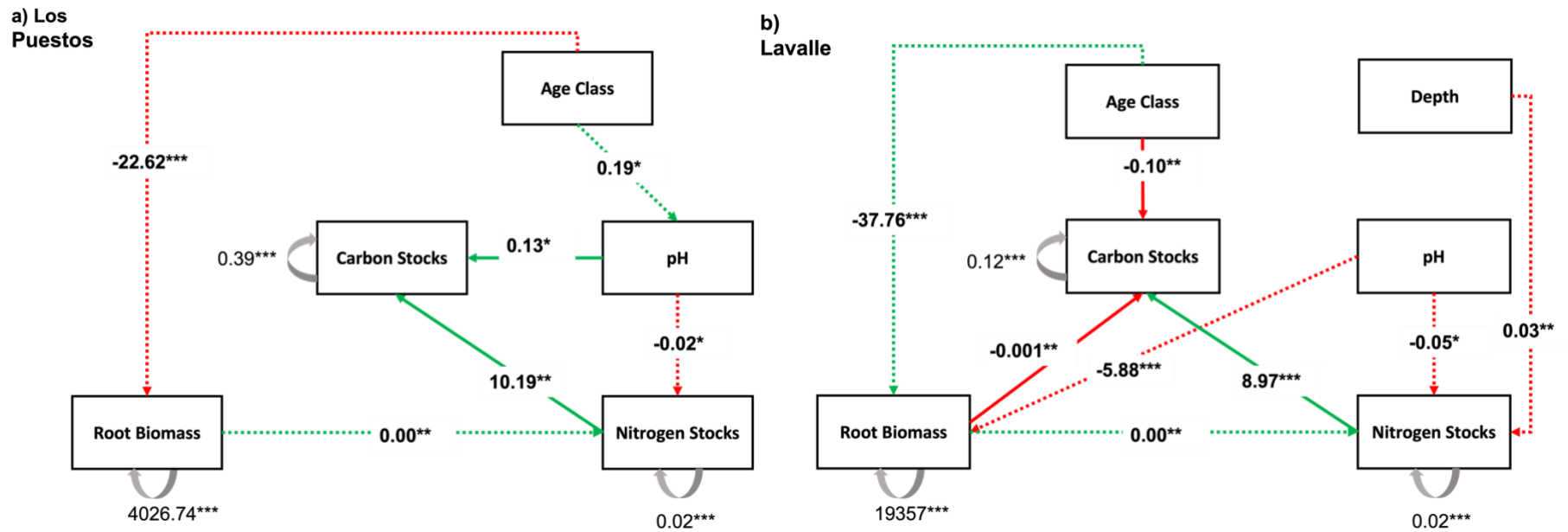


Figure 9. Mediation path analysis diagram of soil and experimental design variables and their direct and indirect effects on soil carbon at both Los Puestos and Lavalle. Solid lines denote direct effects of soil carbon and dashed lines denote indirect effects through effects on other variables. Labels indicate the regression coefficient estimate. * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0005$. Only significant interactions have been depicted.

3.7 Discussion

The effect of deforestation and time since establishment of pastures was found to significantly decrease soil carbon stocks in both the Los Puestos and Lavalle study sites. This loss of carbon was particularly pronounced in the top 10 cm of soil, which is observed to contain less soil carbon stocks than samples from the 10-30 and 30-50 cm depths. These results contrast with other chronosequence studies which have found some recovery in soil carbon stocks, albeit not to pre-land use change condition (Conrad *et al.* 2017; Navarrete *et al.* 2016; Braz *et al.* 2013; Conant & Paustian, 2004). The decrease in soil carbon over time in topsoil within the present study is likely due to the level of degradation of the sites, unlike in previous studies which tend to investigate carbon dynamics in well managed pastures. High levels of compaction, shrubby encroachment and bare patches of soil due to grazing practices, particularly in Los Puestos are likely to have contributed to the carbon losses (Controneo *et al.* 2018; Lohmann *et al.* 2014; Quiroga *et al.* 1999).

Over all depths in Los Puestos, we see a similar effect of land use change on soil nitrogen stocks as to soil carbon, with soil nitrogen decreasing in pastures in comparison to forest soils, but without any difference between pasture sites. However, Lavalle in the 0-10 cm and 10-30 cm depth classes there appears to be a decoupling of soil carbon and nitrogen stoichiometry, with soil nitrogen increasing in the old pasture soils. Previous studies have also observed the decoupling of nutrients, with climate factors appearing to affect soil nutrient interactions. Jiao *et al.* (2016) found that in along a climate transect, nutrient ratios change with decreasing precipitation, increased warming and increased pH. Similarly, in Australia, C:N:P stoichiometry was found to decouple with increasing aridity index, for reasons thought to be due to changes to microbial activity with decreasing precipitation (Delfado-Baquerizo *et al.* 2018). With Lavalle and Los Puestos possessing different climate types (semi-arid and subtropical subhumid respectively), it is possible that differences in precipitation patterns in this study are driving nutrient dynamics in these soils. The positive correlation between nitrogen and carbon stocks in the top 10 cm of soil in both sites is to be expected, with organic matter inputs into soil stimulating nutrient cycling, and in turn increased nitrogen availability stimulating plant growth, which further increases soil carbon inputs into the soil (Barret & Burke, 200). This correlation was also reflected in the path analysis of both sites, with nitrogen stocks having a direct positive effect on soil carbon stocks.

Total phosphorus stocks in Los Puestos were significantly lower in pasture soils compared to forest, but with no difference between age classes. Deforestation can result in increased soil

loss due to erosion, which can lead to nutrient loss, including phosphorus, from the soil. Furthermore, where burning is used after clearance of woody vegetation, phosphorus in vegetation can be lost as ash which can be dissolved and leached out of the soil (Veldkamp *et al.* 2020; Filipelli, 2008). In Lavalle, phosphorus stocks were observed to follow a similar pattern to nitrogen, with a decrease in young and intermediate plots and increase in the old pastures. Studies have found that in calcareous soils, phosphorus is less available for uptake by plants due to the formation of poorly soluble calcium phosphates, which can lead to the accumulation of total phosphorus (Hopkins & Ellsworth, 2005; Johri *et al.* 1999). In the present study, EC, which is an indicator of cation or anion concentration (Friedman, 2005) both increased in old pastures (Table 6) and was found to be correlated with total phosphorus stocks over 0-50 cm. This indicates that increased total phosphorus in old pastures is due to an accumulation of phosphorus salts and decreased plant available phosphorus. In Lavalle total phosphorus stocks were positively correlated with soil carbon in the top 10 cm of soil. Levels of phosphorus in the soil can be influenced by soil organic matter content, with phosphorus being able to bind to SOM, and previous studies have found that 77 – 98% of total phosphorus can be found bound to organic matter (Zhang *et al.* 2014; Yang *et al.* 2012).

Root biomass significant decreased following deforestation at Los Puestos, something which is to be expected, with defoliation and compaction due to grazing practices all previously been shown to decrease belowground biomass (Pineiro *et al.* 2009). Furthermore, the root content of soils was found to be higher in the pastures of Lavalle than Los Puestos, which could be due to climatic differences. Drier climates tend to result in increased allocation of carbon to root biomass to enable soil water uptake where soil moisture is low (Liu *et al.* 2021; Zhou *et al.* 2012; Brown *et al.* 1990). Root biomass was positively correlated with both carbon and nitrogen in the top 10 cm of soil, which generally occurs due to increased root growth increasing carbon inputs to the soil, affecting the amount of carbon available for soil carbon formation (Vaieretti *et al.* 2021; Liang *et al.* 2020; Pineiro *et al.* 2010; Derner *et al.* 2006) Increased root biomass will also increase soil nitrogen due to increased nitrogen uptake by plants and reduced nitrogen mineralization (Pineiro *et al.* 2010; Derner *et al.* 2006). The effect of root biomass on soil nitrogen is reflected in the path analysis as an indirect effect on soil carbon, with increasing nitrogen stocks as a result of increased root biomass further driving soil carbon dynamics.

pH and electrical conductivity were high at both sites and significantly increased with depth. Especially in the Los Puestos sites, soil pH was found to increase to a maximum of 10.6 at 30-50 cm. Such a high pH could characterize these soils as being sodic, and in conjunction with high EC values (>2) they could be further defined as saline-sodic. Lavalle, whilst not possessing as

high pH or EC as Los Puestos, still possesses values in the subsoil (e.g., pH 8.8 and EC 0.9 at 30-50 cm) which would indicate high concentrations of neutral cations such as Ca^{2+} and Mg^{2+} . Such levels of salts would not be quite as detrimental to soil functioning and structure as those in Los Puestos but could still impact plant growth and subsequent organic matter formation. The potential impacts of such pH and electrical conductivity in the soils of the dry Chaco have not yet been researched, and further investigation in this area would be valuable. The path analysis showed that in Lavalle, soil pH was also indirectly affected carbon stocks through decreasing root biomass. High pH values, and the high concentration of salts which cause it, can inhibit root growth and development due to plant salt stress which results in either direct growth reduction, or a slower buildup of toxic ions leading to death of the plant (Isayenkov *et al.* 2019; Roy *et al.* 2014; Rajendran *et al.* 2009). Furthermore, osmotic pressures caused by increases in salt content in the surrounding can also lead to water stress, further impacting plant growth (Isayenkov *et al.* 2019).

Through the laboratory analyses, it became apparent that the soils of this region were highly spatially variable (Supporting Information, Fig 10 – 13). Particularly in Los Puestos, within plot variation was observed to be high in all measured variables, across plots of all age classes, whereas in Lavalle some variation was observed across forest and young plots, but less so in the intermediate and old pastures. Spatial variability could be due to a number reasons, with areas of Los Puestos being subject to flooding in the wet season, both sites having heavily degraded plots with high occurrence of shrubbery, and the potential salinity and sodicity of the soils as previously mentioned.

It has been suggested that degree of soil heterogeneity at field scale can be used as an index for the extent of desertification, and that livestock can introduce soil heterogeneity due to shifts in vegetation abundance (Su *et al.* 2006). The pastures selected for sampling in this study displayed levels of degradation, with bare patches of soil in old pastures, and moderate to heavy encroachment of shrubs in young and intermediate plots. The increased presence of shrubs amongst pasture grasses can result in the uplift of soil, and increased root biomass directly beneath woody patches, which may lead to higher concentrations of C, N and P in these areas (Zhou *et al.* 2017). Soil carbon may also increase beneath or close to woody patches, due to an increased input of woody plant matter which is more resistant to breakdown by microbes than inputs from pasture grasses. In addition, the encroachment of deeper-rooted woody vegetation in degraded pastures can alter the water balance in an area and has been shown to reduce groundwater recharge in dry sub-humid regions. An alteration to hydrological cycles such as this, could potentially impact salt concentrations in the soil profile beneath woody patches of

vegetation, which in turn could impact the spatial pattern of other soil characteristics, such as root biomass and depth, microbial community composition and nutrient concentrations. If this is occurring directly beneath patches of woody vegetation, this could induce spatial variability within large plots where there is substantial space between shrubby patches.

There has been very little research into the spatial variability of soils in the Chaco, particularly with regards to soil chemical characteristics. A recent study, which investigated spatial patterns of salinity in the dry Chaco, observed that clear regional patterns of primary soil salinization which were related to natural occurrence of saline groundwater, annual water budget and topography (Maertens *et al.* 2022). It found that salinization was particularly increased in areas of agricultural production with shallow water tables, similar to the sites of this study, and the periodic flooding brings salts to the soil surface resulting in soil surface salt crusts (Maertens *et al.* 2022). These results are similar to those of Australian saline and sodic soils which have been demonstrated to display spatial 'highs' and 'lows' of salinity indicators such as pH, EC and soluble ions (Semple *et al.* 2004). Furthermore, in spatial areas with high salinity, also known as saline scolds, SOC stocks are lower with increased occurrence of bare soil and decreased vegetation growth (Wong *et al.* 2008). This indicates that areas where salinity or sodicity may be high in the Chaco influencing the spatial variability of these soils. Further investigation in the Chaco would be greatly beneficial, with an in-depth spatial study of individual plots, which would enable the production of spatial mapped soil characteristics of both farms. This may aid in further explaining the variation in other soil chemical and biological variables.

3.6 Conclusion

In conclusion, the present study shows that land use change from forest to grazing systems in the Chaco in Argentina significantly impacts soil carbon stocks, with losses of carbon following deforestation. Furthermore, the removal of forest has been shown to significantly impact other soil variables, with decreases in nutrients and root biomass and increases in soil chemical properties such as pH and electrical conductivity. The results of this study show that changes in soil chemical and biological properties due to land use change can impact soil carbon stocks both directly, and indirectly through effects on intermediate variables (e.g. root biomass both directly through C input and indirectly impacts carbon stocks through interactions with soil nitrogen). This shows that changes in soil carbon are a result of complex interactions in the soil. What is more, through path analyses it is indicated that soil pH and to a lesser extent electrical conductivity, impact soil carbon indirectly via several mechanisms, for example detrimental

impacts on soil nitrogen and root biomass. The importance of high pH and EC is highlighted in this study and further investigation into these impacts would be greatly beneficial because it would allow the identifications of the mechanisms by which causes of high pH and EC in the soil affect soil functioning and ultimately carbon dynamics. Finally, throughout the study, there were marked differences between sample sites is strikingly obvious, with each location displaying different interactions between soil variables. This could be due to a number of key differences; such as differences in precipitation with Los Puestos being found under a sub-humid climate, and Lavalle under semi-arid; and geomorphological and hydrological differences, with Los Puestos being situated in a low lying plain, bringing it close to watertable and susceptible to waterlogging. This study is the first chronosequence investigation of grazing systems in the Chaco, Argentina, and offers an interesting insight into the cycling of carbon over time following deforestation. Future work would benefit from more in-depth studies into soil chemistry (pH and Electrical conductivity, and salinity), microbial activity and plant soil interactions to shed further light on the impacts of land-use change and grazing on soil carbon dynamics.

3.7 Supporting Information

This study uses a chronosequence design to investigate soil nutrient, root and chemical dynamics over time since deforestation and the establishment of pastures. The sampling design involved taking five destructive soil cores across each plot (either pasture or forest plot), at three sampling depths: 0-10 cm, 10-30 cm and 30-50 cm. Sampling points were equidistance from one another across each plot, and avoided edges of plots, tracks and obvious signs of animal disturbance (i.e. livestock manure). Figures 10 – 13 show the spatial variation in each measured characteristic across each age class and site (Los Puestos and Lavalle). The data plotted does not exclude outliers in order to show one value per spatial replicate.

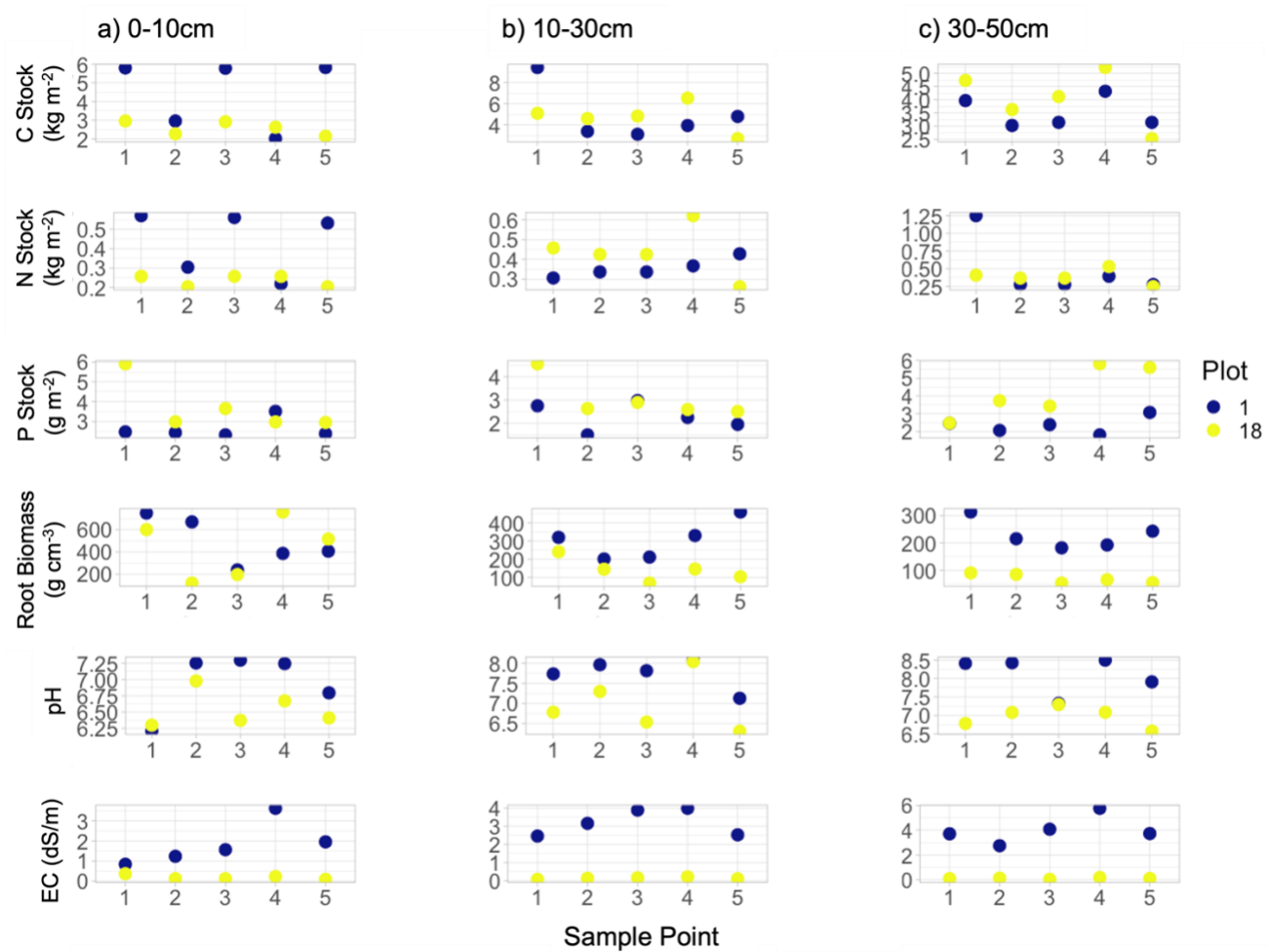


Figure 10. Soil chemical and biological characteristics at each spatial replicate and depths within the forest plots of Los Puestos (1) and Lavalle (18), A) is at 0-10cm, B) is at 10-30cm, and C) is at 30-50cm. Each sample point (1 – 5) is a spatial replicate, of which there is one measurement. Measured variables are Carbon Stocks (kg m⁻²), Nitrogen Stocks (kg m⁻²), Total Phosphorus Stock (g m⁻²), Root Biomass (g cm⁻³), pH, and Electrical Conductivity (EC) (dS/m).

