Fire and herbivory effects on soil organic carbon stocks and soil greenhouse gas emissions from South African grasslands and savannas: Implications for global change.

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Declaration

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Abstract

Understanding drivers of soil organic C (SOC) stocks and soil greenhouse gas emissions in grassland and savanna ecosystems is critical for climate change mitigation considering that soils are large C reservoirs while functioning as sinks and sources of the principal soil greenhouse gases, carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). We however, have limited understanding of how wildlife herbivory, fire and pyric-herbivory influence SOC stocks and soil greenhouse gas emissions in these ecosystems. We hypothesised that, in low nutrient soils, wild herbivores would shift vegetation towards low quality (high C:N) foliage and litter that are resistant to microbial decomposition. This may contribute to the stable SOC pool, while reducing fluxes of all soil greenhouse gases. By contrast, fire was expected to reduce soil N pools by volatilisation, but to increase the stable SOC pool by addition of mostly recalcitrant pyrogenic organic C, thereby lowering CO_2 and N_2O fluxes. CH_4 fluxes would be increased by the fire-induced death of methanotrophic bacteria. To explore this, we examined SOC stocks, soil CO_2, CH_4 and N_2O fluxes including other soil and environmental variables from Long-Term Ecological Research sites within a South African grassland and savanna. Four fire treatments distinguished by fire frequency were used (annual, biennial, triennial and no fire) while the presence or absence of wild herbivores represented the two herbivory treatments. We found that grassland SOC stocks were highest with biennial fires (20.47 ± 2.04 g C m^{-2}, $p < 0.001$) compared to other treatments. In the same site, soil CO_2 fluxes were highest with herbivory plus annual fires (2.28 ± 0.72 µmol m^{-2} s^{-1}, $p < 0.001$). In the savanna, neither SOC stocks nor soil CO_2 fluxes were affected by treatments. Generally, lower frequency fires (biennial or triennial) increased soil CH_4 uptake while annual fires reduced soil CH_4 uptake. From this, we estimated that South African grassland and savanna soils could consume about 0.47% per m^2 of the CH_4 released by global wetlands. These native grassy sites with wildlife exhibited very low fluxes of N_2O whereas tropical grasslands and savannas that include livestock populated rangelands are thought to contribute about 16% to the global terrestrial N_2O emissions. Linear mixed-effects models for SOC stocks showed total soil N was the strongest predictor rather than plant detritus inputs. Thus, we conclude that fire and herbivory drove SOC stocks *via* effects on soil N. After pooling the data for the grassland and savanna to compare with local and global figures, we conclude that South African grasslands and savannas have a small but significant potential for SOC sequestration and reduction of soil greenhouse emissions given that they constitute *ca.* 57% of the country's terrestrial ecosystems.

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Chapter 1

General Introduction

1.1.1 Global greenhouse gas emission trends

Numerous global climate related researches have provided increasingly strong evidence showing that global warming levels exceeding 1.5ºC from the pre-industrial era levels may cause irreversible changes to the global climatic system whose impacts would be detrimental to global social and economic livelihoods (Masson-Delmotte et al., 2018). The global concentration of key greenhouse gases, i.e carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) in the atmosphere has been increasing since 1950, with projections to 2035 showing even further increase (Cubasch et al., 2013). Concentrations of these atmospheric greenhouse gases drive large scale and long-term climate patterns that have both environmental and economic implications (Shresta et al., 2015). Global CO_2 concentration has increased by 40% from an estimated 278 ppm in 1750 (pre-industrial revolution), to 391 ppm in 2011 (Ciais et al., 2013), and most recently, according to the NOAA Earth System Research Laboratory (ESRL), to 410 ppm in 2019 (Fig. 1.1; Dlugokencky and Tans, 2019). Methane concentration increased by 150 % from 722 to 1803 ppb while N_2O increased by 20% from 271 to 324 ppb during the same time interval (Ciais et al., 2013). In lieu of this, many researches have investigated various greenhouse gas sinks and sources and here we focus on soils which represent the largest terrestrial pools of C (Schauffler et al., 2010) and hence are significant potential sinks and sources of greenhouse gases.

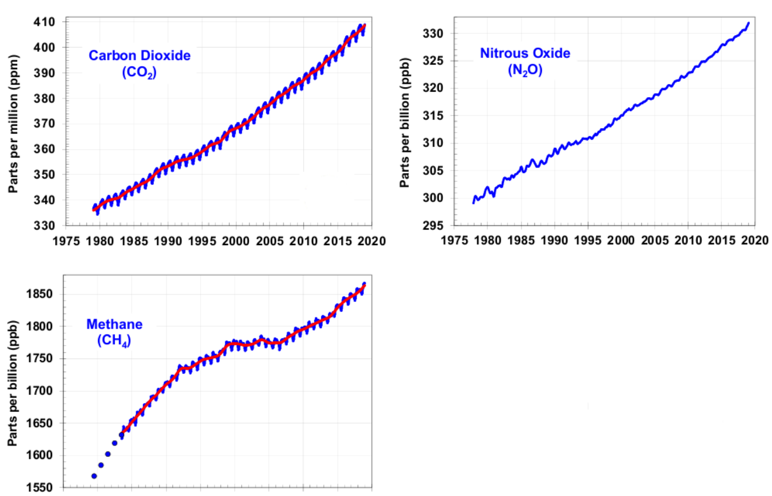

Figure 1.1: Global average abundances of the major biogenic greenhouse gases CO_2, CH_4, N_2O. *Source*: NOAA global air sampling network (Dlugokencky and Tans, 2019).

1.1.2 Soil organic C stocks and soil greenhouse gas emission trends

Soils form a large component of the terrestrial biosphere and are expansive reservoirs of C (FAO, 2017) containing stores that exceed those in vegetation and the atmosphere (Scharlemann et al., 2014). There is general agreement that there are large quantities of C stored as soil organic C, which are more than those in plant biomass (Lal, 2013). However, there is no consensus regarding the size and spatial distribution of global soil organic C stocks and the C emissions from soils as a result of changes in land use and land cover (Gianelle et al., 2010).

Fig 1.2 summarises the different sources and sinks of C and their contributions as part of the global C cycle.

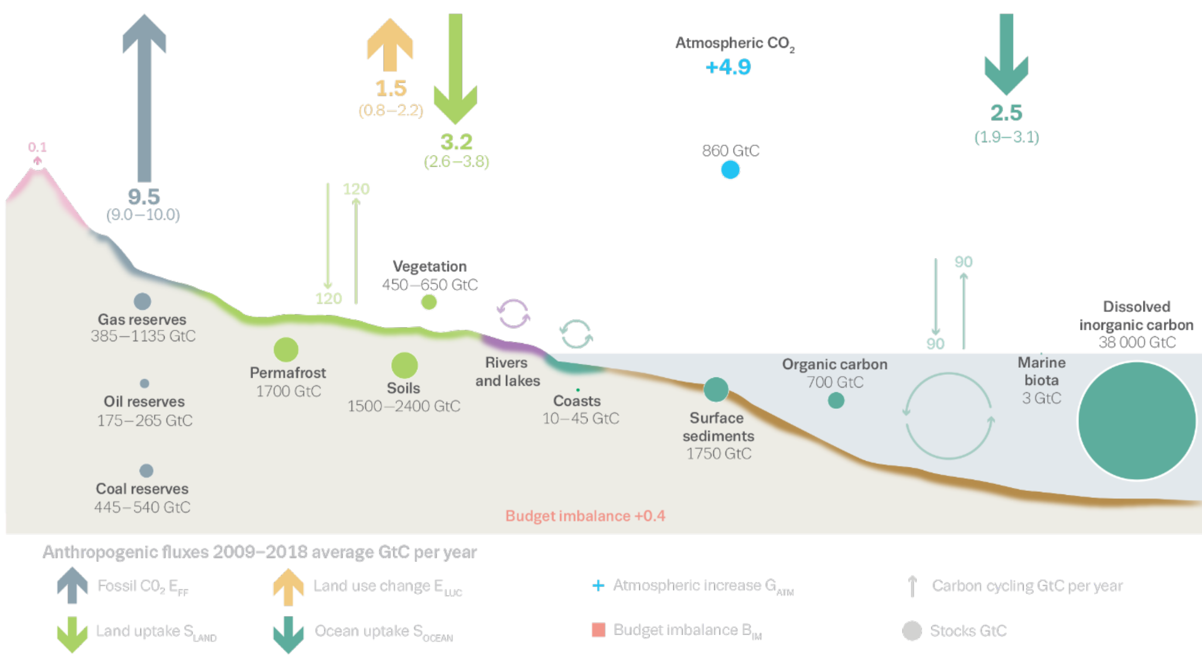

Figure 1.2: Sources and sinks of C and their contributions as part of the global C cycle (Friedlingstein et al., 2019).