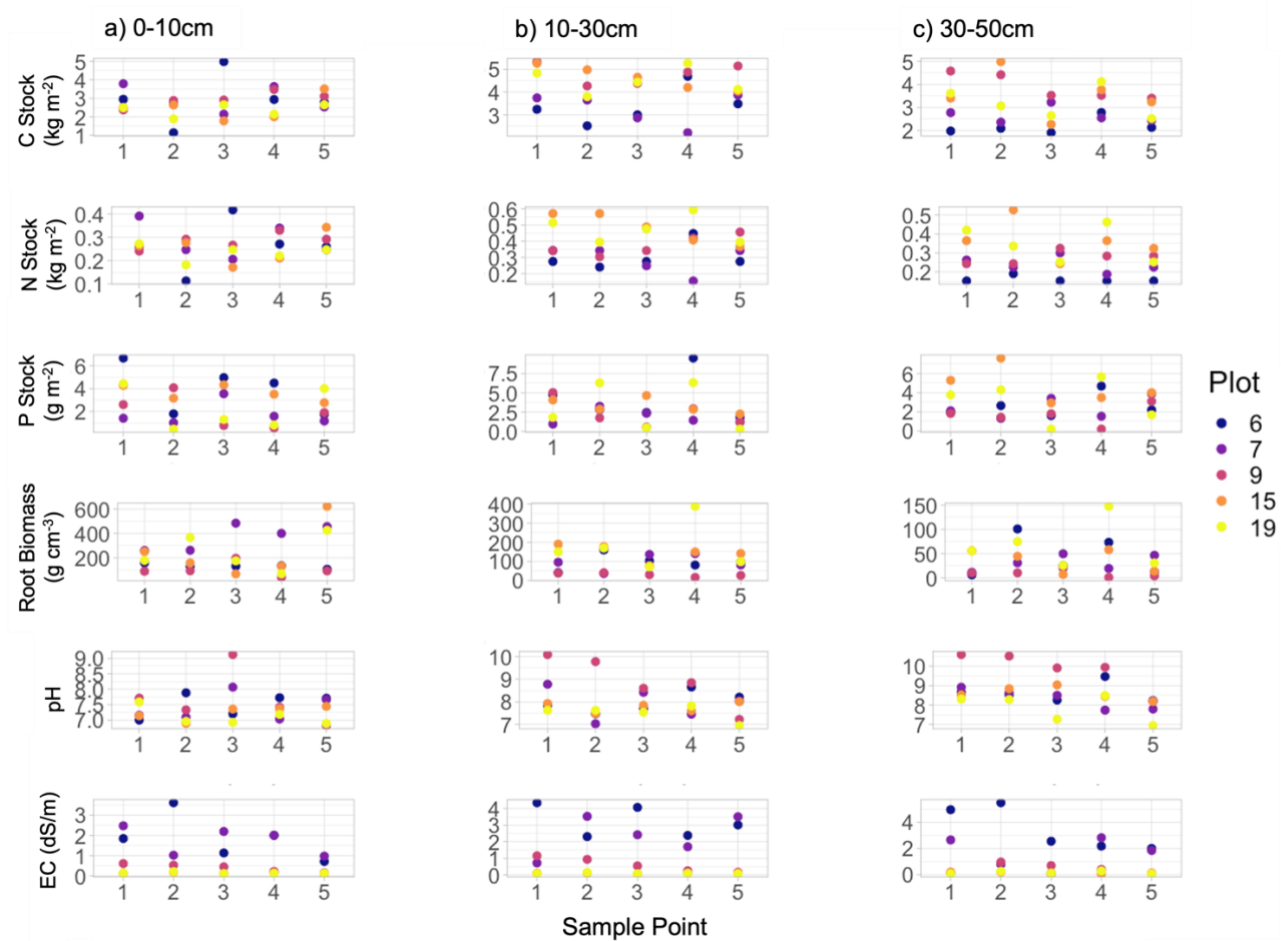


Figure 11. Soil chemical and biological characteristics at each spatial replicate and depths within the Young plots of Los Puestos (6, 7, 9) and Lavalle (15, 19), A) is at 0-10cm, B) is at 10-30cm, and C) is at 30-50cm. Each sample point (1 – 5) is a spatial replicate, of which there is one measurement. Measured variables are Carbon Stocks (kg m⁻²), Nitrogen Stocks (kg m⁻²), Total Phosphorus Stock (g m⁻²), Root Biomass (g cm⁻³), pH, and Electrical Conductivity (EC) (dS/m).

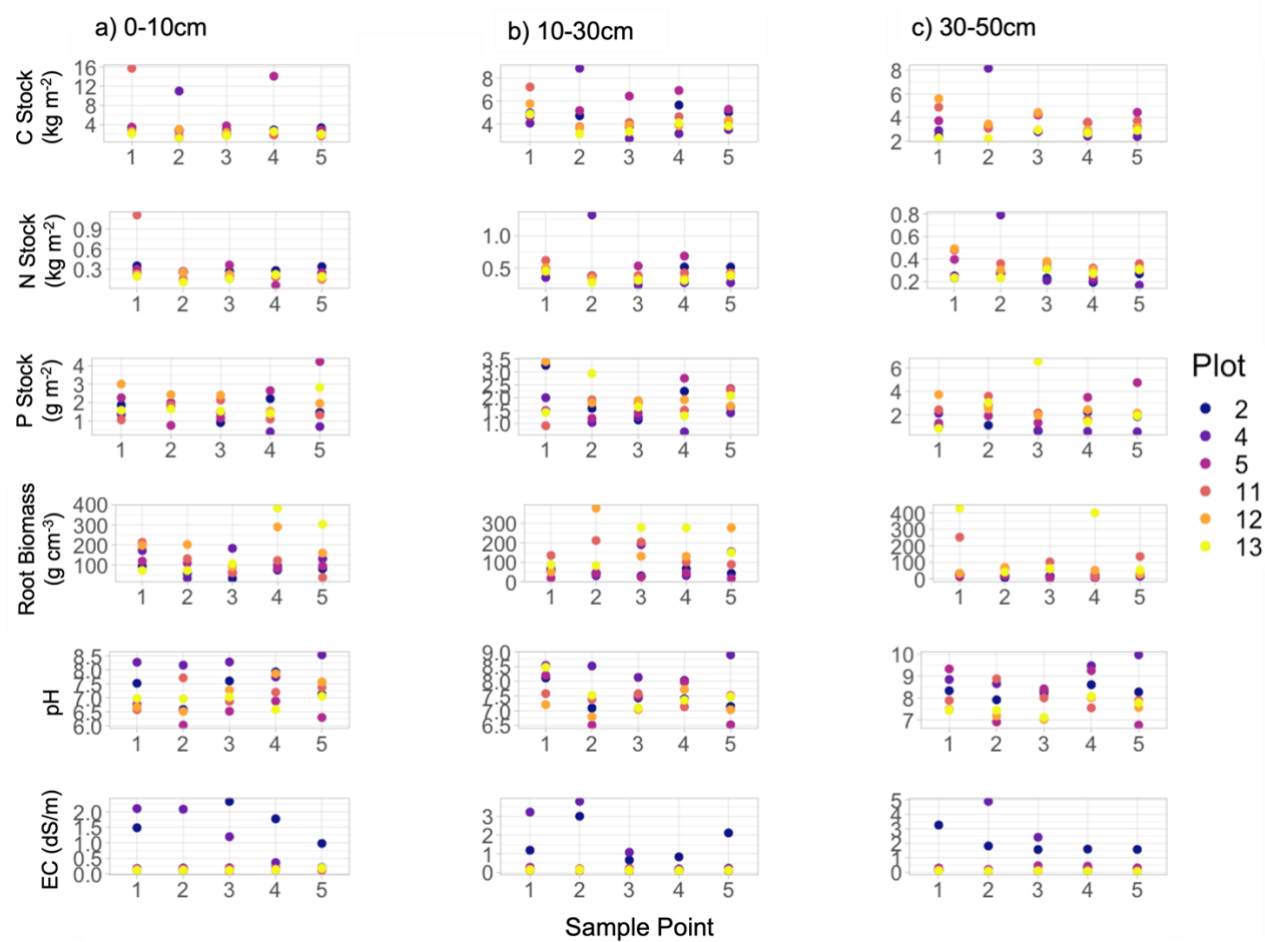


Figure 12. Soil chemical and biological characteristics at each spatial replicate and depths within the Intermediate plots of Los Puestos (2, 4, 5) and Lavelle (11, 12, 13), A) is at 0-10 cm, B) is at 10-30 cm, and C) is at 30-50 cm. Each sample point (1 – 5) is a spatial replicate, of which there is one measurement. Measured variables are Carbon Stocks (kg m⁻²), Nitrogen Stocks (kg m⁻²), Total Phosphorus Stock (g m⁻²), Root Biomass (g cm⁻³), pH, and Electrical Conductivity (EC) (dS/m).

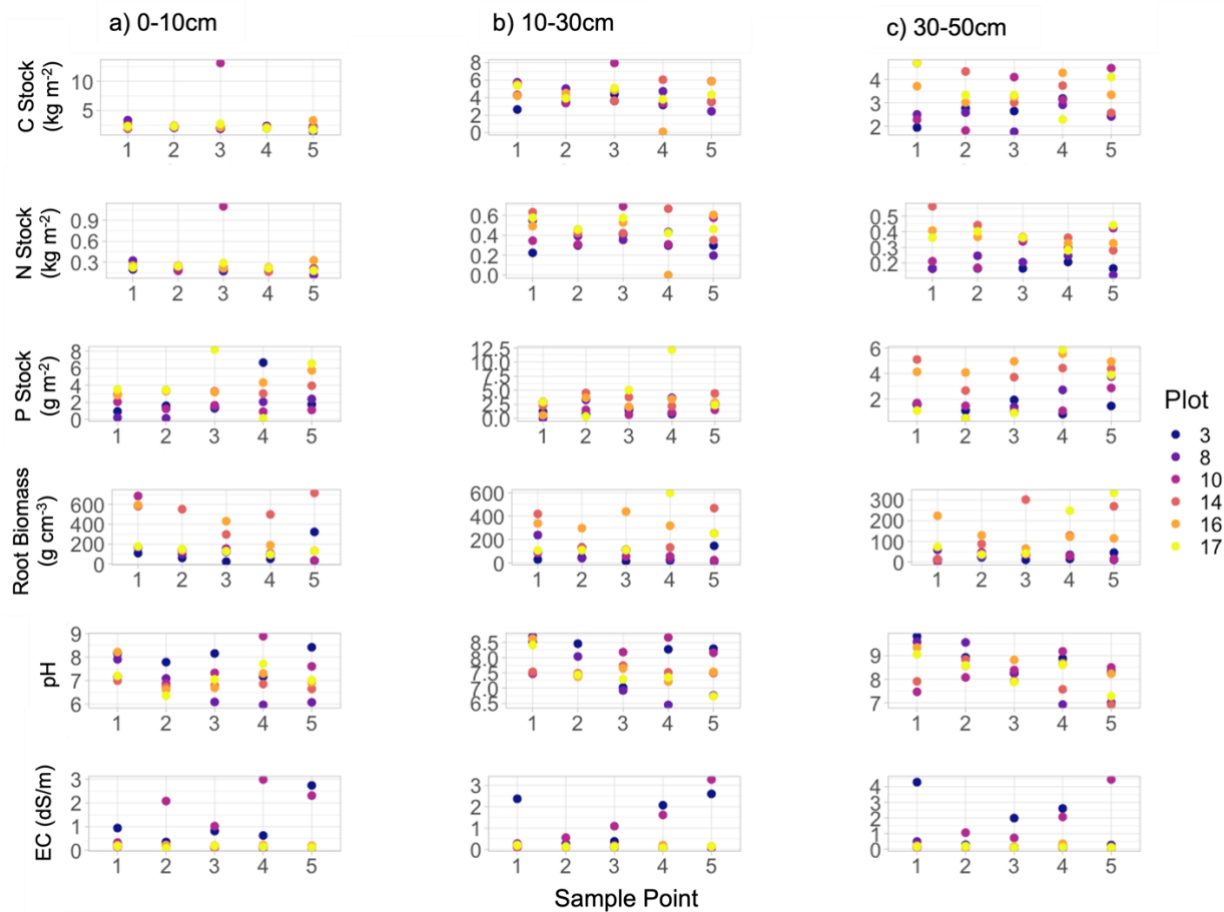


Figure 13. Soil chemical and biological characteristics at each spatial replicate and depths within the Old plots of Los Puestos (3, 8, 10) and Lavelle (14, 16, 17), A) is at 0-10cm, B) is at 10-30cm, and C) which is at 30-50cm. Each sample point (1 – 5) is a spatial replicate, of which there is one measurement. Measured variables are Carbon Stocks (kg m⁻²), Nitrogen Stocks (kg m⁻²), Total Phosphorus Stock (g m⁻²), Root Biomass (g cm⁻³), pH, and Electrical Conductivity (EC) (dS/m).

4 The impacts of climate and land use change on soil greenhouse gas fluxes and microbial community composition in the dry Chaco, Argentina

4.1 Abstract

In the dry Chaco Argentina, there has been widespread deforestation to make way for grazing systems over the last few decades, which has led to the release of large amounts of carbon from the soils of this region. Previous modelling studies have assessed the scale of this land use change (LUC), and the impacts it has had on soil carbon stocks, whilst other studies have investigated the impacts on soil microbial communities. However, there have been no studies which examine the interactions between LUC, soil microbial communities and greenhouse gas emissions. This study aimed to investigate the impacts of deforestation followed by grazing over time on soil greenhouse gas (GHG) emissions (CO_2 , N_2O , and CH_4) microbial community composition, soil chemical characteristics, and interactions between them. Furthermore, it aimed to quantify the effects of climate factors to simulate changing environmental conditions on soil fluxes. The working hypotheses were that; 1) Microbial community composition (Phospholipid Fatty Acids (PLFAs)) will be negatively impacted by deforestation and time since establishment of pastures, due to decreased organic matter availability; 2) Soil GHG fluxes from native vegetation and grazed pastures will be influenced land use change, and by changing environmental conditions, with greater emissions pastures and in warmer and wetter soils; 3) Soil GHG fluxes and microbial community composition will be impacted by soil chemical characteristics, with salinity and sodicity decreasing fluxes and soil Phospholipid Fatty Acids (PLFAs). Intact soil cores and destructive samples were taken along a chronosequence of forest plots and pastures of increasing time since deforestation in north-western Argentina. Destructive samples were analysed for soil PLFAs, soil carbon and nitrogen stocks, pH, electrical conductivity (EC), and cation exchange capacity (CEC) and exchangeable cation percentages (Ca^{2+} , Mg^{2+} , and Na^+). Intact cores were incubated under temperature and moisture treatments; 35°C and 80% Water Holding Capacity (WHC); 35°C and 20% WHC; 10°C and 80% WHC; and 10°C and 20% WHC. Gas samples were taken at 1 hour, 8 hours, 24 hours, 48 hours, 1 week and 2 weeks, and analysed for soil GHG fluxes. Soil fluxes were significantly affected by temperature and moisture treatments, with both CO_2 and N_2O fluxes being highest in Hot Wet conditions, and lowest in Cold Wet conditions. CH_4 fluxes lowest under Hot Wet and highest under Cold incubations. A number of soil CO_2 fluxes were observed to be negative, suggesting the possibility of uptake of CO_2 into the soil. However, additional analyses could not definitively identify the

cause of this apparent uptake. Soil PLFA biomass was found to be increase with increasing soil carbon stocks, CEC, and exchangeable magnesium percentage, and negatively correlated to exchangeable calcium percentage, indicating that increased salinity of these soils is a detriment to microbial communities. The results of this study suggest that climate factors such as moisture and temperature are the predominant drivers of soil GHG fluxes in the dry Chaco rather than land use change and grazing, which suggests that with future climate change soil GHG fluxes will continue to increase. Future research would benefit from the further investigation of the impacts of soil salinity and sodicity on soil microbial community composition and GHG emissions.

4.2 Introduction

Soils are a major source of greenhouse gas (GHG) emissions globally, with land use change (LUC) and agricultural production resulting in large releases of CO₂, CH₄, and N₂O each year (Oertel *et al.* 2016; Bahn *et al.* 2010). A modelling study by Kim *et al.* (2015) found that conversion of land from forest to grassland results an estimated emission of 5.9 t CO₂ eq ha⁻¹ yr⁻¹ which, given that there has been the global loss of approximately 0.8 million km² (80 million hectares) of forest (48% of which equates to agricultural expansion) from 1960 – 2019, indicates high levels of GHG emissions from soil due to LUC (Winkler *et al.* 2021). With increasing demands on food and other agricultural products, deforestation is expected to continue, and it has been predicted that there will be an increase in 100-110% of global crop demand alone between 2005 and 2050 (Meyfroidt, 2018; Tilman *et al.* 2011).

Changes in land cover, such as the removal of forests and establishment of pastures, are one of the major drivers of emissions from soils, with poor land management increasing heterotrophic respiration, and altering microbial communities (Wang *et al.* 2020; Delelegn *et al.* 2017; Zhao *et al.* 2017; Oertel *et al.* 2016). Microbial community composition can play an important role in soil carbon sequestration, and it has been proposed that greater fungal:bacterial dominance favours aggregate formation, the breakdown of organic matter into more stable forms, and greater carbon use efficiency, thus increasing soil carbon sequestration (Malik *et al.* 2016; Strickland & Rousk, 2010). Land use change can alter microbial community composition and structure with the removal of native vegetation and establishment of pastures which changes the composition of vegetation inputs to the soil (Wu *et al.* 2022; Zhang *et al.* 2016; Klumpp *et al.* 2009). Furthermore, the detrimental impacts of deforestation and grazing on soil chemical, and physical characteristics such as decreased nutrient availability, increased alkalinity or acidity, soil compaction, and changes to soil hydrology can all detrimentally impact microbial communities (Zhang *et al.* 2016).

Increased GHG emissions both due to the direct removal of forest biomass and subsequent grazing practices, and the detrimental impacts they have on soil quality are identified to be contributing to global warming and climate change. Such changes to climate can further exacerbate the degradation of soils due to LUC and poor grazing management. Increased temperatures and precipitation can accelerate the breakdown of organic matter due to increased microbial activity, leading to direct positive feedbacks on climate change (Bardgett *et al.* 2008). Increased drought events can cause water stress on both soil microbes and vegetation, reducing nutrient inputs, and sudden precipitation events can disrupt soil

aggregates, increase water erosion and the release microbial carbon stored in the soil from microbial death during periods of drought (Meena *et al.* 2020; Li *et al.* 2018; Sawada *et al.* 2016; Matteucci *et al.* 2015; Rey *et al.* 2011). The impacts of climate change are likely to be particularly detrimental to soils in tropical and subtropical ecosystems, where temperatures are predicted to increase by 1.8 - 5°C over the next century (Jansson & Hofmockel, 2020), and extreme weather events are already becoming more occurrent. Furthermore, as forest systems in these climates typically being the biomes most subjected to land use change (Macchi *et al.* 2013), it is likely that the soils of these regions will be more susceptible to degradation.

The dry Chaco in Argentina is a xerophytic subtropical forest which has been subjected to intensive deforestation in recent years. Between 1985 and 2000, over 1 million ha of the forested area of the dry Chaco was removed for agricultural production (Hoyos *et al.* 2013; Grau *et al.* 2005; Gasparri *et al.* 2013), and deforestation rates continued to increase, reaching a maximum between 2006 and 2012 (Villarino *et al.* 2017; Vallejos *et al.* 2014). A number of studies have used modelling techniques to simulate soil emissions, finding that the removal of forest and establishment of managed grasslands has decreased soil organic carbon and emitted between 23 and 32 Tg C yr⁻¹ from the soil in the years between 1985 – 2013 (Baldassini & Paruelo, 2020; Villarino *et al.* 2018; Baumann *et al.* 2017; Barral *et al.* 2020; Gasparri *et al.* 2008). Furthermore, it has been suggested that the removal of such vast amounts of forest have resulted in increased precipitation in the region (Hoyos *et al.* 2013), and changes to soil physical properties such as water infiltration rate and bulk density (Magliano *et al.* 2016). Whilst the impacts of forest clearance and grazing on soil carbon have been widely reported in the Chaco, the direct impacts of land use change on soil GHG emissions and microbial community composition in this ecosystem remain less researched.

To date, there have been few experimental studies which investigate soil emissions or changes in microbial communities with land use change from forest to pastures in the dry Chaco. In 2001, Abril and Bucher analysed soils for carbon mineralisation rates in degraded pastures, finding that mineralisation rate increased along a grazing gradient and CO₂ production decreased along the same gradient, indicating a reduced humification potential and increased soil carbon loss with increasing degradation. They identified that precipitation and soil moisture was a significant driver of changes in soil GHG emissions in this ecosystem, which was also found by Mazzarino *et al.* (1991) when investigating nitrogen mineralisation, who also indicated that there is high seasonality in mineralisation rates. To date there have been no studies which measure soil methane emissions in the dry Chaco. Soil microbial communities and biomass have been reported more widely with mixed results, with some research indicating that long term

grazing management can increase species richness and microbial biomass due to changes in soil nutrient availability (Viruel *et al.* 2022), yet others showing a decrease in microbial biomass with land use change (Abril & Bucher, 1999). To the best of our knowledge, there has been no investigation into the changes in soil trace gas fluxes and microbial community structure along a land use transition from native vegetation to grazed pasture in the dry Chaco.

This study aims to quantify soil trace gas emissions (CO_2 , CH_4 , and N_2O) in soils incubated under conditions to simulate changes to climate variables (temperature and precipitation) and soil microbial community composition over a chronosequence which shows a land use gradient from native forest to pastures of increasing time since establishment in the dry Chaco. The objectives were to investigate how climate factors (through temperature and precipitation incubations) impact soil respiration along a land use gradient from forest to extensively grazed pastures, and how microbial community composition (through PLFA analysis) changes over time since deforestation. Furthermore, the study sought to identify how soil fluxes and microbial community composition are impacted by soil chemical characteristics such as the soil carbon and nitrogen stocks, pH and electrical conductivity reported in Chapter 3, the samples for which were collected at the same sampling points as those for this study. It will also conduct further analyses on soil cation concentrations with the aim of investigating their influence on soil GHG emissions and microbial populations.

The working hypotheses of this study were that 1) Soil GHG fluxes will be impacted by imposed climate incubation conditions, with greater emissions of CO_2 and N_2O , and uptake of CH_4 in warmer wetter incubations, decreased CO_2 and N_2O emissions and increased CH_4 emissions under cold incubations; 2) Time since deforestation will impact soil GHG fluxes, with greater emissions of CO_2 , N_2O and CH_4 with increasing pasture age within each incubation treatment, 3) Soil GHG fluxes and microbial community composition will be negatively correlated to soil cation concentrations due to an uptake of CO_2 through carbonate precipitation and the detrimental effects of alkalinity on microbial populations

4.3 Methods

The study was carried out in the western dry Chaco region of north-west Argentina, in the Tucuman, Santiago del Estero and Catamarca provinces. The area is characterised by a mean annual rainfall of 500 – 880 mm, 75% of which falls between October and March, with high

summer temperatures (mean: 25°C) and mild (mean: 13°C) dry winters (Banegas *et al.* 2019; ²Banegas *et al.* 2019). Native vegetation is characterised by xerophytic subtropical forest species dominated by the tree species *Schinopsis balansae*, *Schinopsis quebracho-colorado* and *Aspidosperma quebracho-blanco*, typically with a ground layer of grasses and a high presence of halophytic scrub (Bucher, 1982). Pastures are sown with *Chloris gayana*, an African salt-tolerant grass and grazed with beef cattle (Banegas *et al.* 2019). Predominant soil type in the area is eutric fluvisol.

Table 8. Site and soil characteristics at Los Puestos/INTA-IIACS and Lavalle at 0-10 cm of depth (\pm S.E)

Site	Los Puestos	Lavalle
Coordinates	27 11'34" – 27 20'29"S 65 14'46" – 65 01'27"W	28 11'07"S, 65 06'28"W
Mean Annual Precipitation (MAP; mm yr⁻¹)	984.9	527.8
Mean Annual Temperature (MAT °C)	13-25	13-25
Climate (Koppen Classification)	Humid subtropical	Hot semi-arid
Elevation	300-325m a.s.l	450-550m a.s.l
Soil Type	Eutric Fluvisol	Eutric Fluvisol
Clay (%)	12.2 \pm 0.6	13.3 \pm 0.8
Silt (%)	64.7 \pm 1.2	60.8 \pm 0.9
Sand (%)	23.2 \pm 1.1	25.9 \pm 0.5
Soil organic matter (SOM)(%)	6.5 \pm 0.3	4.9 \pm 0.1
pH	5.9-9.1	6.3-8.2
Electrical Conductivity (dS/m)	0.09-3.6	0.03-0.36
Pasture Vegetation	<i>Chloris gayana</i> , <i>Acacia spp</i>	
Native Vegetation	Trees: <i>Aspidosperma quebracho-blanco</i> , <i>Schinopsis quebracho-colorado</i> (Tree), <i>Chorisia speciosa</i> (Tree), <i>Caesalpinea paraguariensis</i> (Legume), and <i>Prosopis spp</i> (Shrub)	

Two sites were used in the study, one spread over two farms in Tucuman (Lago Hondo and (Insituto Nacional de Tecnología Agropecuaria – Instituto de Invetigación Animal del Chaco Semiárido (INTA-IIACs)), referred to here on as Los Puestos which is located on the edge of the

semi-arid and subhumid subtropical climate zone, and the third (Tres Flores/Jai) here on referred to as Lavalle, was split across the border of the Catamarca and Santiago del Estero provinces and sits in a hot semi-arid climate zone (Figure 18). Both sites are found within the subtropics (Table 8).

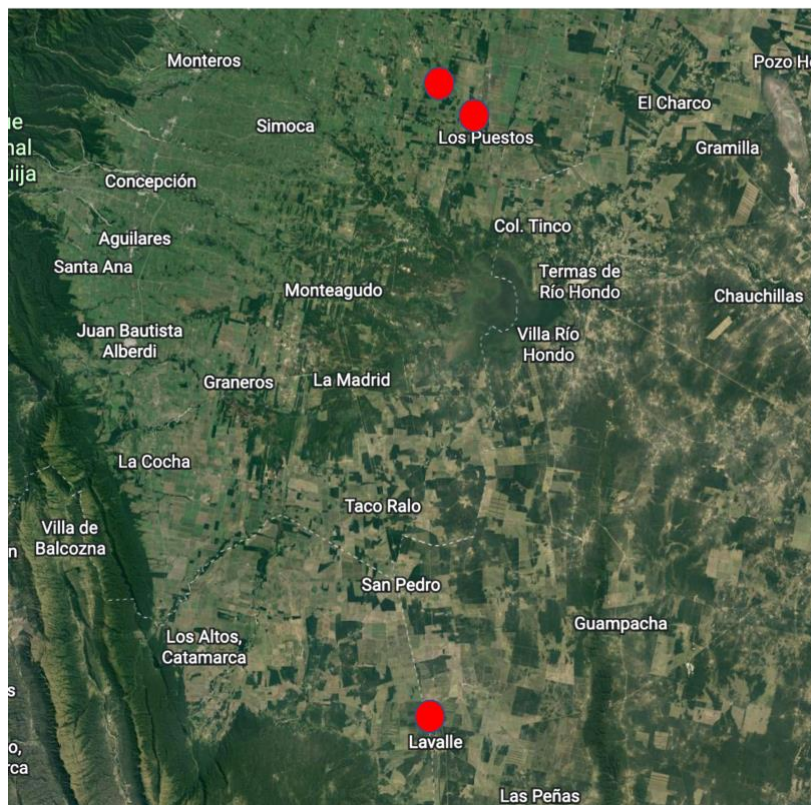
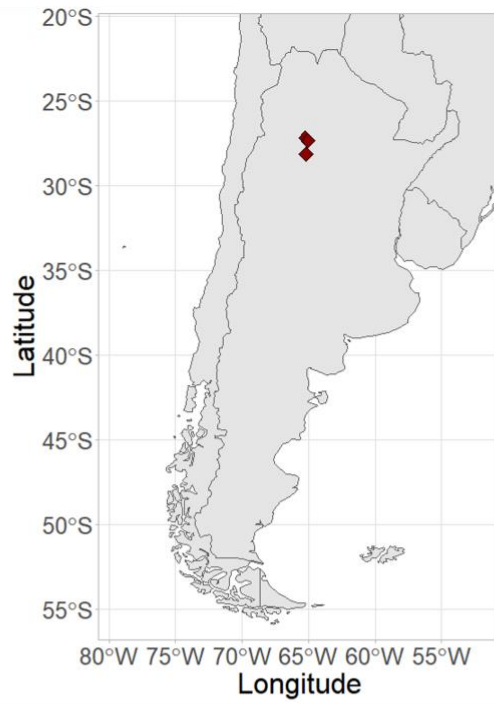


Figure 14 Location of sample sites in the dry Chaco, Argentina

The study followed a chronosequence design replicated six times, which consisted of a site of native forest vegetation followed by pastures of increasing time since deforestation. Age classes of the pastures selected were Forest (Native Vegetation); 0-5 years; 10-15 years; and >20 years. Sites were selected using Google Earth Engine, using a shapefile of the study area which was overlaid with a shapefile which demonstrated deforestation year of individual plots. In addition to this, a timelapse of Landsat imagery from 1984 – 2016 was used which allowed for the visualisation of deforestation year and establishment of pastures. Three repeats of the chronosequence were selected at Lavalle (one patch of forest, nine pastures) and three at Los Puestos/INTA-IIACS (one patch of forest, nine pastures). Due to difficulties in locating intact forest patches, one forest site was selected for each farm and was pseudo-replicated in laboratory analyses (Figure 19).



Figure 15 Newly cleared forest in the dry Chaco, Lavalle

4.3.1 Soil Sampling

Within each plot, intact cores were sampled at 0-10 cm from three points within each plot using 10 cm x 4 cm PVC tubes (10 cm in length, 4 cm in diameter) (Figure 20), which were inserted into the soil with a rubber mallet and extracted, wrapped in film to ensure they remained intact and prepared for transportation. Samples were also taken from three depths: 0-10 cm, 10-30 cm and 30-50 cm using a soil auger for the measurement of soil PLFAs and other characteristics. After sieving to 2 mm and homogenising, samples were analysed for pH and electrical

conductivity. Samples were then refrigerated prior to being shipped to Lancaster University in the UK for analysis. Samples which were to be analysed for PLFA content were freeze dried.



Figure 16 Intact core sampling in Los Puestos

4.3.2 Laboratory Analysis

4.3.2.1 Incubations

CO₂, CH₄ and N₂O emissions were measured from intact soil cores incubated under four different conditions; hot wet, hot dry, cold wet and cold dry (Table 9). Whilst average summer and winter temperatures are 25°C and 13°C respectively, the region can observe temperature highs of >40°C and lows of <0°C across the seasons (Blanco *et al.* 2016), and soils have been recorded anecdotally to reach temperatures of 40°C in the summer and frosts have occurred in winter months. In addition, IPCC scenarios, have predicted that under the “middle of the road” SSP2-4.5 Scenario, mean monthly temperature will increase by a minimum of 1.1°C by 2050, and precipitation will increase in Tucuman in the summer and decrease in winter months, whilst in Catamarca winters will become wetter and summers drier (World Bank, 2021). As a result, the incubation conditions in Table 9 were chosen to represent both the current extreme climate conditions across summer and winter seasons, and the potential future climates which have been predicted according to IPCC climate scenarios. In order to calculate water holding capacity, one core from each point of each site was placed on one end into a tray of water reaching approximately one third up the height of the core. The cores were then left to stand until the

top surface of the soil was saturated, at which point they were weighed, placed in the oven at 105°C for 24 hours and then reweighed. WHC was calculated using the formula:

$$WHC = \frac{\text{saturation weight of soil} - \text{dry weight of soil}}{\text{dry weight of soil} \times 100}$$

Where WHC = Water Holding Capacity

Blanco, L. J., Paruelo, J. M., Oesterheld, M., & Biurrun, F. N. (2016). Spatial and temporal patterns of herbaceous primary production in semi-arid shrublands: a remote sensing approach. *Journal of vegetation science*, 27(4), 716-727.

Table 9. Incubation treatments applied to intact cores

Temperature Water Holding Capacity	10°C	35°C
20%	Cold Dry	Hot Dry
80%	Cold Wet	Hot Wet

Cores from each site were weighed and placed into 1 litre kilner jars and left to acclimatise at room temperature for 48 hours (Figure 21). Samples which were to be used for the dry treatment, were left to air dry until below the desired weight for 20% WHC. Deionised water was added to reach the WHC of the treatment and the cores were left for a further 24 hours. The cores were placed into 2 incubators, one set to 35°C degrees, and the other set to 10°C. Gas samples were taken at 1 hour, 8 hours, 24 hours, 48 hours, 1 week and 2 weeks after being placed in the incubator. Samples were taken with a syringe immediately after sealing the kilner jar, and then again at 20, 40 and 60 minutes. Prior to taking the sample the syringe was first flushed three times with ambient air, and then flushed whilst in the septum of the chamber in order to mix the chamber air. Samples were injected into evacuated exetainer vials and stored

in a cool dark location until analysed. Gas samples were analysed using a Perkin Elmer Trace Gas Chromatograph equipped with a flame ionization detector (FID) lamp for CO₂, CH₄ and N₂O.



Figure 17 Incubated intact cores in kilner jars

4.3.2.2 Soil Phospholipid Fatty Acids (PLFAs)

Soil PLFA profiles were analysed to determine microbial community composition. Soil PLFAs were extracted from freeze dried soil samples according to the procedure by Bligh and Dyer (1959). The lipids were extracted from a soil sample of 1 – 1.5 grams, using an extraction mixture of chloroform, methanol and citrate buffer. The procedure results in the fractionation of the sample, separating the lipids from non-lipids. The lipids can then be separated based on their polarity (neutral lipids, glycolipids and polar lipids). C13 (13:0) and C19 (19:0) were added as internal standards and the samples were analysed using an Agilent PLFA liquid gas chromatograph.

4.3.2.3 Total Carbon and Nitrogen

Total carbon and nitrogen were analysed using dry combustion, using a Vario El Cube organic elemental analyser (Elementar). The samples were sieved to 2mm and air dried, then ground using a ball mill and wrapped into tin cups for analysis. Carbon and nitrogen percentages recorded in the analysis were converted to stocks for statistical analyses.

4.3.2.4 Exchangeable sodium percentage

Sodium concentration of soil was analysed using a prewash of glycol-ethanol to remove soluble cations and leave the exchangeable ions. The plant available sodium was then leached with alcoholic ammonium chloride, with the ammonium displacing the sodium (Rowell, 1994). One gram of air-dried soil was weighed into 50ml beakers, and 4ml of glycol-ethanol added (100ml ethylene glycol:900ml 95% (v/v) ethanol). The soil-solution mixture was stirred for 15 minutes using a magnetic stirrer. The solution was then filtered using Whatmann grade 42 filter papers into 50ml centrifuge tubes, and the glycol-ethanol solution was passed through the soil a further three times. Once the soil had been leached with glycol ethanol three times over, it was then filtered with five successive 10ml volumes of alcoholic ammonium chloride (53.5g NH₄Cl:400ml DI water:600ml 95% (v/v) ethanol, pH adjusted to 8.5) into a fresh centrifuge tube. This solution was then analysed with a Sherwood Flame Photometer 410 which was calibrated using known concentration sodium standards to create a calibration curve.

4.3.2.5 Exchangeable calcium and magnesium

Calcium and Magnesium cations were extracted with 2M KCl solution. 40ml of solution was added to 8 grams of soil, in 50ml centrifuge tubes. The soil-KCl solution was shaken on an orbital shaker for one hour and left to settle overnight. The solution was then filtered through Whatmann grade 42 filter paper. Due to the extremely high concentration of salts in the soil, the samples had to be diluted times 15 in order that they would read within the standards calibration curve. Lanthanum Chloride (LaCl₃) was added to the samples as a releasing agent. Samples were analysed for Ca²⁺ and Mg²⁺ concentration with a Perkin Elmer Atomic Absorption Spectrometer, with a set of known concentration standards made up in KCl solution first run to allow the calculation of a calibration curve.

4.3.2.6 CEC

Due to the soils being alkaline, cation exchange capacity was assumed to be the sum of Calcium, Magnesium and Sodium cations (Rowell, 1994).

4.3.3 Statistical Analyses

Statistical analyses were carried out using a mixture of linear and non-parametric Generalised Linear Model (GLM) models in R. Post hoc comparison of means were carried out on categorical explanatory variables in order to ascertain differences between treatment levels.

4.4 Results

4.4.1 Soil chemical characteristics

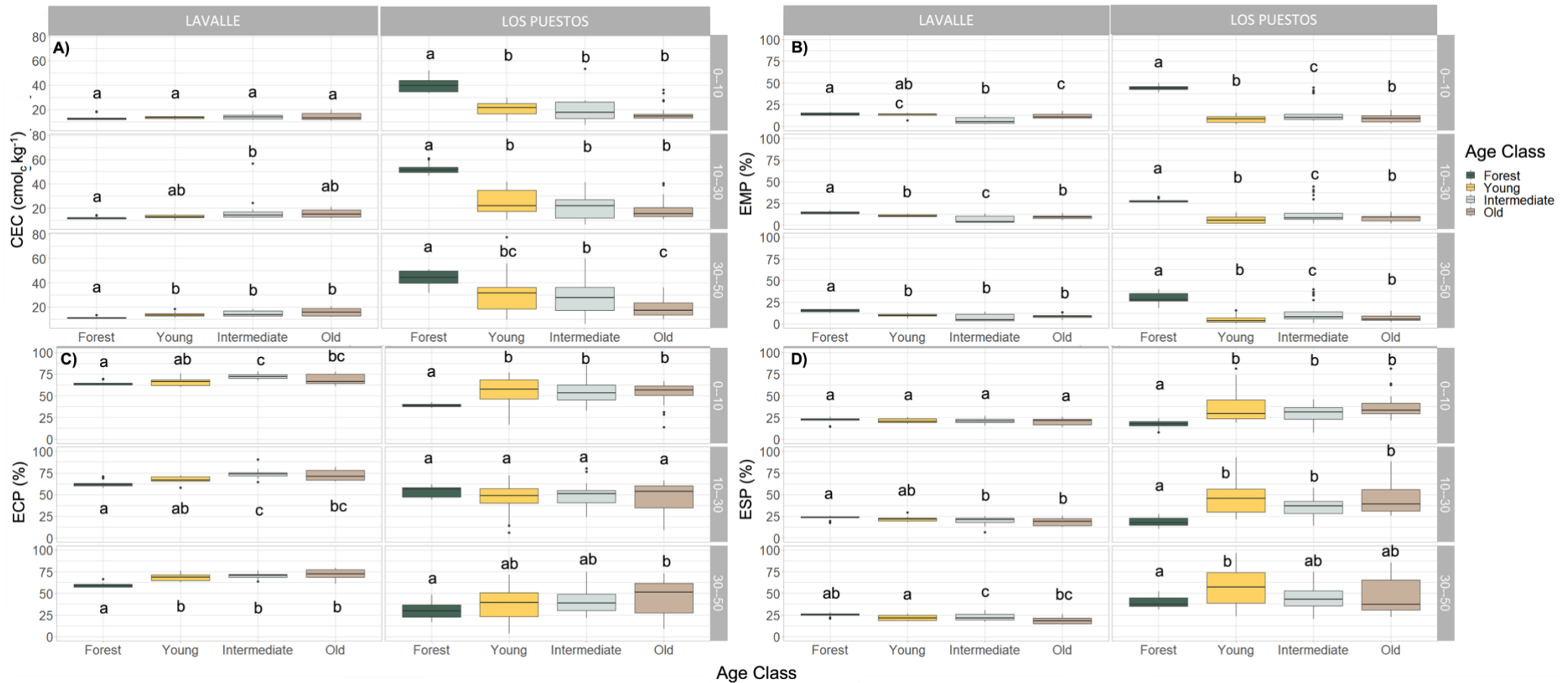
Soil pH was spatially variable within each site and was higher in Los Puestos than in Lavalle ($p < 0.001$). Land use change affected pH only in Lavalle, with pasture soils having higher values than forest (Table 10, $p < 0.05$) over all depths. In both sites, pH increased with depth in forest and pasture soils ($p < 0.001$). Electrical conductivity also spatially variably within each site (Supplementary Materials; Figures 26 – 33) and was significantly greater Los Puestos than Lavalle ($p < 0.001$). Whilst there was no impact of deforestation on EC in Lavalle, there were differences between pastures at each sample depth (0-10, Young-Old; 10-30, Young-Old, 30-50; Intermediate-Old). In Los Puestos, EC was significantly decreased in the subsoil of pastures in comparison to forest soils (>10 cm, $p < 0.05$).

Table 10. Mean soil pH and EC (\pm S.E) observed in the research sites of Los Puestos and Lavalle, within each age class and sampling depth. Letters (a,b,c) denote significant differences at each depth, and site ($p < 0.05$). Age classes which have different letters are significantly different.