Many estimates of global soil organic C stocks have been published and most studies report a global soil organic C stock estimate of approximately 1500 Pg of C (Hiederer and Kochy, 2011; FAO, 2009). Estimates of global soil organic C stocks have more recently, been based on more detailed data and the most recent is a global soil organic C map which reported total stocks of 1267 Pg of C from soil grids and modelling (FAO, 2018). Significant disparities in sampling periods, sampling intensities, spatial resolution of soil profile databases and perhaps different calculation approaches are the cause of this particularly wide variation in estimates of global soil organic C stocks and some studies included inorganic soil C, or varying levels of stone content (Batjes, 1996) or disturbance (Meentemeyer et al., 1985), eliciting further variation. More fine scale data is thus needed for more reliable estimates of global soil organic C stocks and the incorporation of the effects of different land-uses in the estimates is particularly important. Globally, soils play an important role as both sinks and sources of the three key greenhouse gases depending on different biotic and abiotic conditions (Oertel, et al., 2016). Carbon dioxide, released from autotrophic and heterotrophic soil respiration, is by far the largest component of greenhouse gas emissions from soils, however due to their global warming potentials, CH_4 and N_2O are equally important. When converted to CO_2 equivalents, they have more potent radiative effects per molecule than CO_2, e.g 1 kg of CH_4 released is equivalent to *ca.* 28 kg of CO_2 and 1 kg of N_2O is equivalent to *ca.* 265 kg of CO_2 (Myhre, 2013). Oxidation reactions in the soil largely from methanotrophic bacteria, result in soils generally becoming net sinks of CH_4 (Dutaur and Verchot, 2007), excepting wetland soils which are strong sources of CH_4 due to anaerobic reactions taking place (Tian et al., 2016; Hashimoto, 2012). Nitrous oxide is generally released from soil surfaces into the atmosphere (Chapuis-Lardy et al., 2006) and is a product of nitrification and denitrification processes in the soil (Soussana et al., 2010).

1.1.3 Effects of land use on soil physio-chemical properties

Grasslands and savannas comprise *ca.* 40% of the global terrestrial area (Suttie et al., 2005; Wang and Fang, 2009), *ca.* 33.5% of Africa's terrestrial biomes (White, 1983; Parr et al., 2014) and in South Africa, they cover *ca.* 60.4% of the country (Mucina and Rutherford, 2006). Across their range, grassland and savannas support a growing proportion of the global population (Scholes, 1997) by providing livelihoods to about 180 million people, including more than 20 million pastoralists in Africa (Swallow and Bromley, 1995). They are however, becoming increasingly threatened by land-cover change and transformation, unregulated burning and grazing and climate change (Osborne et al., 2018). These threats potentially affect the sink or source role of grassland and savanna soils, in-turn affecting local, regional and global C budgets. Le Quere et al. (2018) provide an estimate of the global C budget based on CO_2 emissions from fossil fuels and industry, land-use change and sinks that include the ocean and land. For the 2007 to 2016 decade, estimations of combined emissions from fossil fuels and industry were 9.4 ± 0.5 Pg C yr^{-1} while 1.3 ± 0.7 Pg C yr^{-1} was emitted from land-use change, with a global CO_2 concentration growth rate of 4.7 ± 0.1 Pg C yr^{-1}. Approximately 2.6 ± 0.5 Pg C yr^{-1} and 2.7 ± 1.0 Pg C yr^{-1} were absorbed into ocean and land sinks, respectively. Tian et al. (2016) estimated the overall terrestrial CH_4 emissions to be 0.33 ± 0.04 Pg C yr^{-1} where natural wetlands contribute *ca.* 40% to 50%, rice cultivation *ca.* 10%, and the remainder of the terrestrial CH_4 emissions are from ruminants (*ca.* 20%), landfills and waste (*ca.*14%), biomass burning (*ca.* 4% to 5%), manure management (*ca.* 2%) and termites, wild animals and others (*ca.* 6% to 10%). Global N_2O emissions were estimated between 0.013 to 0.015 Pg N yr^{-1}, with natural ecosystems contributing 55% to 65%, and the remaining coming from agricultural soils (*ca.* 25% to 30%), biomass burning (*ca.* 5%), indirect emissions (*ca.* 5%), manure management (*ca.* 2%) and human sewage (*ca.* 2%). Although the effects of different land-uses on soil greenhouse gas emissions have been extensively studied, the nexus between land-use, soil organic C sequestration and soil greenhouse gas emissions is yet to be ascertained. A change in the size of soil organic C stocks, due to above ground disturbances such as fire and herbivory, can significantly affect atmospheric CO_2 concentrations (Raich and Potter, 1995). There is evidence to suggest that C availability may influence N_2O emissions and, in turn, N availability can influence the production of CO_2 in soils (Piao et al., 2013). It is, therefore, possible that the modification of the C:N ratios of detritus inputs as an influence of disturbances such as fire and herbivory, might alter the soil organic C storage and fluxes of the three most key greenhouse gases, with far reaching consequences at a global change scale.