Age	Depth	Lavalle				Los Puestos			
		Carbon (%)	Nitrogen (%)	pH	EC	Carbon (%)	Nitrogen (%)	pH	EC
Forest	0-10	2.4 \pm 0.1 ^a	0.22 \pm 0.01 ^a	6.5 \pm 0.1 ^a	0.18 \pm 0.03 ^a	4.8 \pm 0.4 ^a	0.47 \pm 0.04 ^a	7.0 \pm 0.2 ^a	1.83 \pm 0.48 ^a
Young		1.9 \pm 0.1 ^a	0.19 \pm 0.01 ^{ab}	7.2 \pm 0.1 ^b	0.13 \pm 0.01 ^{ab}	2.6 \pm 0.2 ^b	0.25 \pm 0.02 ^b	7.5 \pm 0.2 ^a	1.02 \pm 0.2 ^a
Intermediate		2.5 \pm 0.7 ^a	0.16 \pm 0.01 ^b	7.1 \pm 0.1 ^b	0.12 \pm 0.01 ^b	3.6 \pm 0.8 ^{ab}	0.22 \pm 0.01 ^b	6.9 \pm 0.2 ^a	0.71 \pm 0.17 ^a
Old		1.8 \pm 0.1 ^a	0.19 \pm 0.01 ^{ab}	7.0 \pm 0.1 ^b	0.16 \pm 0.01 ^{ab}	2.3 \pm 0.8 ^b	0.16 \pm 0.01 ^b	7.2 \pm 0.2 ^a	0.81 \pm 0.17 ^a
Forest	10-30	1.5 \pm 0.1 ^a	0.13 \pm 0.01 ^a	7.0 \pm 0.1 ^a	0.14 \pm 0.01 ^a	1.7 \pm 0.2 ^a	0.13 \pm 0.02 ^a	7.7 \pm 0.2 ^a	3.21 \pm 0.32 ^a
Young		1.1 \pm 0.1 ^b	0.12 \pm 0.01 ^a	7.6 \pm 0.1 ^{bc}	0.09 \pm 0.01 ^b	1.1 \pm 0.1 ^b	0.09 \pm 0.01 ^a	8.3 \pm 0.2 ^a	1.53 \pm 0.32 ^b
Intermediate		1.2 \pm 0.1 ^b	0.11 \pm 0.01 ^a	7.4 \pm 0.1 ^{ab}	0.11 \pm 0.01 ^{abc}	1.3 \pm 0.1 ^{ab}	0.11 \pm 0.01 ^a	7.4 \pm 0.2 ^a	0.87 \pm 0.25 ^b
Old		1.2 \pm 0.1 ^b	0.13 \pm 0.01 ^a	7.6 \pm 0.1 ^{bc}	0.14 \pm 0.01 ^{ac}	1.1 \pm 0.1 ^b	0.10 \pm 0.01 ^a	7.9 \pm 0.2 ^a	0.82 \pm 0.21 ^b
Forest	30-50	1.0 \pm 0.1 ^a	0.09 \pm 0.01 ^a	7.0 \pm 0.1 ^a	0.11 \pm 0.01 ^{ab}	0.9 \pm 0.1 ^a	0.13 \pm 0.05 ^a	8.1 \pm 0.2 ^a	4.01 \pm 0.49 ^a
Young		0.8 \pm 0.1 ^a	0.09 \pm 0.01 ^a	8.2 \pm 0.2 ^{bc}	0.14 \pm 0.01 ^{ab}	0.7 \pm 0.1 ^a	0.06 \pm 0.01 ^b	8.9 \pm 0.2 ^a	1.35 \pm 0.35 ^b
Intermediate		0.9 \pm 0.1 ^a	0.09 \pm 0.01 ^a	7.7 \pm 0.1 ^b	0.08 \pm 0.001 ^a	0.9 \pm 0.1 ^a	0.07 \pm 0.01 ^{ab}	8.1 \pm 0.3 ^a	0.97 \pm 0.28 ^b
Old		0.9 \pm 0.1 ^a	0.09 \pm 0.01 ^a	8.3 \pm 0.2 ^c	0.15 \pm 0.02 ^b	0.7 \pm 0.1 ^a	0.05 \pm 0.01 ^b	8.6 \pm 0.2 ^a	1.05 \pm 0.29 ^b

Soil cation exchange capacity was observed to be significantly higher ($p < 0.001$) and with greater variability in Los Puestos than Lavalle. Furthermore, exchangeable cation percentages were more variable in Los Puestos, and Na^+ cations were significantly higher ($p < 0.001$), whereas in Lavalle soils had significantly greater Ca^{2+} ($p < 0.001$). Depth had an effect on both sites, with CEC being higher in the subsoil of forest soils in both ($p < 0.01$), and in young pastures and intermediate pastures in Los Puestos ($p < 0.01$). In Los Puestos, the removal of forest significantly reduced CEC ($p < 0.001$), which corresponded to a loss of Mg^{2+} cations with deforestation throughout the soil profile ($p < 0.001$, Figure 22). Following deforestation, CEC in Los Puestos moves from being dominated by Mg^{2+} cations in forest soils, to dominance of Ca^{2+} and Na^+ , both of which significantly increase in pasture soils ($p < 0.05$). Proportion of exchangeable cations changed with depth, with exchangeable Ca^{2+} decreasing down the soil profile, whilst exchangeable Na^+ increased with depth ($p < 0.05$).

In Lavalle, CEC was not impacted by deforestation in the topsoil, but at 30-50 cm it was significantly higher in pastures than in forest soils ($p < 0.001$). Cation exchange sites were dominated by Ca^{2+} cations throughout the soil profile, which significantly increased deforestation in the topsoil (higher in intermediate and old pastures than in forest). Deeper in the soil profile, exchangeable calcium was significantly higher in all pastures than in forest ($p < 0.001$). Sodium and magnesium cations, which were lower in Lavalle than Los Puestos ($p < 0.001$) were both impacted by land use change, with Mg^{2+} cations observed to be significantly different in pastures than forest soils over all depths, and Na^+ being significantly different in the subsoil ($p < 0.05$).



4.4.2 Soil trace gas fluxes

Fluxes were 9.3%, 241% and 250% and significantly higher in Los Puestos than in Lavalle for all three of the measured trace gases ($p < 0.001$) (CO_2 , N_2O , and CH_4 respectively). However, soil trace gas fluxes were not significantly affected by age of pasture, and there was no difference between fluxes from forest and pasture soils (Figure 23).

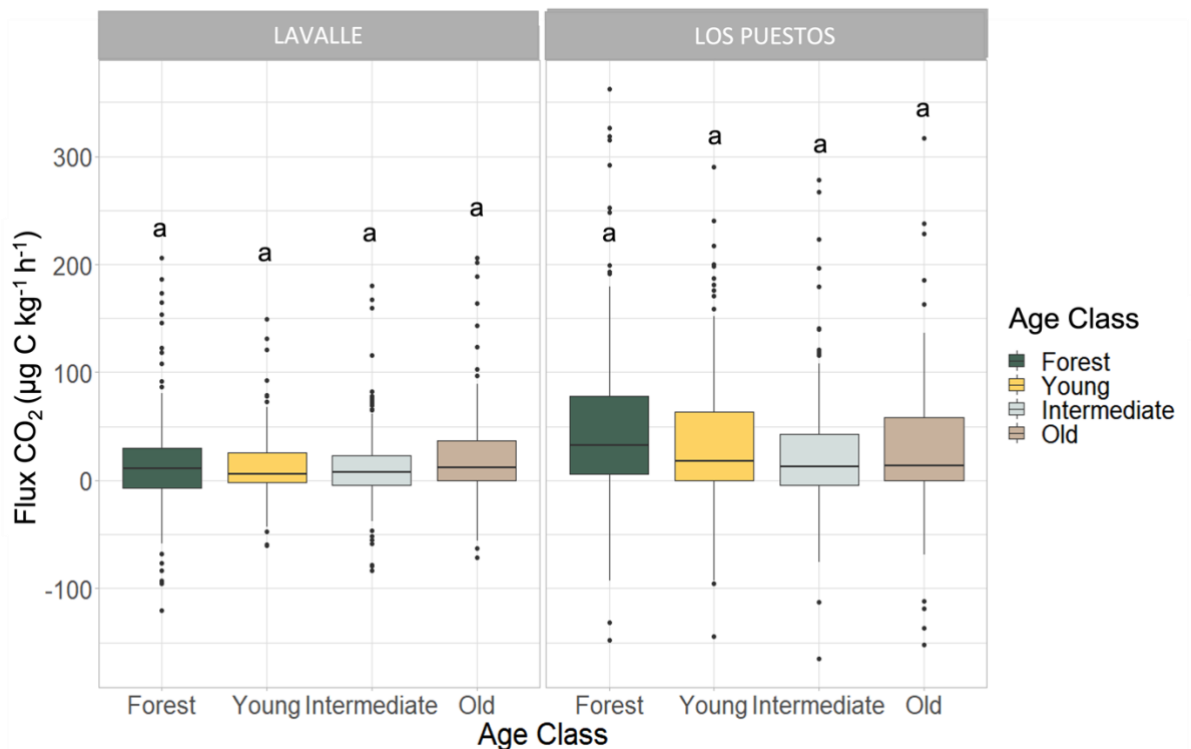


Figure 19 Soil CO_2 fluxes grouped by age class at Los Puestos and Lavalle, with all incubation treatments together. Letters denote significant differences between age classes within each site. Age classes denoted with the same letter are not significantly different

Contrastingly, environmental conditions significantly affected soil gas emissions in both sites (Figure 24). It was observed that over all treatments, in both sites CO_2 fluxes were greatest under hot wet incubation conditions and lowest under cold wet treatments ($p < 0.05$). N_2O flux was significantly higher under hot wet conditions than the other incubation treatments in Los Puestos, whilst in Lavalle emissions were different under Hot Dry, Hot Wet and Cold treatments ($p < 0.05$). Finally, CH_4 emissions in Los Puestos were significantly lower under Hot than Cold treatments and there was no effect of moisture under either temperatures ($p < 0.05$), whilst in

Lavalle CH₄ flux was lowest under Hot Wet treatments and highest under Cold conditions which had no difference between moisture treatment ($p < 0.05$).

Statistical analyses of the effect of age class within each treatment showed that in Los Puestos, intermediate pasture soils emitted 28% less CO₂ than forest soils under hot wet conditions, and under hot dry conditions CO₂ flux decreased 23-73% with age ($p < 0.05$) (Forest – Young – Intermediate - Old). CH₄ emissions were 20% lower in intermediate pastures than in forest soils under the cold wet treatment in Lavalle ($p < 0.05$), and in Los Puestos, CH₄ fluxes were 9% and 130% significantly higher in pastures than in the forest under cold dry conditions ($p < 0.05$), but there was no difference between pasture age classes. Finally, under the hot wet treatment, N₂O emissions were 32% and significantly higher in forest soils than in young pasture soils in Los Puestos ($p < 0.05$), and in Lavalle, forest N₂O emissions were 106% and significantly higher than in young pastures under cold wet conditions ($p < 0.05$).

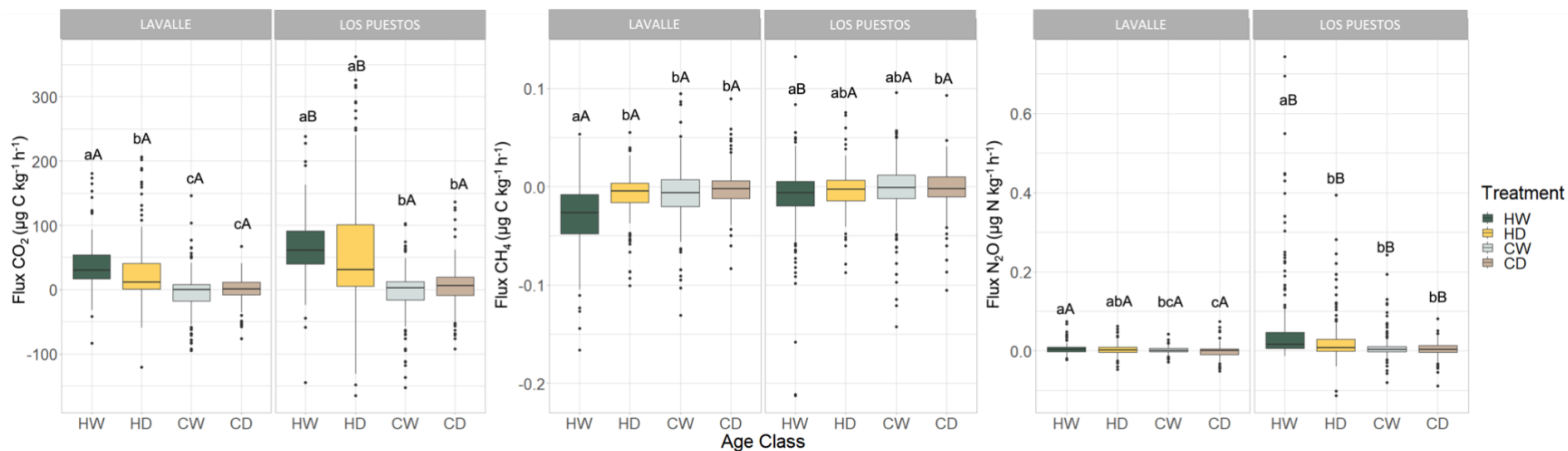


Figure 20 Soil trace gas fluxes (CO_2 , CH_4 and N_2O in $\mu\text{g kg}^{-1} \text{h}^{-1}$) under all age classes and grouped by incubation treatment (HW: Hot wet, HD: hot dry, CW: cold wet, and CD: cold dry) at Los Puestos and Lavalle. Letters denote significant ($p < 0.05$) differences between treatments and sites. Treatments labelled with the same letter (a, b, c, d, e) are not significantly different. Letters AB denote significant ($p < 0.05$) differences within treatments, between the Los Puestos and Lavalle sites (where letters of the same treatment are the same, there is no significant difference).

Time since application of treatment was found to significantly affect soil gas fluxes under certain incubation treatments (Figure 21). Under hot dry conditions, time point was found to have a consistent impact on CO₂ fluxes with, CO₂ decreasing significantly with time. This effect was found in both the Los Puestos and Lavalle sites. Time since application of incubation treatments was not found to have a significant impact on CH₄ emissions. With N₂O emissions, time point had a significant effect on fluxes under hot wet conditions in Los Puestos. The results showed that N₂O flux peaked at 8 hours, and then decreased with time (Figure 21) ($p < 0.05$).

Generalized Linear Models (GLM) were carried out between fluxes of CO₂, CH₄ and N₂O and total soil carbon and nitrogen, with the fluxes split between site and incubation treatments (Table 11). The analyses found that soil carbon stock and CO₂ flux were negatively correlated under hot wet conditions in Lavalle ($p < 0.01$). No other significant correlations were found between carbon, nitrogen and soil CO₂ flux. Similarly, CH₄ was only significantly positively correlated to nitrogen ($p < 0.05$) under hot wet conditions, yet this relationship was not found in Los Puestos. N₂O was found to be significantly negatively correlated to both total carbon and total nitrogen in hot dry and hot wet conditions (Table 11).

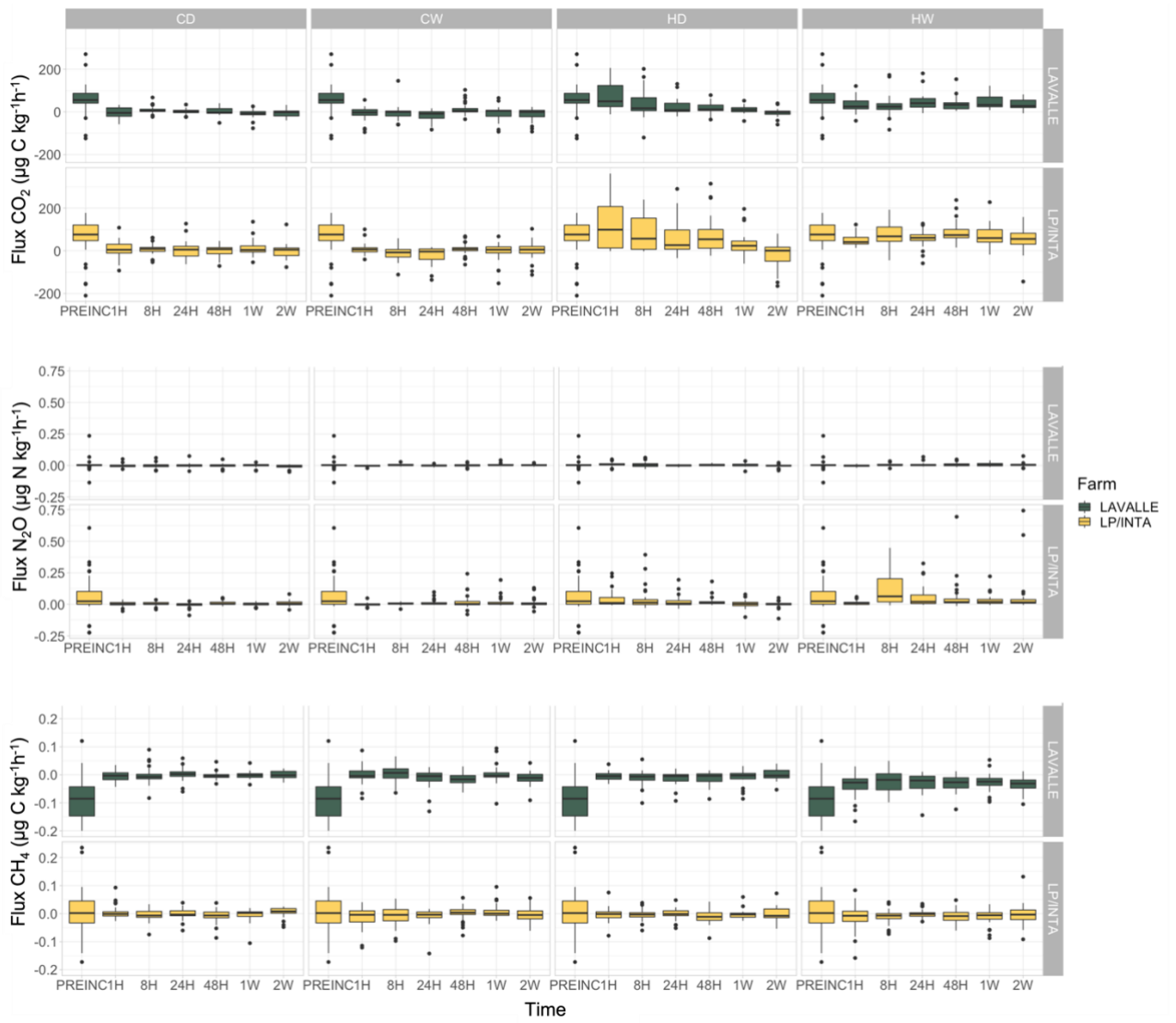


Figure 21 Soil trace gas fluxes (CO₂, CH₄ and N₂O in $\mu\text{g kg}^{-1} \text{h}^{-1}$) under all age classes and grouped by time point at Los Puestos and Lavalle.

Table 11. Generalised linear model outputs of relationship between soil trace gas emissions (CO₂, CH₄, and N₂O) and total carbon and nitrogen (kg m⁻²), showing R Sq values and Coefficient of each model. Models were carried out on each incubation treatment, with all age classes together. *** = p < 0.001, ** = p < 0.01, * = p < 0.05

Treatment	Los Puestos				Lavalle			
	Carbon		Nitrogen		Carbon		Nitrogen	
	Coeff	R sq	Coeff	R sq	Coeff	R sq	Coeff	R sq
CO₂								
Hot Wet	-0.75	0.03	-7.60	0.03	-2.33	0.04**	-11.92	0.008
Hot Dry	0.40	0.002	3.09	0.009	-1.41	0.006	-12.89	0.004
Cold Wet	0.66	0.04	6.61	0.04	-1.16	0.01	-3.57	0.009
Cold Dry	-0.25	0.005	-2.69	0.006	1.00	0.02	7.56	0.007
CH₄								
Hot Wet	7.5x10 ⁻⁴	0.04	0.009	0.005*	-2.2x10 ⁻⁴	3.2x10 ⁻⁴	0.003	5.3x10 ⁻⁴
Hot Dry	-2.0x10 ⁻⁵	9.6x10 ⁻⁵	-2.0x10 ⁻⁴	9.6x10 ⁻⁵	-5.4x10 ⁻⁴	0.004	-0.006	0.004
Cold Wet	6.4x10 ⁻⁴	0.90	0.006	0.90	3x10 ⁻⁴	0.001	0.003	0.90
Cold Dry	-1.8x10 ⁻⁴	0.007	-0.002	0.90	-5.6x10 ⁻⁴	1.01	7.0x10 ⁻⁴	0.90
N₂O								
Hot Wet	-7.2x10 ⁻⁴	0.004	-0.01	0.005	4.0x10 ⁻⁵	7.3x10 ⁻⁵	-0.002	0.001
Hot Dry	-0.002	0.09**	-0.02	0.09**	-2.5x10 ⁻⁴	0.9	0.001	8.9x10 ⁻⁵
Cold Wet	-4.7x10 ⁻⁴	0.02	-0.004	0.02	-2.3x10 ⁻⁴	0.006	-0.004	0.02
Cold Dry	-4.4x10⁻⁴	0.07*	-0.004	0.07*	3.8x10 ⁻⁴	0.003	0.01	0.005

The results found a number of negative CO₂ emissions (n = 398). Such negative CO₂ fluxes were predominantly observed under Cold conditions, with 177 observations under Cold Wet conditions, and 136 under Cold Dry conditions. In Lavalle, negative fluxes were found to be significantly lower under Cold Wet conditions than Cold Dry (p<0.01), but there was no effect of treatment on negative flux in Los Puestos. Furthermore, negative fluxes were significantly different between Forest cores and pasture cores, with young and intermediate pastures both having greater fluxes (p<0.05). There was no effect of age class on negative fluxes in Los Puestos.

Statistical analyses were used to test for interactions between the negative soil CO₂ fluxes and soil chemical characteristics (pH, EC and Cations), in order to investigate what might be causing these negative fluxes. In both sites (Los Puestos and Lavalle), interactions were found between

soil chemical characteristics and CO₂ fluxes within specific age classes and under specific incubation conditions. These interactions did not appear to follow any definitive trend (Table 12). Analysis of interactions between CH₄ and N₂O fluxes also found a number of significant interactions in both Lavalle and Los Puestos, within specific age classes and incubation conditions. As with the analysis of negative fluxes, there was no trend or pattern observed in the significant interactions.

Table 12. Generalised linear model outputs of relationship between negative soil Carbon Dioxide flux (CO₂) and soil chemical characteristics (CEC (cmol_c kg⁻¹), Exchangeable Sodium Percentage (Na⁺), Exchangeable Calcium (Ca²⁺), Exchangeable Magnesium (Mg²⁺), Electrical Conductivity (dS/m), showing P-value and Coefficient of each model. Models were carried out on each age class within each incubation treatment, with all age classes together. *** = p < 0.001, ** = p < 0.01, * = p < 0.05

Interaction	Site	Age Class	Treatment	Coefficient	P-value
CO ₂ ~ CEC	Lavalle	Young	Cold Wet	11.3	0.004**
CO ₂ ~ Ca ²⁺	Lavalle	Young	Cold Wet	3.3	0.0008***
CO ₂ ~ Mg ²⁺	Lavalle	Young	Cold Wet	-4.5	0.008**
CO ₂ ~ Na ⁺	Lavalle	Young	Cold Wet	-7.7	0.0004***
CO ₂ ~ Na ⁺	Los Puestos	Intermediate	Cold Dry	-0.7	0.03*
CO ₂ ~ EC	Los Puestos	Forest	Cold Wet	-26.5	0.01*
CO ₂ ~ EC	Los Puestos	All	Cold Dry	-14.4	0.05*
CO ₂ ~ EC	Lavalle	All	Cold Dry	-71.1	0.009**

4.4.3 Soil PLFAs

Total soil PLFAs, total bacterial PLFAs, and total gram-positive bacterial PLFAs were not significantly affected by pasture age in either site at any depth (Figure 25). In Los Puestos, both total fungal PLFAs and total gram-negative bacteria are lower in intermediate pastures than in forest sites at 0-10 cm (p<0.05), and at 30-50 cm total fungal PLFAs are significantly lower in intermediate than young pastures (p<0.05). In Lavalle, total gram-positive PLFAs were significantly higher in old than intermediate pastures at 0-10 cm. At 30-50 cm, both total bacterial, and total gram-negative PLFAs were greater in intermediate pastures than forest sites (p<0.05).

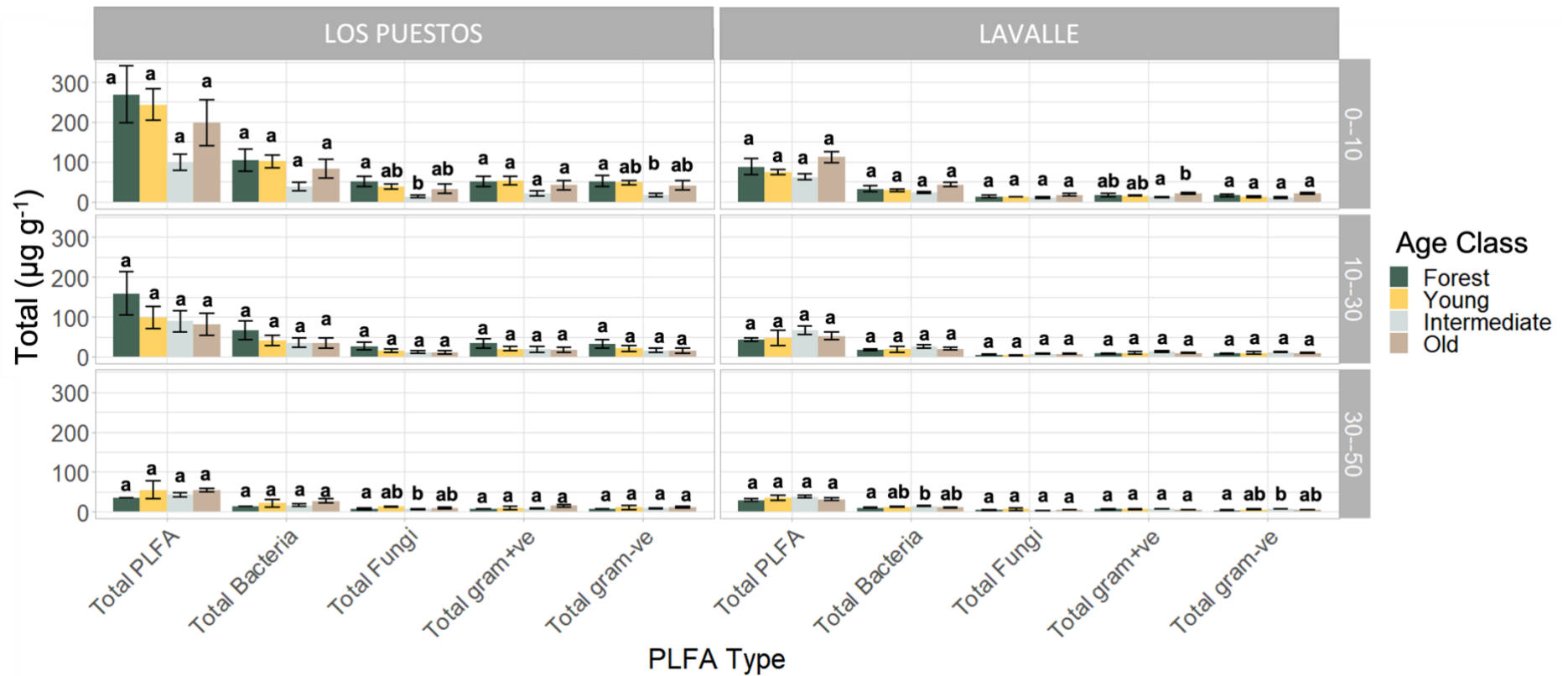


Figure 22 Biomass of PLFA types (total fungi, total bacteria, total gram-positive bacteria, and total gram-negative bacteria) group by age class, sample depth and site. Letters denote significant ($p < 0.05$) differences in biomass of each PLFA type, between age classes at each depth within individual sites. Treatments labelled with the same letter (a, b) are not significantly different.

All PLFA types in Los Puestos significantly decreased between the 0-10 cm depths and 10-30 and 30-50 cm depths ($p < 0.001$), but there was no difference between 10-30 and 30-50 cm. The same was observed in Lavalle, with all PLFA types decreasing after 0-10 cm ($p < 0.001$). Contrastingly, there were also significant differences between 10-30 cm and 30-50 cm ($p < 0.01$). There was no significant effect of either age class or depth on fungal:bacteria or gram+ve:gram-ve ratios (Table 13).

Table 13. PLFA ratios in both Lavalle and Los Puestos. Fungal:Bacteria and Gram Positive:Gram Negative ratio from both sites, within each age class and sample depth. Values within the same age class and depth which share letters are not significantly different.

Age	Fungal:Bacteria	Gram+ve:Gram-ve	Fungal:Bacteria	Gram+ve:Gram-ve	Fungal:Bacteria	Gram+ve:Gram-ve
	0-10cm		10-30cm		30-50cm	
Los Puestos						
Forest	0.48±0.00 ^a	1.01±0.03 ^a	0.41±0.01 ^a	1.05±0.01 ^a	0.49±0.09 ^a	0.92±0.14 ^a
Young	0.38±0.03 ^a	1.08±0.09 ^a	0.35±0.03 ^a	1.01±0.12 ^a	0.46±0.10 ^a	0.78±0.14 ^a
Intermediate	0.30±0.04 ^a	1.18±0.10 ^a	0.35±0.03 ^a	1.12±0.13 ^a	0.37±0.03 ^a	0.95±0.10 ^a
Old	0.36±0.04 ^a	1.04±0.03 ^a	0.32±0.05 ^a	1.01±0.12 ^a	0.42±0.10 ^a	1.32±0.18 ^a
Lavalle						
Forest	0.39±0.04 ^a	1.07±0.05 ^a	0.28±0.04 ^a	0.98±0.13 ^a	0.40±0.12 ^a	1.84±0.34 ^a
Young	0.42±0.05 ^a	1.20±0.11 ^a	0.20±0.02 ^a	1.01±0.00 ^a	0.52±0.24 ^a	1.18±0.23 ^a
Intermediate	0.45±0.06 ^a	1.14±0.06 ^a	0.26±0.04 ^a	1.19±0.09 ^a	0.20±0.06 ^a	1.07±0.04 ^a
Old	0.40±0.02 ^a	1.04±0.06 ^a	0.36±0.03 ^a	1.03±0.08 ^a	0.50±0.10 ^a	1.14±0.19 ^a

Total fungal, total bacteria, gram positive bacteria and gram-negative bacteria were all found to significantly positively correlate to soil carbon ($p < 0.05$). These correlations were found both in the 0-10 cm depth, and over all measurements. No correlation was found between soil PLFA types and trace gas emissions.

Statistical analyses of PLFA types and soil chemical characteristics found that over all data, total fungal, total bacteria, gram-positive bacteria and gram-negative bacteria were all significantly positively correlated to both CEC and exchangeable magnesium percentage (Table 14), and negatively correlated to exchangeable calcium percentage. No significant correlations were found

between PLFAs and exchangeable sodium, and only gram-positive bacteria were found to be correlated to pH (Table 14).

Table 14. Generalised linear model outputs of relationship between soil PLFA types (Total Fungi, Total Bacteria, Total Gram-Positive Bacteria and Total Gram-Negative Bacteria) and soil chemical attributes, showing R Sq values and Coefficient of each model. Models were carried out all age classes and sample depths together. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$

PLFA type	pH		Exchangeable Na ⁺		Exchangeable Ca ²⁺		Exchangeable Mg ²⁺		CEC	
	Coeff	R sq	Coeff	R sq	Coeff	R sq	Coeff	R sq	Coeff	R sq
Fungal	-3.5	0.05	0.14	0.02	-0.33	0.15**	0.47	0.13**	0.50	0.18***
Bacteria	-8.44	0.06	0.36	0.03	-0.75	0.14**	0.95	0.10*	1.10	0.15**
Gram+ve	-4.63	0.06*	0.16	0.02	-0.36	0.12**	0.50	0.10*	0.53	0.14**
Gram-ve	-3.77	0.05	0.20	0.04	-0.38	0.15**	0.45	0.10*	0.56	0.17***

4.5 Discussion

Fluxes of all trace gases (CO_2 , CH_4 , and N_2O) were not affected by age class, indicating that there was no effect of land use change on soil fluxes. This is contrasting to a number of previous studies which have all found that the removal of native vegetation for the establishment of pasture have led to increased emission of both CO_2 and CH_4 (Kooch *et al.* 2016; Ribeiro *et al.* 2016; Grover *et al.* 2012). Fluxes from each age class in this study had high variation, for example, ranging from -16.92 – 49.67 in forests and -25.35 – 56.06 in old pastures in Los Puestos, and -20.73 – 33.87 in forests and -11.40 – 35.31 in old pastures in Lavalle. This high variability in fluxes could be due to high spatial variability in the soils of this area (Supplementary Materials Figures 26 – 33) and could be the reason that no significant difference is observed between age classes. Other studies have noted a high variability in the soils of the Cordoba region of Argentina. Site-specific modelling of previously collected experimental data predicted that short term carbon mineralisation over 28 days of incubation is highly variable across the Cordoba region, likely due to differences in site specific soil, climatic and management variables (Koritschoner *et al.* 2022). This result could also indicate that soil CO_2 , CH_4 and N_2O fluxes are driven by other soil or environmental variables. For example, in South African grasslands, Mujonji *et al.* (2020) identified that CO_2 emissions are not influenced by grazing practices (in comparison to livestock exclusion or native vegetation) and are in fact driven predominantly by soil moisture and precipitation patterns.

Soil CO_2 , N_2O , and CH_4 emissions were all significantly affected by soil incubation treatment. Carbon dioxide trace gas emissions were found to be greatest under hot wet conditions, and lowest under cold wet conditions within both sites. Greater CO_2 emissions under hot wet conditions has been widely reported in several studies, with increased temperature and moisture content stimulating an increase in microbial activity and thus organic matter decomposition (Chen *et al.* 2021; Leiros *et al.* 1999; Kirschbaum, 1995). In the dry Chaco in particular, Abril and Bucher (1999) found that soil moisture content and precipitation was the major driver of soil gas fluxes in this ecosystem. However, it should be noted that the “Wet” treatment in this study was at 80% water holding capacity, which could have had some impact on fluxes through causing anoxic conditions. This is corroborated by the greater N_2O fluxes under hot wet conditions under both sites, with N_2O production occurring during anaerobic denitrification processes. The production of N_2O from nitrifying bacteria and some fungi can occur as both aerobic nitrification and anaerobic

denitrification, with the latter being highly dependent on both soil moisture and temperature (Chen *et al.* 2015; Pihlatie *et al.* 2004). Fluxes of nitrous oxide through denitrification have previously been found to peak at 80-90% water-filled pore space, which indicates that in the present study, the wet treatment is around the optimal level for denitrifying bacteria (Chen *et al.* 2015; Pihlatie *et al.* 2004; Klemmedtsson *et al.* 1988). N₂O has also been found to peak at higher temperatures, with Lai *et al.* (2018) measuring a highest rate of emission in incubated soils at 35°C.

The opposite effect of treatment was seen on CH₄ emissions, with the greatest emissions in Lavalle being found under cold dry conditions and the lowest under hot wet. In Los Puestos only cold dry and hot wet treatments were significantly different, with hot wet having lower CH₄ emissions. Furthermore, treatments from both sites had negative emissions within the hot wet treatment, indicating an uptake of methane. Whilst soil methane uptake is influenced by increased soil moisture content (McLain *et al.* 2006), due to increased methane and oxygen diffusion (Maucieri *et al.* 2017; Fest *et al.* 2015; Inubushi *et al.* 2005), studies show that the optimum range of soil moisture for methane uptake is at a medium soil water content, and above and below which it begins to decrease (Maucieri *et al.* 2017; Wu *et al.* 2010; Van den Pol-van Dasselaar *et al.* 1998). As this range is below the water holding capacity used in this study, it is likely moisture does not drive the methane uptake. Indeed, high water contents of soils can increase methane production, due to increasing anaerobic conditions (Van den Pol-van Dasselaar *et al.* 1998; Yavitt *et al.* 1995). It likely that methane uptake is increased in the hot wet treatment of this study due to the high temperatures of the incubations. Oxidation of methane has been shown to increase with temperature (Wu *et al.* 2010; Sjogersten & Wookey, 2002; Grosso *et al.* 2000; Van den Pol-van Dasselaar *et al.* 1998) with higher temperatures increasing gaseous exchange between the atmosphere and the soil profile and increasing enzymatic processes associated with oxidation (Tate, 2015; Shukla *et al.* 2013).

A number of negative CO₂ fluxes were observed in the present study, indicating an uptake of CO₂, but statistical analyses found that these interactions did not follow any identifiable trend, and so were unable to definitively identify what factor could be causing this uptake of CO₂. Negative CO₂ fluxes, whilst uncommon, have been observed in a number of previous studies and have been attributed to abiotic processes involving soil minerals and the soil solution. One proposed explanation for CO₂ uptake into the soil, is through dissolution of CO₂ into the soil solution (Ma *et al.* 2013). This movement of CO₂ into the soil solution is highly dependent on temperature and pH,

as per Henry's Law, and at lower temperatures and more alkaline pH values the dissolution of CO₂ could be expected to increase (Sagi *et al.* 2021; Ma *et al.* 2013; Hamerlynck *et al.* 2013). As the present study observed negative fluxes predominantly in Cold incubation treatments and in Lavalle the volume of CO₂ uptake was greater in Cold Wet than Cold Dry conditions it could be possible that CO₂ uptake through dissolution into soil solution is occurring here. Furthermore, the negative CO₂ fluxes may be visible due to the low temperatures and high alkalinity both reducing biotic respiration, such as in the study by Ma *et al.* (2013), which found CO₂ influx to be predominant in "extreme cases". However, as the statistical analyses carried out on the negative CO₂ fluxes did not yield any clear trend, this cannot be identified as a process which occurs in this study, and further research needs to be undertaken to investigate this further. This could involve the measurement of soil carbonates (CO₃²⁻, HCO₃⁻), which have been identified to be positively correlated to CO₂ influx, due to remove CO₂ from the soil solution through carbonate precipitation which increases the volume of CO₂ which can move into the soil (Sagi *et al.* 2021; Far *et al.* 2016; Hamerlynck *et al.* 2013; Roland *et al.* 2013). In addition, further incubations could be carried out with the measurement of fluxes from sterilised versus unsterilised soils which will remove the contribution of microbial activity to net CO₂ flux (Gao *et al.* 2021; Liu *et al.* 2015; Ma *et al.* 2013).

Soil PLFAs were not significantly impacted by land use change, which is an unexpected result as it would be presumed that microbial community composition would alter due to changes in soil substrate supply (Wu *et al.* 2020; Zhang *et al.* 2016; Klumpp *et al.* 2009). Contrastingly the results have indicated that all soil PLFA types (Fungi, Bacterial, Gram-positive and Gram-negative) have a positive relationship with total carbon concentration likely due to increasing organic matter providing more substrate for the respiration and growth of microbial communities (Ramirez *et al.* 2020; De Graaff *et al.* 2010; Cookson *et al.* 2005; Drenovsky *et al.* 2004). Previous studies in semi-arid ecosystems have found similar results, with soil organic carbon being identified as the key driver of soil microbial biomass in semi-arid and arid grasslands (Rosenzweig *et al.* 2018; Hu *et al.* 2014; Murphy *et al.* 2011). The lack of interaction between land use change and microbial community composition yet positive interaction between soil carbon and PLFAs is surprising, as it would be presumed that a decrease in soil carbon due to land use change would in turn affect soil microbes. These findings could be due to the degradation of some pastures, which saw high levels of shrubby encroachment. In semi-arid climates the presence of shrubs in pastures has been shown to alter microbial community composition and can in some cases cause a shift from a community typical of grasslands to one more like forest vegetation (Lan *et al.* 2021; Li *et al.* 2017; Yannarell *et al.* 2014).

Whilst shrubby encroachment is a sign of degradation and desertification in semi-arid and arid ecosystems (Chandregowda *et al.* 2018; Mureva *et al.* 2018; Cabral *et al.* 2003), it could be the case that in the dry Chaco that the invasion of shrubby species may be partially reversing the impact on microbial communities associated with deforestation.

Soil chemical characteristics had a significant impact on soil PLFAs, with CEC, exchangeable magnesium percentage and exchangeable calcium percentage all significantly affecting fungal, bacterial, gram-positive and gram-negative PLFAS. With regards to CEC, the positive correlation found in the results likely relates to the effect of organic matter availability on PLFA concentrations. Cation exchange capacity of soils is highly dependent on organic matter content, with previous studies finding that CEC increases with OM content due to interactions between carboxyl groups and clay particles (Aprile & Lorandi, 2012; Parfitt *et al.* 1995).