1.1.4 Effects of herbivory and fire on soil physio-chemical properties in grasslands and savannas

Grasslands and savannas dominate many African rangelands and may sequester more C than wooded ecosystems (Soussana et al., 2010). The conservation of the dynamic and vulnerable C pool is critical in avoiding emissions from dry grassland and savanna ecosystems, despite low C stocks per unit area (Brandt et al., 2018). Herbivory and fire are common features of these grassland and savanna ecosystems and are major forms of ecological disturbance that influence vegetation and soil properties (Mcsherry and Ritchie, 2013; Allen et al., 2014). Due to this, management practices such as pyric-herbivory have emerged which utilize the interactive effects of herbivory and fire to facilitate structural heterogeneity and biodiversity conservation

(Fuhlendorf et al., 2009). These practices have gained traction in opposition to species-centric focuses on restoring wild grazed ecosystems by favouring certain herbivore species and thereby overly underestimating the intricacies of herbivory and fire interaction in complex landscapes (Donlan et al., 2005). Some studies however, argue that the effects of fire and herbivory cannot be equated because herbivores cannot consume all the available vegetation unlike fires, moreover, their products i.e dung and urine versus pyrogenic C compounds and ash are different (Polis, 1999). Bond and Keeley (2005), however liken fires to a non-selective herbivore with a broad dietary preference, in this case fuel types, that span the spectrum of both living and dead organic material at a broad scale.

Fires affect about 4.64 million km^2 of biomass per year, corresponding to about 4% of the earth's vegetated surface (Randerson et al., 2012). Various physical effects of fire on soil properties have been reported and these include breakdown of soil structure, reduced moisture retention capacity and development of water repellency (Stavi et al., 2017). Most studies on effects of fire on soil have focused on immediate system responses to fire such as changes in soil temperature, moisture and nutrients (Savadogo et al., 2017). Fire intensity and frequency have been found to have more profound effects on soil. Repeated annual exposure to fires has cumulative long-term effects that include reduction in soil organic matter, changes in species composition and modification of C allocation by plants to the soil (Stoof et al., 2010). Fires in grasslands with fuel loads of less than 1 Mg ha^{-1} usually have ground-level temperatures of less than 250°C, although higher instantaneous temperatures have been measured (Raison, 1980). Fast moving fires on grass may be intense in terms of energy release per unit area, but do not transfer the same amounts of heat to mineral soil or soil organisms as do slow moving fires in moderate to heavy fuels (Trollope. 2007). Low intensity fires generally result in mineral soil temperatures that typically do not exceed 100°C at the surface and 50°C at 5-cm depth (Campbell et al., 1995). Trollope (2007) reports that in grasslands fires generally burn as surface fires, either as back or head fires. Backfires are more intense than head fires as they tend to have a residence time of 20 s longer than head fires resulting in critical threshold temperatures of *ca.* 95ºC, hence causing more heat release at ground level (Trollope, 1978). Generally, temperatures between 40ºC and 70ºC are adequate to start biological tissue disintegration and desiccation, where death of roots can occur between 48ºC and 54ºC, between 70ºC and 90ºC for seed mortality and between 50ºC and 121ºC for microbial mortality