Similarly, soil PLFAs were also positive correlated to exchangeable magnesium percentage. Magnesium is essential for plant growth and plays an important role in many physiological and biochemical processes and has been found to increase soil microbial biomass and alter bacterial community structure (Yang *et al.* 2021; Senbayram *et al.* 2015). Whilst this effect of Mg^{2+} on soil microbial communities is typically observed in acidic soils (where Mg^{2+} is limited), it could be the case that in these soils exchange sites are dominated by sodium and calcium, which will displace Mg^{2+} from exchange sites, rendering it unavailable to plants and microbes (Yang *et al.* 2021; Rengasamy *et al.* 1986). If Mg^{2+} were to dominate exchange sites as plant available magnesium, it would likely result in decreased PLFAs and microbial biomass, due to toxic stress (Yuan *et al.* 2007). Contrastingly, exchangeable calcium was observed to decrease soil PLFAs, which is likely due to the increased salinity which will accompany an increase in Ca^{2+} cations in the soil. The dissolution of $CaCO_3$ in water in the soil profile and subsequent binding of Ca^{2+} to cation exchange sites will result in the release of OH^- ions and increase the pH of the soil water matrix (Brady & Weil, 2008). If the pH rises to a point the soil becomes saline ($pH > 7$), it can become toxic to both plants and soil microbes, thus decreasing microbial biomass in the soil due to toxic stress (Jobbagy *et al.* 2017).

The study found that the sites studied have high salt concentrations within their soils. Particularly in Los Puestos which has a pH of up to 10.5 deeper in the soil profile, the high concentration of both sodium and calcium indicate that the soils at this site are saline-sodic. Lavelle, which has lower pH values and low sodium concentrations, are saline deeper in the soil profile but not in the topsoil. This indicates that there is an accumulation of soluble salts in the soil profiles of these sites, due to

biological activity and chemical weathering (Jobbagy *et al.* 2017). The removal of deep-rooted woody systems in the dry chaco has altered the hydrological balance of the region (Kim *et al.* 2020; Marchesini *et al.* 2017; Jayawickreme *et al.* 2011). Where soils have a deep-water table and deep drainage, the accumulation of salts tends to not be an issue due to sufficient leaching and soil acidification (Jayawickreme *et al.* 2011). However, where land use change has resulted in a negative water balance, due to lower evapotranspiration and shallow rooted systems it results in the movement of the water table upwards (Jobbagy *et al.* 2017; Marchesini *et al.* 2017; Jayawickreme *et al.* 2011). This upward moving wetting front brings with it a high concentration of salts which concentrate and precipitate in the soil profile, affecting plant growth and microbial activity (Jobbagy *et al.* 2017).

Whilst there are no strong effects of salt concentrations on soil GHG fluxes, it could be possible that they are having a greater impact on the soil than is reported in this study. High sodium concentrations are associated with the degradation of soil structure, with the breakdown of soil aggregates leading to a greater release of CO₂ and breakdown of organic matter due to lack of protection from microbial activity (Jobbagy *et al.* 2017; Laurenson *et al.* 2011). Furthermore, high concentrations of salts beyond a toxic level can decrease microbial activity and plant growth through osmotic effects and ion toxicity, which is highlighted by the negative correlation between exchangeable calcium percentage and soil PLFAs (Jobbagy *et al.* 2017). To date, there have been no studies within the dry Chaco which investigate the specific impacts of salts on soil respiration and microbial community composition. This would be an interesting and important avenue for future research in this region, as it is clear that factors driving soil nutrient cycling in these soils are highly complex, and the impacts of soil chemical characteristics on the soil need further investigation.

4.6 Conclusion

In conclusion, the present study shows that land use change from forest to pasture does not increase soil greenhouse gas fluxes or influence microbial community composition in the dry Chaco. The chronosequence design did not show significant changes in the stated variables with forest removal and grazing over time, which is contrary to findings from other studies in semi-arid and subhumid grasslands. The predominant driver of soil GHG fluxes has been found to be climate factors, with *ex situ* soil incubations finding that warmer, wetter conditions favour both CO₂ and N₂O emissions,

whilst also increasing CH₄ oxidation. Soil microbial community composition, whilst not affected by land use change, was significantly impacted by soil chemical variables, and PLFA types increased with increasing soil carbon, cation exchange capacity and exchangeable magnesium, but significantly decreased with exchangeable calcium percentage. This study found that the soils of this region have high pH and electrical conductivity, which is associated with high concentrations of soluble salts in the soil profile (in this case sodium and calcium cations). The sample sites are not saline, saline-sodic or sodic, but they are spatially variable with areas of high salt accumulation within each site. Furthermore, there is high variability between each site which could in part be due to a combination of climate and topographic location (i.e., Los Puestos is low lying). The lack of effect of land use on soil fluxes and microbial community composition is unexpected as it would be expected that with the removal of forest vegetation, the decrease in soil carbon would detrimentally impact microbial populations. This would benefit further investigation, perhaps through *in situ* GHG measurements and further investigation into seasonal climate effects. In addition, whilst no significant effect of soil chemistry was found on soil fluxes, negative fluxes were observed which in previous studies have been identified to occur in soils of similar characteristics to those of this ecosystem and have been found to be directly related to soil chemical characteristics such as alkalinity and salinity. Thus, further investigation into the impacts of salinity and sodicity on soil GHG emissions would be greatly beneficial.

4.7 Supporting information

This study uses a chronosequence design to investigate incubated soil greenhouse gas emissions, soil PLFAs and chemical dynamics over time since deforestation and the establishment of pastures. Part of the sampling design involved taking five destructive soil cores across each plot (either pasture or forest plot), at three sampling depths: 0-10 cm, 10-30 cm and 30-50 cm. Sampling points were equidistant from one another across each plot, and avoided edges of plots, tracks and obvious signs of animal disturbance (i.e. livestock manure). Figures 23 – 26 show the spatial variation in each measured soil chemical characteristic (pH, EC, CEC, exchangeable Ca^{2+} percentage, exchangeable Na^+ percentage, and exchangeable Mg^{2+} percentage) across each age class at each site (Los Puestos and Lavalle). The data plotted does not exclude outliers in order to show one value per spatial replicate.

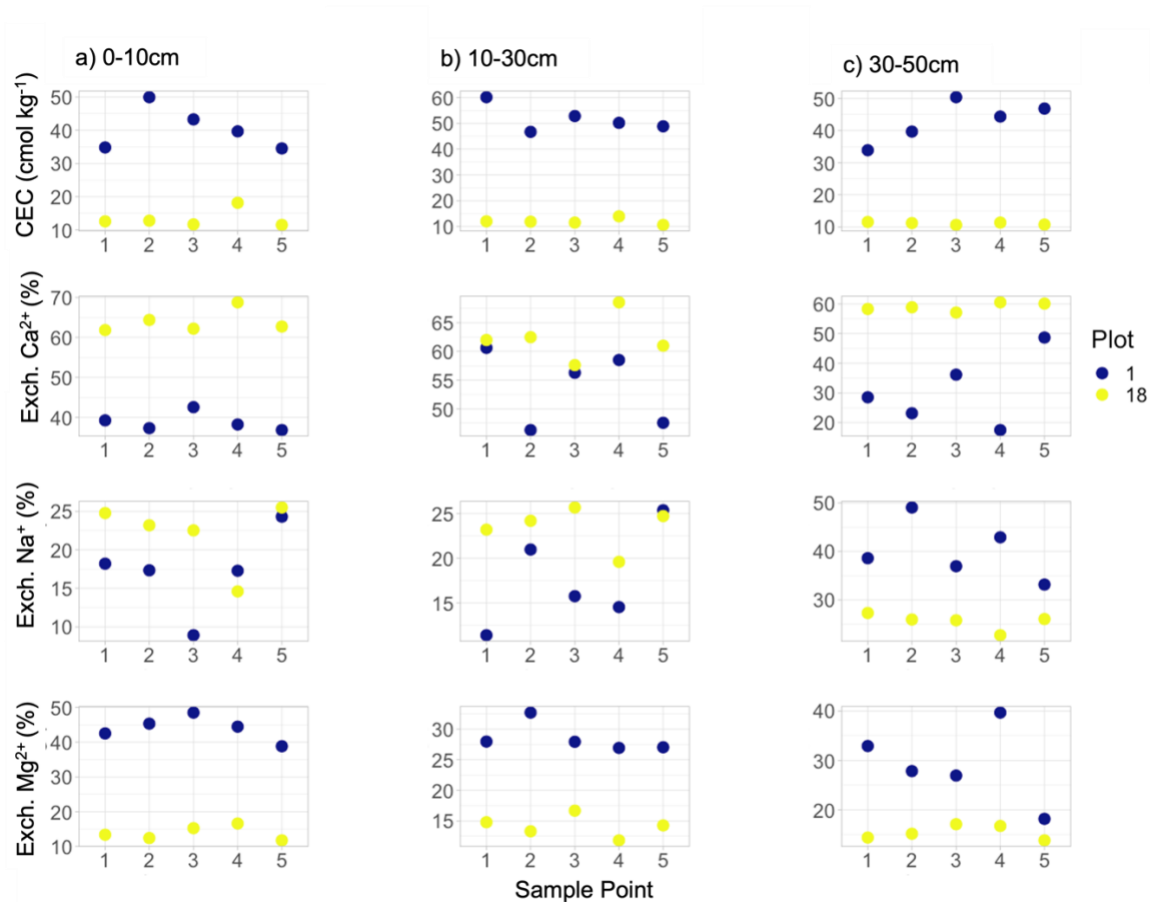


Figure 23 Soil chemical characteristics at each spatial replicate and depths within Forest plots of Los Puestos (1) and Lavalley (18), A) is at 0-10cm, B) is at 10-30cm, and C) is at 30-50cm. Each sample point (1-5) is a spatial replicate of which there is only one measurement. Measured variables are cation exchange capacity (CEC) (cmol kg⁻¹), exchangeable Ca²⁺ percentage (%), exchangeable Na⁺ percentage (%), and exchangeable Mg²⁺ percentage (%).

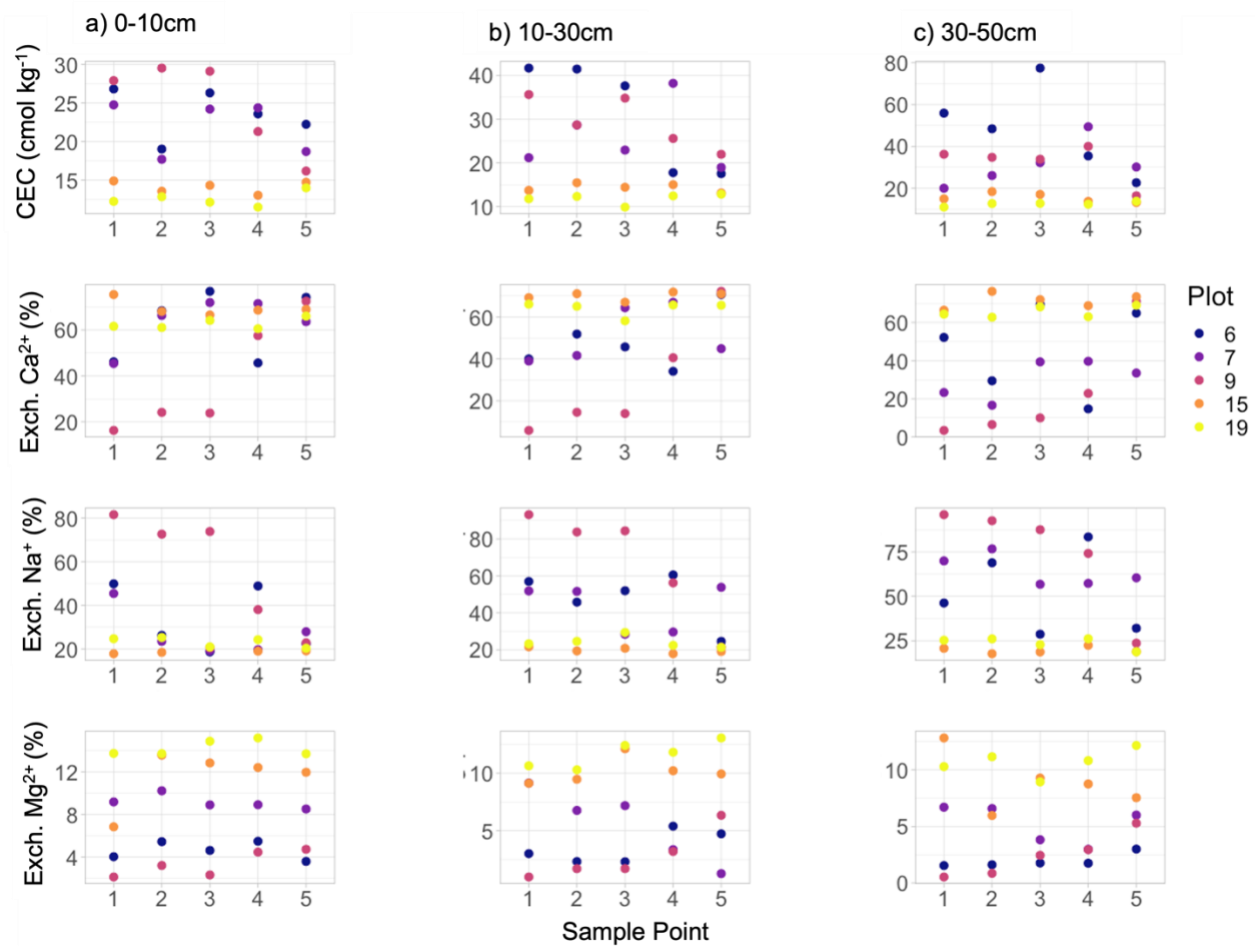


Figure 24 Soil chemical characteristics at each spatial replicate and depths within Young plots of Los Puestos (6, 7 and 9) and Lavalle (15, 19), A) is at 0-10cm, B) is at 10-30cm, and C) is at 30-50cm. Each sample point (1 – 5) is a spatial replicate, of which there is only one measurement. Measured variables are cation exchange capacity (CEC) (cmolkg⁻¹), exchangeable Ca²⁺ percentage (%), exchangeable Na⁺ percentage (%), and exchangeable Mg²⁺ percentage (%).

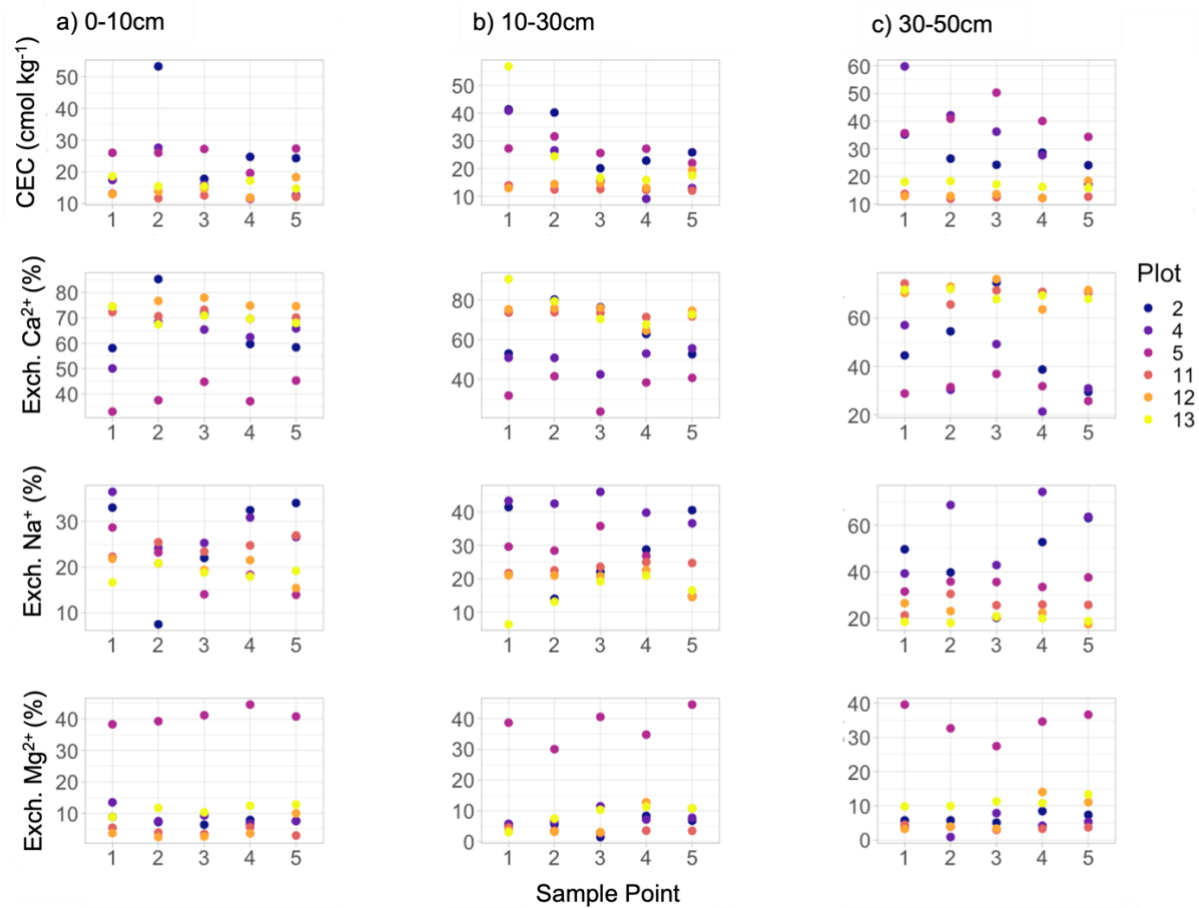


Figure 25 Soil chemical characteristics at each spatial replicate and depths within intermediate plots of Los Puestos (2, 4, 5) and Lavalle (11, 12, 13), A) is at 0-10cm, B) is at 10-30cm, and C) is at 30-50cm. Each sample point (1 – 5) is a spatial replicate, of which there is one measurement. Measured variables are cation exchange capacity (CEC) (cmol kg⁻¹), exchangeable Ca²⁺ percentage (%), exchangeable Na⁺ percentage (%) and exchangeable Mg²⁺ percentage (%).

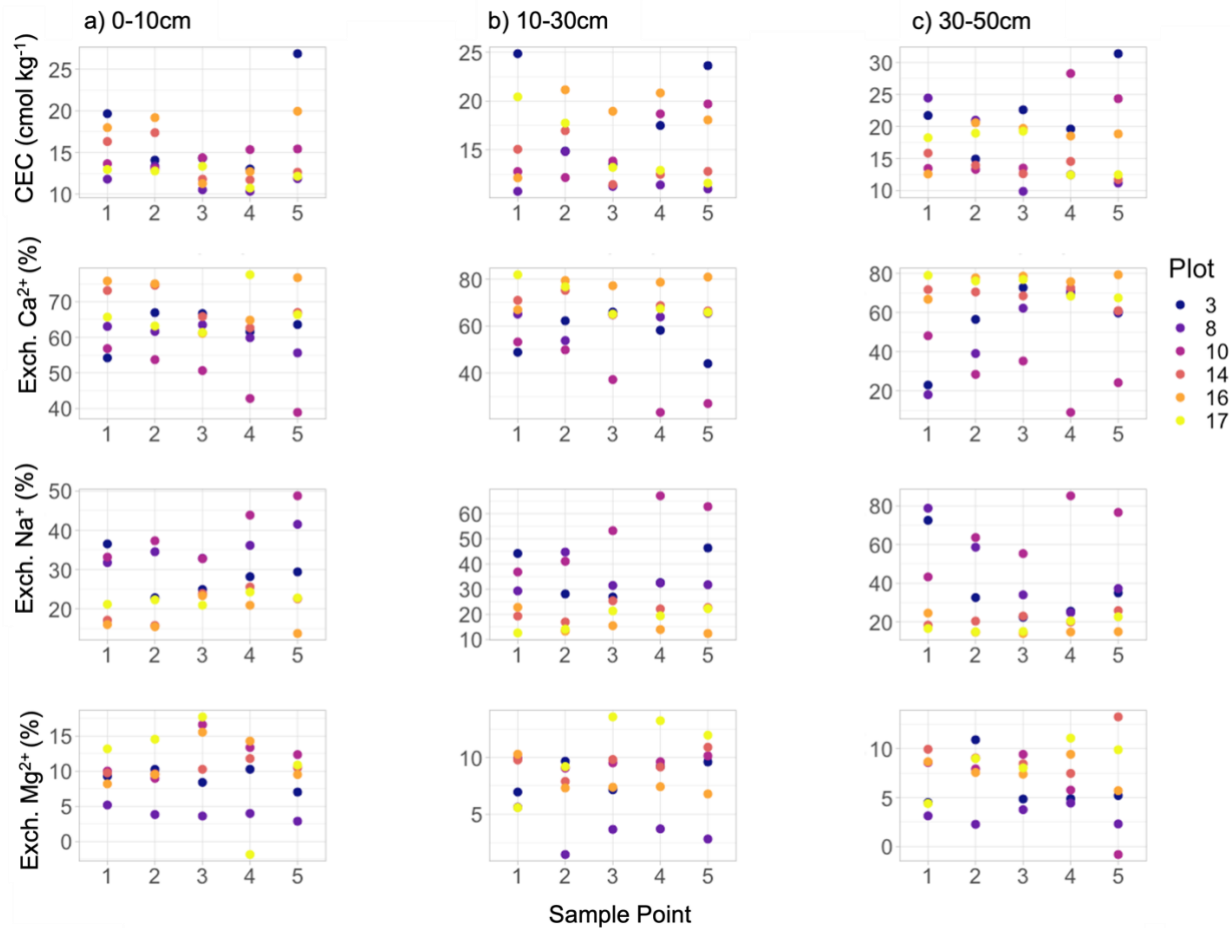


Figure 26 Soil chemical characteristics at each spatial replicate and depths within old plots of Los Puestos (3, 8, 10) and Lavelle (10, 14, 15), A) is at 0-10cm, B) is at 10-30cm, and C) is at 30-50cm. Each sample point (1 – 5) is a spatial replicate, of which there is one measurement. Measured variables are cation exchange capacity (CEC) (cmol kg⁻¹), exchangeable Ca²⁺ percentage (%), exchangeable Na⁺ percentage (%) and exchangeable Mg²⁺ percentage (%).

5 Discussion

5.1 Summary

The thesis has carried out an in-depth study into the land use conversion followed by grazing practices on soil carbon dynamics in both the tropics and subtropics, and then in further detail into the dry Chaco in Argentina. As the largest dry forest ecosystem in South America, yet one of the most understudied forest ecosystems globally (Caldas *et al.* 2015; Bucher & Huszar, 1999), this research shows insight into the complex nutritional, biological and chemical interactions which are present in the soils of this region. This discussion will give a general overview of the key results of this thesis, followed by reflection into the broader implications of the findings including what they mean for the future of livestock production in tropical and subtropical climates. It will go into detail about the limitations of the research presented and unanswered questions which have arisen from the findings of this thesis, and how they could be addressed or improved in future work. Finally, there are the final conclusions to this thesis.

In order to investigate the impacts of both land use conversion followed by grazing on soil carbon stocks, and the key drivers of soil carbon dynamics in tropical and subtropical ecosystems, the following objectives were set:

1. To evaluate and compare through a meta-analysis the effects of grazing on soil organic carbon in subtropical and tropical grazing systems and to identify key biotic and abiotic variables which influence soil carbon dynamics (Chapter 2).
2. To quantify the impact of deforestation and grazing over time since removal of native vegetation on soil properties and identify interactions between soil carbon stocks and other soil chemical and biological characteristics (Chapter 3).
3. To quantify soil greenhouse gas fluxes (CO₂, CH₄, N₂O) in forest and pastures of increasing time since deforestation under moisture and temperature incubation treatments and identify the impact of land use change on key soil biological and chemical properties (Chapter 4).

5.1.1 Chapter 2: Unequal effects of livestock grazing on soil organic carbon in tropical and subtropical grasslands

Chapter two carried out an in-depth meta-analysis which collected data regarding soil characteristics (soil carbon, nitrogen, phosphorus, pH, bulk density, and texture) and grazing and land management variables in tropical and subtropical grasslands. The study compared the impacts of grazing and land management variables on soil carbon and other soil variables between the two climates, aiming to identify the key drivers of soil carbon dynamics in the tropics and subtropics. This research, which addressed Objective 1, observed the following key findings:

- The impacts of grazing on soil carbon stocks were found to differ between the tropics and subtropics, with soil carbon being observed to decrease by 18% under high intensity yet increase by 14% under low intensity grazing in the tropics, whilst in the subtropics soil carbon decreased under all intensities by 18-28%.
- Vegetation was observed to play a role in driving soil carbon dynamics, with pastures in the tropics being found to have higher carbon stocks under pastures with C4 grasses than those with a mixture of C3/C4 grasses.
- There were more studies undertaken in in the tropics (n = 40) than the subtropics (n = 14), highlighting that data in the subtropics is lacking and further work is needed in the subtropics to further understand drivers of soil carbon in this climate.
- Few studies investigated the microbial characteristics of soil (such as microbial biomass or PLFAs), which highlighted an important gap in the literature as microbial communities play a key role in the cycling of nutrients in soil.

5.1.2 Chapter 3: Land use change effects on soil carbon dynamics and soil quality in the dry Chaco, Argentina

Chapter three investigated the impacts of deforestation and pasture age on soil nutrient dynamics, and soil biological and chemical characteristics in the dry Chaco in northwestern Argentina. Through employing a chronosequence design, two sites in the dry Chaco (Lavalle and Los Puestos), were sampled for soils at three depths (0-10 cm, 10-30 cm, and 30-50 cm), from forest plots and pastures of increasing time since deforestation (from 0-5 years up to over 20 years) and analysed for total carbon (C), total nitrogen (N), total phosphorus (P), root biomass, pH, electrical conductivity (EC),

and texture. A mediation path analysis was carried out to investigate the direct and indirect effects of changes in soil biological and chemical characteristics on soil carbon stocks. The research, which addressed Objective 2, found that:

- Soil C stocks were 17% higher in Los Puestos than in Lavalle in the topsoil, and in both sites soil C, N and P stocks were significantly reduced by 16 – 53%, 4-55%, and 6-68% respectively over all sample depths due to deforestation. Only P stocks in Lavalle were impacted by pasture age, with young and old pastures having 36 – 52% higher total P than intermediate pastures and 0-10 cm, and an increase of 46% from intermediate to old at 10-30 cm (Figure 34),
- Root biomass significantly decreased by 68 - 82% with the removal of forest in Los Puestos, yet pasture age was not observed to have a significant impact. In Lavalle, there are significant differences between forest and intermediate pastures in the top 10 cm of soil (-62%), and forest and old pastures at 10-30 cm (48% increase) (Figure 34).
- EC and pH were 93% and 7% respectively, higher in Los Puestos than in Lavalle. In Los Puestos, EC significantly decreased by 28-69% post deforestation, but there was no impact of LUC on pH. In Lavalle, pH and EC were both significantly impacted by land use change, with pH increasing by 8 – 13% in pastures, and EC decreasing in young pastures after deforestation by 47% and were 34% higher in old pastures than young (Figure 34).
- This study also found pH (>8) and EC (> 2 dS/m) values which indicates that the soils of this region are saline and sodic in parts due to the presence of high concentrations of soil cations.
- Mediation path analysis found that in Los Puestos soil carbon is directly driven by changes in nitrogen stocks and pH, and indirectly driven by root biomass, pH and age class.
- In Lavalle, mediation path analysis indicated that soil carbon stocks were impacted directly by root biomass and nitrogen stocks, and indirectly by root biomass, pH, depth and age class.

5.1.3 *Chapter 4: The impacts of climate and land use change on soil greenhouse gas fluxes and microbial community composition in the dry Chaco, Argentina*

The research presented in Chapter 4 quantifies the impacts of imposed climate factors on emissions from incubated soils from the dry Chaco, and the impacts of land use change (LUC) from forest to pasture of increasing time since establishment and soil chemical characteristics on microbial activity and community composition. The work uses the same chronosequence design as stated in Chapter 3 to measure soil trace gas fluxes from intact cores incubated under Hot Wet (35°C, 80% WHC), Hot Dry (35°C, 20% WHC), Cold Wet (10°C, 80% WHC), and Cold Dry (10°C, 20% WHC) conditions. Destructive samples were collected at 0-10, 10-30 and 30-50 cm, and were analysed for soil phospholipid fatty acids (PLFAs), cation exchange capacity (CEC), exchangeable cation concentrations (Ca^{2+} , Mg^{2+} , N^+), pH and electrical conductivity (EC). This study, which addressed Objective 3, found that:

- Soil greenhouse gas emissions were significantly affected by incubation treatment, with highest CO_2 and N_2O emissions under hot wet conditions and lowest emissions under cold wet conditions.
- Over all incubation treatments, age class had no effect on soil fluxes. However, soil greenhouse gas fluxes were impacted by land use change from forest to pasture within incubation treatments. In Los Puestos, under hot wet conditions intermediate soils emitted 28% less CO_2 than forest soils, and under hot dry conditions CO_2 flux decreased 23 – 73% with age (Forest – Young – Intermediate – Old).
- N_2O emissions were observed to be 32% higher in forest than in young soils under hot wet incubations in Los Puestos, and 106% higher in forest than in young soils under cold wet conditions in Lavalle.
- CH_4 was observed to lowest under hot wet, and highest under cold wet and dry conditions over all treatments, whilst under cold wet treatments CH_4 emissions were 20% lower in intermediate pastures than forest soils in Lavalle, and between 9 and 130% higher in pastures than forest under cold dry conditions in Los Puestos.
- Soil exchangeable cations were significantly impacted by land use change, with the removal of forest in Los Puestos significantly reducing CEC which also moved from being dominated by Mg^{2+} cations in forest soils to a dominance of Ca^{2+} and Na^+ in pastures, both of which significantly increase with LUC. In Lavalle, cation exchange sites were dominated by Ca^{2+}

throughout the soil profile and significantly increased with deforestation, whilst CEC significantly increased in the subsoil after LUC (Figure 34).

- Soil PLFAs were positively correlated with exchangeable Mg^{2+} ($p < 0.01$), soil carbon stocks ($p < 0.01$) and CEC ($p < 0.01$), and were negatively correlated with exchangeable Ca^{2+} ($p < 0.01$)

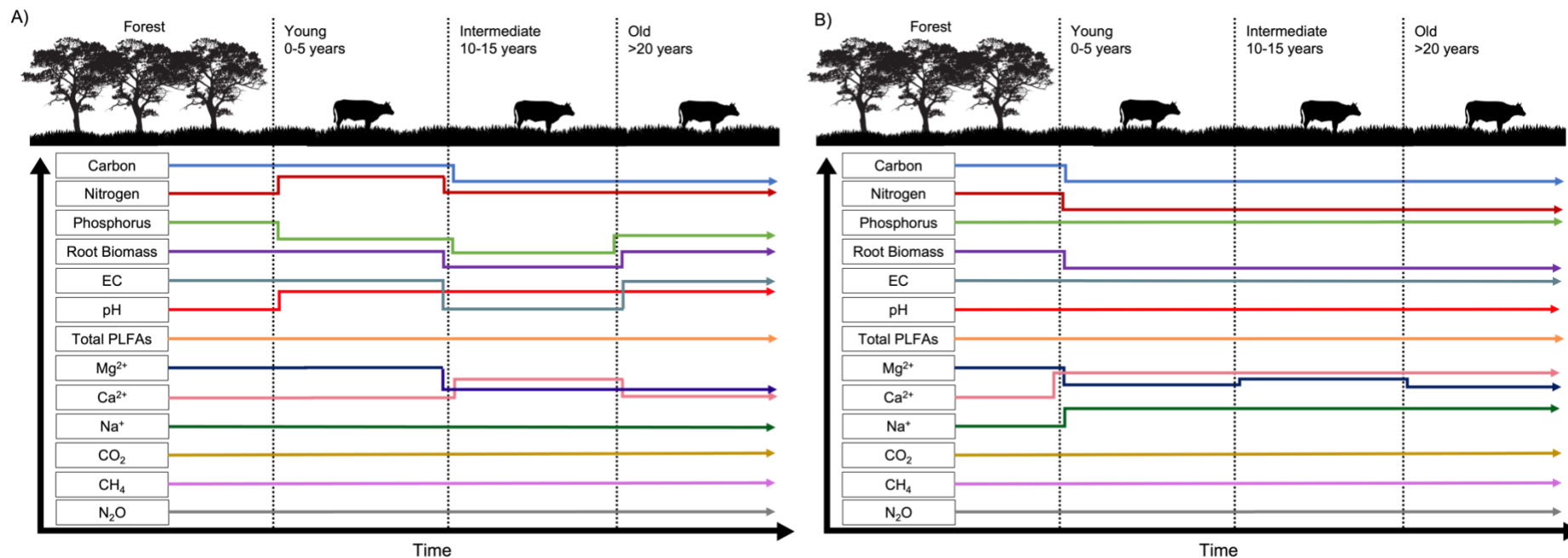


Figure 27 Changes to soil chemical and biological characteristics at 0-10cm with deforestation and time since the establishment of grazing practices in the dry Chaco (Chapters 3 & 4). Represented increases and decreases in soil variables are not to scale and lines are used to demonstrate where changes in each variable occur over time. Left hand side shows changes in Lavalle, right hand side shows changes in Los Puestos.

Discussion of key findings

Impacts of land management on soil carbon cycling

This thesis used a combination of field, laboratory and meta-analyses to explore the relationship between land use change and soil carbon and other soil characteristics. Focusing solely on the tropics and subtropics the research directly compared the responses of soil carbon to land management practices and land use change between the two climate zones (Chapter 2). The analysis collated existing data from a total of 54 studies and carried out statistical analyses on soil carbon stock changes transformed to response ratios. The key findings of this research were that low intensity grazing increased soil carbon stocks in the tropics by 14% whilst high intensity grazing corresponded to a decrease of 18% in soil carbon. Contrastingly in the subtropics grazing of all intensities was observed to decrease carbon stocks. The latter of these findings is further demonstrated in Chapter 3 which used a chronosequence design to demonstrate that soil carbon stocks decreased following land use change and with grazing over time by 16 - 21% in the subtropical dry Chaco. In the context of wider research, grazing impacts in the tropics have tended to be mixed, with both increases in soil carbon (Navarette *et al.* 2016; Moguees *et al.* 2013; San Jose *et al.* 2003) and decreases (Schulz *et al.* 2016, Trilleras *et al.* 2015; Northup *et al.* 1999) being observed. Over all treatments and depths, the soil carbon response ratio in Chapter 2 was -0.04, which corresponds to a slight decrease in soil carbon of 4%. Thus, it could be concluded from this that whilst there are grazing management practices which can increase soil carbon they are either underutilized or do not exceed the losses from grazing and the overall impact of grazing in the tropics is a loss in soil carbon stocks.

In all three chapters vegetation management and change was considered as a driving factor for soil carbon stock changes and other soil processes. Chapter 2 investigated the impacts of photosynthesis pathway, previous native vegetation and pasture vegetation composition on soil C, whilst Chapters 3 and 4 examined the removal of native vegetation and its impacts on soil C, and the effects of pasture age on root biomass. Land use change from forest to pasture caused a decrease in soil carbon stocks in the subtropics (Chapter 3) of 16-23%, across all depths. This corresponds to the findings of the meta-analysis in Chapter 2, which observed decreases soil carbon in grazed pastures converted from forest lands in both the subtropics and tropics. Deforestation and the removal of native vegetation would be expected to reduce soil carbon stocks due to the removal of belowground plant biomass, the alteration of soil microbial communities, decreased

quality and quantity of soil inputs, changes to hydrological cycles and decreased soil structure, all of which contribute to the sequestration of carbon belowground (Wienkenkamp *et al.* 2020; Borrelli *et al.* 2017; Crowther *et al.* 2014).

The vegetation composition of pastures was reflected in the soil carbon changes with grazing in Chapter 2. In both the tropics and subtropics pastures with C4 vegetation showed an increase in soil carbon of 0.6% - 20% with grazing whereas those with mixed (C3&C4) and C3 vegetation demonstrated decreases of 13-15%. The presence of C3 vegetation in C4 pastures is likely representative of shrubby encroachment into grazing lands, which is a sign of degradation. Encroaching shrubs into managed pastures can compete with improved grasses for soil nutrients, in turn reducing the productivity of pasture vegetation (Turpin-Jelfs *et al.* 2019; Sankey *et al.* 2012; Rivest *et al.* 2011). In the sites selected in Chapters 3 and 4, it was noted (section 3.3.1, Chapter 3), that the level of degradation varied between sites, with intermediate pastures of Lavalle displaying moderate shrubby encroachment and the young and intermediate pastures of Los Puestos displaying high encroachment. However, analyses were not conducted to investigate the impact of encroachment on soils and further research would benefit from this in these sites.

Impacts of land use change and grazing on biogeochemical cycles

In this thesis Chapters 2, 3 and 4 evaluated whether land use change and grazing impacted soil biogeochemical cycling, and the interactions between soil characteristics. Soil Nitrogen and Phosphorus both significantly decreased immediately post-deforestation in the Chaco, and were observed to correlate to soil carbon stocks at 0-50cm. In Lavalle, soil C:N:P stoichiometry decoupled in the subsoil (10-30cm and 30-50cm), with increases in N and P stocks in old pastures and decreases in soil C. Similarly in Chapter 2 increasing phosphorus decreased the carbon response ratio in the tropics, and thus soil carbon stocks. It would be expected that soil C, N and P would maintain close linkages throughout the soil profile due to the dependence of plant growth and subsequent soil carbon inputs to soil on nitrogen and phosphorus availability (Zhang *et al.* 2014; Barret & Burke, 2000). However, there are instances in which the decoupling of nutrient cycles can occur. In the context of the dry Chaco, this could be occurring either due to the climatic conditions of the region or salinity of the study area as identified in Chapter 4. In both Chapters 3 and 4 we identified that in Lavalle and Los Puestos, soils had high concentrations of soluble cations. Particularly in Lavalle, cation exchange sites were dominated by Ca²⁺ ions, which

increased post-deforestation. Soil nutrients can form complexes with cations in the soil solution to form salts such as Calcium Phosphate or Calcium Nitrate which are less available for plant uptake and can lead to the accumulation of nutrients in the soil (Hopkins & Ellsworth, 2005; Johri *et al.* 1999). Alternatively, in areas of low rainfall, climate factors can affect nutrient interactions with increasing aridity in turn leading to a decoupling of C:N:P stoichiometry (Jiao *et al.* 2016). Increased pH, decreased precipitation and warm temperature associated with this region can all lead to changes in nutrient ratios, and in particular decreased rainfall can limit microbial activity which will inevitably restrict nutrient cycling (Dalfado-Baquerizo *et al.* 2018; Jiao *et al.* 2016).

The meta-analysis in Chapter 2 identified that there was limited evidence in the tropics and subtropics on the impacts of LUC and grazing on soil microbial communities. Investigating this further in Chapter 4 found that whilst land use change did not have any impact on total PLFAs, soil microbial communities decreased with increasing exchangeable Ca^{2+} in the soil and increased with increasing Mg^{2+} . This finding could be indicative of the impacts of increased pH and EC with land use change, which was observed in Chapter 3, on soil microbial communities. In Lavalle, pH increased following deforestation by 8-13% and whilst there was no difference between forest and pasture in Los Puestos, pH values were on average 7% higher than in Lavalle. Such high pH values suggest high concentrations of soluble cations such as Ca^{2+} and Na^+ in the soil profile, which in turn can become toxic to both plant and microbial populations (Jobbagy *et al.* 2017). Salinity and sodicity beyond the threshold of which microbes can persist, can result in a build up of organic matter in the subsoil due to limited microbial activity and in turn limit nutrient cycling (Jobbagy *et al.* 2017).

The effects of climate variability on soil functioning

The impacts of climate on soil functioning were key to the research questions which underpinned this thesis, with Chapter 2 comparing soil carbon responses to LUC and grazing in the subtropics and tropics, and Chapter 4 investigating the impacts of climate manipulations on soil GHG emissions. The loss of soil carbon in the subtropics could be due to the dominance of studies in semi-arid and arid precipitation zones ($n = 47$) in Chapter 2, and the semi arid climate of the dry Chaco. The incubation experiment in Chapter 4 found that emissions of CO_2 at 80% water holding capacity, and lowest at 10C and 20% WHC. This is to be expected, with warm and moist climate conditions are identified to be prime conditions for microbial respiration, with increased

temperature and precipitation being widely reported as the key driver for microbial activity across all climate zones (Liu *et al.* 2016; Butenschoen *et al.* 2011). Taking this into account, it is likely the impact of hot wet incubation conditions on CO₂ emissions indicate that carbon losses from semi-arid and arid soils are not due to microbial breakdown of organic matter. Chapter 2 identified that soil carbon increased in areas with humid and subhumid precipitation patterns, and whilst these regions have high levels of microbial respiration, the climate conditions also promote carbon sequestration with increased vegetation productivity and plant inputs into soil and increased fungal activity which metabolises organic matter into more stable forms belowground (Hayakawa *et al.* 2014; Raich *et al.* 2006). Contrastingly, regions with low rainfall are not conducive to carbon sequestration, with systems in arid and semi-arid regions becoming increasingly water limited and subsequently having decreased vegetation and plant inputs (Li *et al.* 2018). Furthermore increasing aridity promotes conditions for soil carbon loss, such as reduced abundance of fungal bacteria, increased vulnerability to erosion and decreased aggregate stability (Li *et al.* 2018; Sawada *et al.* 2016; Heisler-White *et al.* 2008).