(Knicker, 2007). Although organic matter is commonly volatilized at temperatures between 200ºC and 315ºC, considerable amounts of C can be lost at lower temperatures (Lide, 2004). Carbonization processes start above 200ºC (Freitas et al., 1999) and almost all unprotected soil organic matter is combusted under aerobic conditions at around 460ºC (Knicker, 2007). The release of CO_2 *via* soil respiration can be offset by the reincorporation of the CO_2 into biomass in the post-burn recovery period when vegetation regrows, however, in instances where soil respiration is higher than photosynthesis, there is net emission of CO_2 (Hao et al., 1990; Houghton, 1991). Recent studies also suggest that up to 15% of fire affected biomass (Santín et al., 2015) is converted into pyrogenic organic C through incomplete combustion, also referred to as fire-derived organic matter, charcoal or black C (Hammes and Abiven, 2013). Pyrogenic C can bind and deactivate phenolic compounds in the soil (Wardle et al., 1998). It is considered one of the most stable components in soil and can represent more than 30% of total soil organic C, however, few estimates of global pyrogenic organic C stock or distribution exist and thus pyrogenic organic C is not included in any global C cycle models, despite its potential major relevance for the soil C pool (Reisser et al., 2016). The changes in biological activity in the soil environment from biomass burning can affect localized atmospheric C budgets as CO_2 is released through soil respiration and decomposition of dead organic matter produced by the action of fire (Zepp et al., 1996). Other essential elements are also affected by fire for instance, volatilization of N begins at 200ºC and above 500ºC half of the N in organic matter is lost to the atmosphere (Knicker, 2007), while P and K are normally volatilized at temperatures above 760ºC (Lide, 2004). The effects of fire on N loss from soils to the atmosphere have been widely debated and low intensity burning transforms organic N into more readily available forms like NH_4^+, NO_3^- and dissolved organic matter (Prieto-Fernaˊndez et al., 2004). Ash, which is usually rich in cations, phosphate and trace elements, is also a product of vegetation fires and according to Toma et al. (2010), burning of grasslands can lead to ash buildup on the soil surface, thereby contributing to the overall soil C accumulation. Ash has also been reported to cause an increase in pH in soils, which plays a pivotal role in the biological recovery of soils after fires (Chambers and Attiwil, 1994). Significant increases in pH however, only occur at temperatures beyond 450ºC (Knicker, 2007), and it is therefore important to consider the intensity and duration of fires when determining their effects on soils.

Herbivory has major effects on the chemical composition and decomposability of plant litter from which soil organic matter is formed (Bardgett and Wardle, 2003). Ungulates alter the quality of plant litter mainly by shifting species compositions of plant communities (Díaz et al., 2007). Over 50% of the earth's surface is chronically grazed or browsed by both wild and domesticated ungulates and therefore herbivores are likely to have an important role in the governing of the global carbon cycle (Menke and Bradford, 1992). The effects of livestock on soil health have been widely studied (e.g Fleischner, 1994; Jones, 2000; Golluscio et al., 2009; Eldridge et al., 2011), however there is little that is known about effects of free ranging wild megaherbivores such as elephants, wildebeest and zebras found in South African grasslands and savannas. Most estimates indicate that about 25%, 20%, and 15%, respectively, of N, P and K contained in forages consumed by grazing cattle is retained in their bodies for support of their various metabolic processes and this means that about 75%, 80%, and 85%, respectively, of N, P and K passes through the animal and are excreted in urine and feces (Wells and Dougherty, 1997). The deposition of dung and urine coupled by the trampling effect of hooves affect nutrient cycling in the soil and initiate responses such as increased activity from soil microbes (Taboada et al., 2011). This deposition of excreta increases N and extractable P, while losses of these nutrients occur when animals feed and from erosion that results from trampling however, such processes are a function of stocking densities and initial nutrient status of the soils (Lavado et al., 1995). According to Barthelemy (2016), grazing-induced increase in above ground plant quality, drove N cycling in arctic grasslands through provision of litter of higher quality (lower C:N) to the below ground system, increasing organic matter deposition and thus enhancing soil nutrient availability. The rate of exudation of compounds (rhizodeposition) such as carbohydrates, sugars and amino acids by grass roots is increased by herbivory and these become readily accessible to rhizospheric microbes (Bardgett and Wardle, 2003; Frank and Groffman, 2009). Under moderate grazing regimes in resource-rich grasslands, herbivores increase the abundance of relatively palatable and decomposable forages that facilitate decomposition (Díaz et al., 2007; Bakker et al., 2009). Contrastingly, in resource-poor grassland or under excessive grazing pressure, herbivores often increase the abundance of relatively unpalatable forage species that have relatively high secondary compounds such as lignin and tannins, resulting in recalcitrant litter that degrades at much slower rate (Augustine and McNaughton, 1997; Díaz et al., 2007). Ganjegunte et al. (2005) also found that moderate grazing pressure increased soil C stocks compared to zero grazing or heavy grazing while Eldridge et al. (2016) found grazing to have no effect on soil stability and soil nutrients under high productivity. This was also supported by Loreau (2010) who suggest that soils are expected to be more resistant to herbivore impacts under high productivity due to greater plant cover and richness, thus translating to more stable soils. A greater stability of soils would suggest that more C stays in the soil and hence less is emitted as greenhouse gases to the atmosphere.

These effects of herbivory on soil, in turn, can affect soil greenhouse gas fluxes *via* stoichiometric gradients in C and N availability due to the intrinsic linkage between microbial C and N metabolism (Robertson and Groffman, 2007). Microbial and plant respiratory processes are intrinsically dependent on soil nutrient availability and hence, natural C and N content in soil and atmospheric deposition, manure or fertilizer applications play an important role (Oertal et al., 2016). Nitrous oxide emissions negatively correlate with the C:N ratio (Pilegaard et al., 2006), with N_2O emissions being lowest at C:N ratios ≥ 30 (limited disintegration of organic material) and highest at a C:N value of 11 (optimum disintegration and humus build-up) (Gundersen et al., 2012). Combined with drought and low pH values, N_2O emissions can be significantly suppressed even at soil C:N ratios below 20 (Gundersen et al., 2012). Emissions of CO_2 and CH_4 positively correlate with the soil C:N ratio (Shi et al., 2014; Weslien et al., 2009). Liang et al., (2015) found that depending on the C:N stoichiometry of organic resources to the soil, the effect of the secondary resource (C or N) can either enhance or inhibit the emission of gases. In their study, total greenhouse gas emissions increased linearly with C additions and with additional N while emissions increased minimally with N additions and no C additions. Substrate availability and quality are therefore critical in driving soil nutrient sequestrations and gaseous emissions.

Soil physical properties, most importantly soil texture, have also been shown to be equally important in soil organic C sequestration. Soil texture is an important abiotic factor that influences mineral distribution, organic matter decomposition, microbial biomass, and other soil properties (Scott and Robert, 2006). Several studies have linked fine textured soils to greater soil organic C stocks in contrast to coarse textured soils (Hassink, 1997; Bird et al., 2000). A study conducted by Parton et al. (1987) using 560 soil profiles showed significant correlation between soil texture and soil organic C stocks with fine textured soils sequestering more soil organic C compared to sandy textured soils. Clayey soils have a greater stabilizing influence on soil organic C than sandy soils, probably due to a large surface area, which form stable organo-mineral complexes that protect C from microbial decomposition (Feller and Beare, 1997; Six et al., 2000). Soil texture may interact and be confounded with other environmental factors such soil temperature and moisture, which may profoundly affect soil organic C depletion in grassland soil (Feller and Beare, 1997). Soil pore space size and distribution have a major impact on hydrodynamics and therefore on the abundance of microbes and may contribute to the rate of C mineralization (Kuka et al., 2007; Cai et al., 2016). Generally, direct N_2O emissions increase with clay content in the soil in most cases because anaerobic denitrification occurs more frequently in fine textured soils than in coarse textured soils (Skiba and Ball, 2002; Gaillard et al., 2016). Negative CH_4 fluxes are prevalent in sandy soils where aerobic conditions dominate and favour methanotrophic bacteria while they are positive in clayey soils that often get waterlogged and favour anaerobic conditions which are optimal for methanogenic bacteria (Oertal et al., 2016).

Fire and herbivory are inevitable occurrences in savanna and grassland ecosystems and have historically shaped the present-day status of these ecosystems. Their independent and interactive effects on key determinants of net primary productivity such as plant biomass are important. According to Piao et al. (2006), rising CO_2 levels, increased rainfall in dry ecosystems such as semi-arid savannas, increasing temperatures in previously low temperature areas and extra N-deposition can cause significant spikes in net primary productivity. Such an increase in net primary productivity can induce higher soil organic C stocks by increasing C inputs to the soil (Eglin et al., 2010). The effects of herbivory and fire on soil rhizospheric processes are complex and varied in different ecosystems and as has been shown, depend on various factors including ecosystem productivity. Many studies often decouple the effects of these two disturbances without adequately addressing their interaction, thereby underestimating the complexity of their impacts in natural ecosystems. This thesis examines the effects of herbivory, fire and pyric-herbivory on soil organic C stocks and soil CO_2, CH_4 and N_2O emissions in South African grasslands and savannas and their overarching implications for global change.