5.2 Broader implications of the findings

5.2.1 Land use change and grazing in the tropics and subtropics

In both the tropics and subtropics, increased global demand for livestock products has led to the widespread removal of native vegetation and establishment of both extensive and intensive grazing systems (Erb *et al.* 2018; Godde *et al.* 2018; Asner *et al.* 2004). Such scales of land use change have detrimentally impacted soils, with increased soil greenhouse gas emissions, decreased soil carbon sequestration rates and soil degradation (Pulido *et al.* 2018; Smith *et al.* 2016; Lal, 2015). In the tropics, management practices have been identified which can promote carbon sequestration in grazing systems, such as low intensity grazing (Chapter 2), whereas in the subtropics, soil carbon stocks consistently decrease with land use change and grazing, regardless of grazing practice (Chapter 2 & 3). However, Chapter 2 of this thesis demonstrates that there is a lack of research surrounding practices in the subtropics which can mitigate against soil degradation. The higher proportion of research in the tropics is likely due to heavy deforestation for grazing establishment in regions such as the Amazon which are high profile and receive substantial public and scientific attention (Dos Santos *et al.* 2015), yet ecosystems like the Chaco in Argentina, or the Caatinga in

Brazil are relatively unknown so have been less researched. Given that these ecosystems have been subjected to high rates of deforestation over the last decades (de Espindola *et al.* 2021; ²Baumann *et al.* 2017), further research, such as that presented in this thesis is necessary to fully understand the impacts of grazing systems on soil health in the subtropics.

In the tropics soil carbon can be sequestered where low extensive grazing (e.g. 0.8 – 1.0 heads of forage-fed livestock per hectare) is used, as demonstrated in Chapter 2. However extensive grazing requires large amounts of space, which likely will lead to further deforestation where demand for livestock products increases (Pereira *et al.* 2020). Attention should also be paid to the land use change which occurs besides that of forest to pasture. Increasing the production of livestock and establishment of pastures brings with it a need for improved infrastructure, such as establishment of roads for transport of agricultural equipment or products, which will ultimately result in further land use change (Jusys, 2016; Laurance *et al.* 2014). This thesis also demonstrates that in the subtropics, grazing practices result in the loss of carbon and other nutrients from soils, even at low grazing intensities (Chapters 2 and 3). With an increasing population and subsequently potential future demand of livestock, one alternative to prevent further deforestation and mitigate against subsequent greenhouse gas emissions would be the intensification of current systems, i.e. producing more per given area of land (Cohn *et al.* 2014; Burney *et al.* 2010). This can range from increasing stocking rates of livestock in an area (Grau & Aide, 2008), to establishing integrated cop-livestock systems, where livestock are in rotation with annual crops (Alves *et al.* 2017). Such practices can be beneficial when managed appropriately, with increasing stocking rates resulting in avoided deforestation, and integrated crop-livestock systems improving soil quality (Carvalho *et al.* 2018; Moraes *et al.* 2014). However, it should also be noted agricultural intensification can also be harmful to mitigation efforts, with the potential for intensification to degrade soils further and increase GHG emissions, and it is important to consider the trade offs between agricultural production and environmental impacts when managing these systems (Carvalho *et al.* 2018; Bonaudo *et al.* 2014)

5.2.2 Impacts of land use change and grazing in the dry Chaco and wider context

Following forest clearance and establishment of pastures, soil nutrients decrease in the dry Chaco (Chapter 3). The soils of semi-arid ecosystems are typically low in nutrients, due to low organic matter content and water availability which limits plants growth (Hag Husein *et al.* 2021), and the decrease of soil nutrients following land use change may further exacerbate this by leading to decreased productivity of pasture grasses, resulting in increased bare soil, poor soil structure, and further GHG emissions from soils (Lal, 2015). This is further reflected in Chapter 3, which demonstrates a 62 - 82% decrease in root biomass along the land use gradient. Despite this, soil GHG fluxes and microbial communities are not strongly impacted by land use change, indicating that whilst carbon inputs decrease organic matter breakdown continues, further limiting nutrient availability (Chapter 4). The loss of nutrients from soils in this region will ultimately decrease agricultural productivity due to inadequate nutrition for livestock, which may in turn lead to further deforestation to establish new, more productive pastures, and the extent of degraded soils will grow.

It is likely that seasonality plays an important role in the biogeochemical cycling in subtropical soils. Warm and moist summers will increase soil respiration rates, increasing CO₂ and N₂O emissions from soils whereas dry cold winters will decrease microbial activity and organic matter decomposition (Chapter 4). Furthermore, the higher temperatures and precipitation during summer months will promote the sequestration of carbon, as shown in positive interactions between soil carbon and mean annual precipitation and temperature in Chapter 2, due to increased plant growth and in turn carbon inputs to the soil (Del Grosso *et al.* 2008). The role of seasonality is highlighted in the dry Chaco, in the waterlogging of pastures in Los Puestos site, which is on the border of the subhumid climate zone and which occurs with heavy rain in summer months. Such stagnation of the landscape is likely to exacerbate the seasonal differences in soil functioning, with waterlogging promoting anaerobic conditions and subsequent CH₄ emissions, and restricting vegetation growth further (Welch *et al.* 2019).

In the Tucuman and Catamarca provinces, Argentina, mean monthly temperature is predicted to increase year-round by a minimum of 1.1°C by 2050 (under the IPCC “middle of the road” SSP2-4.5 Scenario), and precipitation will increase in summer months and decrease in winter months in Tucuman, whilst in Catamarca winters will become wetter, and summers drier (World Bank, 2021).

Such projected changes to climate will undoubtedly impact ecosystem and soil functioning, with wetter, warmer winters in Catamarca increasing net primary productivity and carbon inputs (Liu *et al.* 2020; Wang *et al.* 2011; Del Grosso *et al.* 2008), and soil respiration during these months (Chapter 4), whilst in the summer plant growth may decrease due to decreased water availability which will in turn decrease carbon inputs to the soil (Lei *et al.* 2020). In Los Puestos, warmer and wetter summers will likely lead to increased periods of waterlogging in pastures which will exacerbate further problems associated with sodicity and salinity, and warmer drier winters will result in increased organic matter decomposition, greater soil GHG emissions as was shown in Chapter 4, and low net primary productivity due to low water availability (Lei *et al.* 2020; Del Grosso *et al.* 2008).

Forests typically have deep rooted vegetation which maintain deep drainage of water in the soil and once removed this alters the hydrological balance of an area (Jobbagy *et al.* 2017; Jayawickreme *et al.* 2011), which in can result in increased pH and EC due to increased concentrations of soluble salts (Chapter 4) (Osman, 2018). Soil salinity and sodicity is a growing problem, with 73.3 Mha of grasslands estimated to be salt-affected (Hassani *et al.* 2020). Saline soils which are characterized by excess accumulation of soluble salts in the root zone, can cause decreased vegetation growth and microbial biomass as shown in the decrease in microbial PLFAs with increasing exchangeable Ca²⁺ in chapter 4. Sodicity on the other hand, which is caused by excess concentrations of sodium cations in relation to other exchangeable cations, can lead to dispersion of soil particles and disruptions of aggregates, decreases in soil infiltration capacity, and increases in wind and water erosion (Hassani *et al.* 2020; van de Craats *et al.* 2019). The accumulation of salts can occur both naturally through weather events such as El Nino and La Nina, and prolonged periods of drought, and through anthropogenic processes such as native vegetation clearance, which causes groundwater tables to rise and waterlogging of soils (Hassani *et al.* 2020; Ahmad *et al.* 2012), as was observed in this research (Figure 18). Often referred to as secondary dryland salinity and sodicity, the accumulation of salts as a result of human-induced change (excluding irrigation) is widespread, and impacts vast areas of Australia, USA, India, South Africa, Thailand and Turkey (Pannell & Ewing, 2006).



Figure 28 Soil sample from 10-30cm in Los Puestos. Water tables had risen to ~10cm depth and soil samples were saturated.

At present pastures are able to remain productive in the Chaco, despite high concentrations of sodium and calcium cations, in part mitigated through the establishment of salt tolerant grasses (*Chloris gayana*) (Taleisnik *et al.* 2021). This is demonstrated in this research, with measurements of root biomass at 30-50 cm (Chapter 3), indicating continued growth where conditions are saline (ESP < 15, EC > 2), saline- sodic (ESP > 15 , EC > 2), or sodic (ESP > 15 , EC < 4) (Chapter 3), and the persistence of microorganisms and microbial activity throughout the soil profile, albeit at low concentrations (Chapter 4). Whilst this raises questions of how pastures can withstand such conditions, it also begs the question of the implications of continued increases in salinity and sodicity. Climate change has the potential to significantly increase both the severity and extent of soil salinity and sodicity, with increased frequency of extreme weather conditions such as excessive

rainfall following periods of drought, increased temperature and evaporation rate creating conditions for salinization, as observed in Los Puestos (Mukhopadhyay *et al.* 2021). On top of this, further human-induced change, such as continued deforestation will expand areas with groundwater recharge and stagnation, and in turn expand areas of salt-affected soils, and ultimately increase soil degradation and desertification, particularly in semi-arid and arid regions (Jobbagy *et al.* 2017; Gimenez *et al.* 2016).

5.3 Limitations to the research

Whilst this thesis has displayed valuable findings, there are areas in which the research could have been improved and the present findings have a degree of limitation. The samples collected for analysis in both chapters 3 and 4, were collected over a three month period in Argentina, and the transported back the UK for analyses in the laboratory. This meant that some samples had to be shipped fresh (intact cores and samples for PLFA analysis). Ideally, these samples would have been freeze dried prior to shipping, or analysed in country, however limitations upon equipment and time meant that there was no other option than to ship the samples by plane to the UK. This process may have impacted the soils, with a long period of time between leaving Argentina and arriving to the laboratory in the UK, and temperature changes during shipment. Despite trying to mitigate these risks as much as possible, by securing express shipping, and storing the soil samples in large insulated cool boxes, it was impossible to completely avoid and there may have been some impact on soil microbial communities and organic matter.

Due to heavy deforestation in the dry Chaco, untouched forest areas are sparse and hard to find in this region, particularly in the provinces of the sampling region (Tucuman, Catamarca and Santiago del Estero). It is worth noting that part of the forest sampled at Lavalle was removed between the two sampling periods (first for Chapter 3 and second for Chapter 4), which was merely a few weeks. As a result of this, it was only possible to sample one area of forest per farm. Of course, the best-case scenario would have been to sample 3 separate forest plots per site. In order to ensure that the results could be analysed statistically, each forest sample from each farm was pseudo-replicated in the laboratory 3 times for each of the chemical, biological and physical analyses. Employing this strategy could have led to a less representative view of forests in the area, particularly noting the clear spatial variability that exists in this ecosystem. Being able to accurately represent how soil

chemical and biological characteristics vary over space in the forest sites could help to give more insight into soil carbon dynamics under native vegetation in the dry Chaco.

Chapter 4 gives insight into the impacts of climate factors on soil greenhouse gas emissions, displaying the impacts of both high and low temperatures and high and low moisture contents on CO₂, CH₄, and N₂O emissions from the top 10 cm of soil. Whilst the results have presented interesting findings, they present the impacts of two extreme climate scenarios on the soil. By imposing 80% and 20% of water holding capacity the soils have been subjected to both drought and near saturation. These treatments were selected to represent the impacts of increased and decreased rainfall, and due to both time and equipment limitations no other treatments were imposed. This study would have benefitted with the use of further WHC levels, such as a medium moisture content of 55%, which has been demonstrated in previous studies to be the optimum moisture content for microbial activity (Howard & Howard, 1993).

Similarly, due to time constraints, samples for PLFA analysis were bulked, with spatial replicates from each depth of each plot being bulked and extracted, resulting in one data point per depth per site. It is clear from the results of other analyses (such as exchangeable Na⁺, EC, soil trace gas fluxes and exchangeable Ca²⁺), that the soils studied are highly spatially variable (Supporting Information, Chapter 3 and 4), with samples from individual plots having large ranges of values. By bulking the samples, it is possible that the PLFA analyses has missed the opportunity to observe how concentrations of PLFAs and microbial community composition varies within plot, rather than just between plots. Furthermore, by analyzing spatial samples, this may have given more insight into the relationships between soil chemical characteristics and the occurrence of microbial PLFA types. The present research did not find any interactions between PLFA ratios (fungi:bacteria, and gram positive:gram negative) and soil nutrients and other chemical characteristics. It is possible that significant relationships between these variables were missed due to the bulking of samples. To strengthen these results and the statistical analyses and the further investigate microbial community dynamics in these soils, carrying out a more in-depth investigation of spatial variability with more spatial replicates per plot, would add to the study of the impacts of deforestation and grazing over time on dry Chaco soils.

5.4 Recommendations for further research

The presented research has set out an initial understanding of the drivers of soil carbon dynamics and other soil variables in the extensive grazing systems of the dry Chaco, Argentina. However, it is clear from the results that the soils of this region are highly complex, with a high degree of variability in chemical and biological characteristics and with a great deal of interacting factors which impact soil carbon cycling. This thesis has highlighted the need for further research into the functioning of these soils, and has identified the following specific questions which need to be answered in order to further our understanding of the soils of this ecosystem:

1. What is the mechanism is causing the apparent uptake of carbon dioxide in these soils?
2. What are the impacts of high pH, sodicity and salinity on soil functioning?
3. How does the pronounced seasonality of the Chaco impact soil carbon dynamics, and interactions between soil variables?
4. What is the driving factor for the high spatial variation of soil characteristics in this ecosystem, and how does it impact soil carbon dynamics overall?

5.4.1 What is the mechanism driving the apparent uptake of carbon dioxide, causing negative emissions?

A soil incubation experiment which monitored soil CO₂ emissions observed negative fluxes, suggesting an uptake of CO₂ into the soil, and as these fluxes were greater than the limits of a minimum detectable flux, they could be due to a chemical process. However, the study presented in Chapter 4 could not identify any significant cause of these negative fluxes, thus further investigation would be greatly beneficial. A valuable first step in investigating the negative fluxes further would be in the measurement of carbonates in the soil. Furthermore, as the uptake of CO₂ into soil is likely a chemical process and not microbial activity, these negative fluxes could be further investigated with a comparison of fluxes between fresh and sterilised soil cores. This would work under the assumption that sterilisation through autoclaving with pressurized steam will remove microbial biomass and thus activity from the samples (Gao *et al.* 2021; Liu *et al.* 2015; Ma *et al.* 2013), allowing to see a) whether negative fluxes are occurring in these soils and b) the magnitude of these fluxes.

5.4.2 What are the impacts of high pH, sodicity and salinity on soil functioning?

Chapters 4 and 5 discovered that the soils of this region have high concentrations of soluble cations (Na^+ , Ca^{2+} and Mg^{2+}), which can indicate the presence of saline, sodic or saline-sodic soils. The presence of such high concentrations of these cations can cause detrimental impacts on soil functioning and have been recognized to cause severe issues in many regions globally. Sodium ions in the soil are known to disrupt soil structure, through dispersion, which forces soil particles apart and can lead to an increase in erosion and soil loss (Hassani *et al.* 2020). Further work would benefit from the measurement of aggregate stability which would help to identify the scale of impact high sodium content is having on these soils.

The PLFA data collected in chapter four demonstrated that soil microbes are able to withstand such high pH, EC and cation concentrations in the soil. Further work here could involve seeking further details about microbes present, more in depth study with DNA analyses and enzyme analysis in order to identify specific species, and characteristics which may enable the persistence of microbial communities in saline and sodic conditions.

5.4.3 How does pronounced seasonality of the dry Chaco impact soil carbon dynamics, and interactions between soil variables?

The results of Chapter 5 of this thesis showed that soil trace gas emissions are impacted by changes in soil moisture and temperature, which leads to the question of the impact of seasonality on the soils of the Chaco. The Chaco is in the subtropical climate type, so had pronounced seasons, with heavy rainfall and high temperatures in the summer, and low rainfall and temperatures in the winter, which is shown to have an impact on soil GHG emissions in Chapter 4. As this thesis investigated soil GHG emissions *ex situ*, from incubated cores, further investigation could benefit from *in situ* GHG gas measurements using a portable Infrared Gas Analyser (IRGA) during both summer and winter months. This would also allow the observation of soil microbial activity under the waterlogging and stagnation of the landscape in Los Puestos in the summer months which does not occur in the winter when there is less rainfall. Due to the waterlogging of Los Puestos during periods of intense precipitation, it may also be valuable to investigate the seasonal differences in soil chemical characteristics, such as exchangeable cations, pH, EC and soil nutrients. It may be the

case that these characteristics vary between summer and winter due to increased immobilization of soluble salts in the soil profile in summer during frequent waterlogging and drying of the soil profile when temperatures are high.

5.4.4 What is the driving factor for the high spatial variation of soil characteristics in this ecosystem, and how does it impact soil carbon dynamics overall?

Previous studies in the Chaco have recognized that the soils of this regions are highly spatially variable (Koritschner *et al.* 2022), which was further proven in the results of this thesis (Supporting information, Chapter 3 and Chapter 4), which observed high variation between sites and within sampling plots. Whilst this research showed that soil chemical characteristics are spatially variable, the sampling design did not involve extensive sampling of individual plots, with five spatial replicates being taken across each. Future work could involve undertaking an in-depth spatial study taking many samples across a few sites in order to gain a more detail spatial picture of soil carbon dynamics. This could involve the construction of a digital elevation model, or another spatial modelling technique, to visually display soil chemical characteristics post deforestation.

5.5 Conclusions

This research has investigated the impacts of deforestation followed by grazing practices on soil carbon dynamics in the tropical and subtropical climates. Increases in soil carbon under low intensity grazing in the tropics indicates the mitigation of greenhouse gas emissions from land use change, however, the widespread deforestation associated with extensive grazing systems outweighs these benefits and may lead to further GHG emissions. Furthermore, with an increasing population, demand for livestock products will also increase, which will inevitably lead to further deforestation. Mitigation options for this potentially devastating land use change could include intensification through integrated-crop livestock systems or increased stocking rates, but such practices must be managed appropriately in order to avoid further deforestation. The results of this thesis have demonstrated that in subtropical ecosystems, the removal of forest and establishment of grazing systems decreases soil carbon and other nutrients, and that more research is needed in order to establish management practices which can restore soil quality.

In the dry Chaco, Argentina, the removal of forest to make way for grazing systems has detrimentally impacted soil health, with decreased nutrient availability, reduction in plant growth (decreased root biomass), and increased salinity and sodicity. Contrastingly, soil greenhouse gas fluxes (CO₂, CH₄ and N₂O), and soil microbial communities were not impacted by land use change, but by other factors such as temperature, moisture and soil chemical characteristics. The degradation of soils in the dry Chaco, has not yet obviously impacted agricultural productivity, and livestock production can continue. However, with projected changes to the climate under IPCC scenarios, it is likely that such degradation will intensify in the coming decades. Salinity, and sodicity, which are a growing problem worldwide, will likely increase with increasing temperatures and rainfall and in turn soils will continue to experience the detrimental impacts. In addition, changes to precipitation and seasonal temperatures could directly impact vegetation growth and soil biological activity, reducing carbon inputs and in turn sequestration. With increased degradation, it is inevitable that agricultural productivity will be impacted in this ecosystem unless mitigation strategies are put in place. Improved land management strategies have the potential to mitigate both against the degradation of soils, and by increasing carbon sequestration, reduce the emission of greenhouse gases and mitigate against climate change. However, it is imperative that the carbon dynamics of these soils are completely understood and further investment in research is necessary in this subtropical region.

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