1.1.5 Hypotheses and thesis outline

Overall, herbivory was expected to increase soil organic C stocks while fire was expected to initially reduce soil organic C stocks but eventually increase stocks over time. Both herbivory and fire were expected to have a dual effect of either increasing or decreasing soil greenhouse emissions. Fig 1.3 below is a conceptual diagram summarises the hypotheses and mechanisms involved.

Specific hypotheses:

1. Selective grazing of relatively nutrient-rich (low C:N) plants by herbivores on low nutrient soils was expected to initially provide high quality inputs from excreta but to shift vegetation towards high C:N ratios in plant species in the long-term, with subsequent low quality detrital inputs as litter that would reduce the total soil N accumulation. This would result in microbial immobilization of soil N and the remaining low quality soil organic matter becomes more resistant to microbial decomposition and therefore potentially contributes to the stable soil organic C pool (Chapter 2).
2. Fire was expected to reduce soil N pools by volatilization but to increase the stable soil organic C pool by addition of mostly recalcitrant pyrogenic organic C (e.g charcoal, tar or soot) (Chapter 2).
3. Reduced soil N accumulation and increased soil organic C sequestration would result in reduced CO_2, CH_4 and N_2O emissions (Chapter 3).
4. Fire was expected to reduce both soil CO_2 and N_2O emissions, due to increased stable pyrogenic organic C and N volatilization respectively, exacerbated by fire-induced microbial death. Conversely CH_4 emissions were expected to increase due to fire-induced decreases in populations of methanotrophic bacteria (Chapter 3).

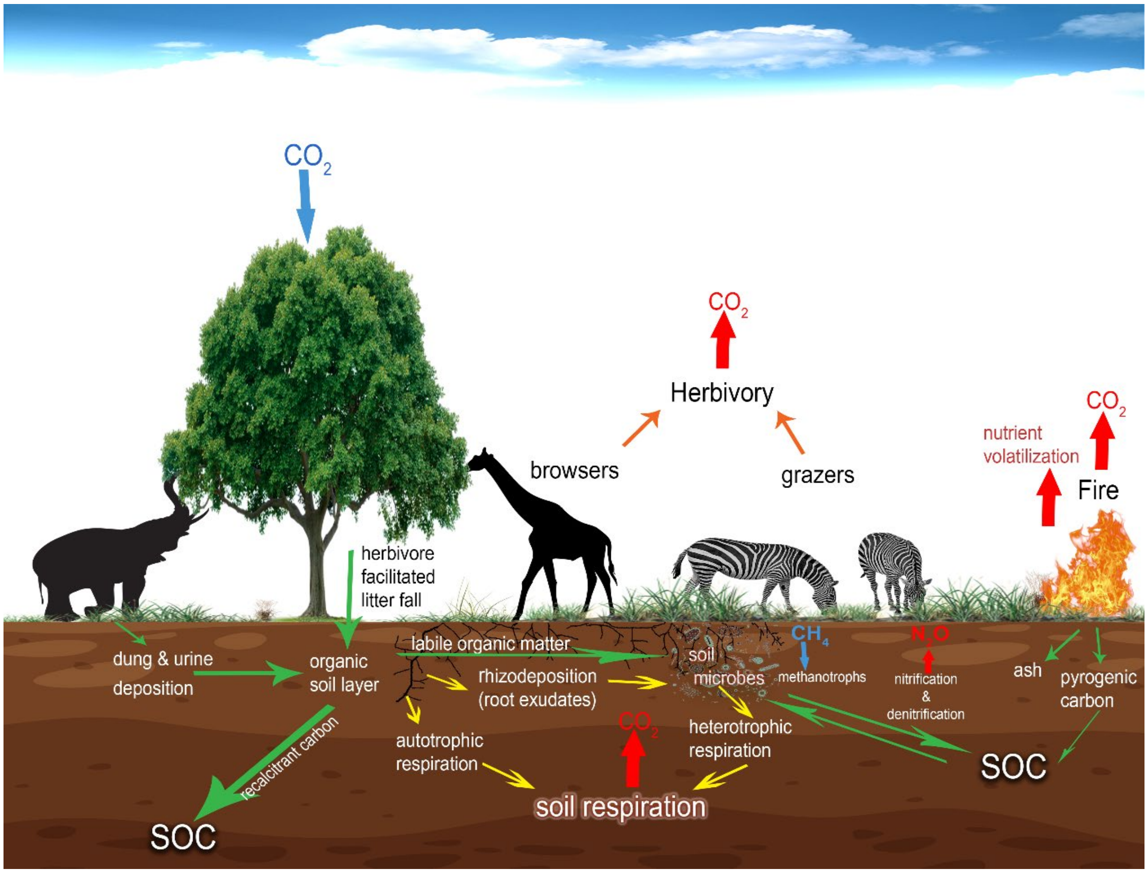

Figure 1.3: Conceptual diagram of above and below ground (rhizosphere) processes driving soil organic C sequestration and key soil greenhouse gas fluxes in grassland ecosystems.

Chapter 2

Fire and herbivory can drive soil organic C stocks *via* effects on soil N in South African grasslands and savannas.

2.1 Abstract

Unregulated herbivory and burning threaten biodiversity and climate resilience of grasslands and savannas. We have limited understanding of how herbivory, fire and pyric-herbivory influence the coupling between C and N of soil organic matter inputs and the resultant implications for soil organic C sequestration. We hypothesised that, in low nutrient soils, herbivory would ultimately shift vegetation towards low quality (high C:N) foliage and detrital inputs as litter that are resistant to microbial decomposition and thus contribute to the stable soil organic C pool. Contrastingly, fire was expected to reduce soil N pools by volatilisation but to increase the stable soil organic C pool by addition of mostly recalcitrant pyrogenic organic C from biomass burning. We measured herbaceous biomass, foliar and litter C and N, total soil organic C and N, and other soil and environmental variables from a range of fire and herbivory treatments within Long-Term Ecological Research sites in one grassland and two savanna areas in South Africa. In the grassland, soil organic C stocks were highest with biennial fire (20471 ± 2040 g C m^{-2} within the top 20-cm) compared to other treatments (17885 ± 1914 g C m^{-2}, $P < 0.001$). Total soil N stocks were also higher with fire compared to other treatments in the same site (1309 ± 68.7 to 1049 ± 82.9 g N m^{-2}, $P < 0.001$). In the savanna, fire and herbivory treatments did not affect either soil organic C or total soil N stocks. In the grassland, 97% of the variation in soil organic C stocks was predicted by treatments, total soil N stocks, herbaceous biomass and bulk density. Total soil N stocks were an especially strong predictor of soil organic C stocks, indicating a close coupling between soil organic C and soil N. Overall fire and herbivory drove soil C stocks mainly *via* effects on soil N. This relationship was clear in grasslands but less evident in the savanna, possibly due to some threshold low level of soil N in our sites. The unique fire and herbivory management histories of the sites influence soil organic C and it is therefore important to include site history in future soil organic C sequestration research. Globally, efforts to increase soil C stocks should consider not only management but that the inherent capacity to sequester C is dependent on soil N.

2.2 Introduction

Grasslands, including savannas, comprise *ca.* 40% of the world's land surface (Suttie et al., 2005; Wang and Fang, 2009) and across their range they support livelihoods of a growing portion of the global population (Ryan et al., 2016). Given the global importance of soil C sequestration for climate mitigation, the extant and potential soil C stocks of grasslands, savannas and other natural ecosystems have been widely assessed (Hiederer and Kochy, 2011; Scharlemann et al., 2014, Batjes and van Wesemael, 2014). Within grasslands and savannas, fire and herbivory are major ecosystem components and are often managed to regulate their impacts on these ecosystems. In savannas for instance, herbivory and fire partially determine the relative proportions of herbaceous (mainly grasses) and woody components (Bond et al., 2003; Sankaran et al., 2005). Studies on wildlife herbivory in the savanna Serengeti (McNaughton, 1985), have shown that herbivores can modify both plant composition and biomass to benefit themselves and in turn modify the quality and quantity of organic matter input to the soil. Krumins et al. (2015) pointed out that feeding mechanisms of herbivory differ where for example, some herbivores can partially consume biomass (sloppy feeding), leaving behind large quantities of biomass as detritus while some consume most biomass. As such, some plants may benefit from herbivore consumption as this may reduce plant competition. However, herbivores may also selectively feed on plants with N-rich (low C:N) tissues which are more palatable resulting in N-poor (high C:N) plants dominating above-ground (Ritchie et al., 1998; Tuomi et al., 2018). This phenomenon as illustrated in Fig 2.1, has a direct effect on whether microbes mineralize or immobilize soil nutrients based on available fresh organic matter quality (Schimel and Bennett 2004; Mooshammer et al., 2012; Cherif and Loreau, 2013).

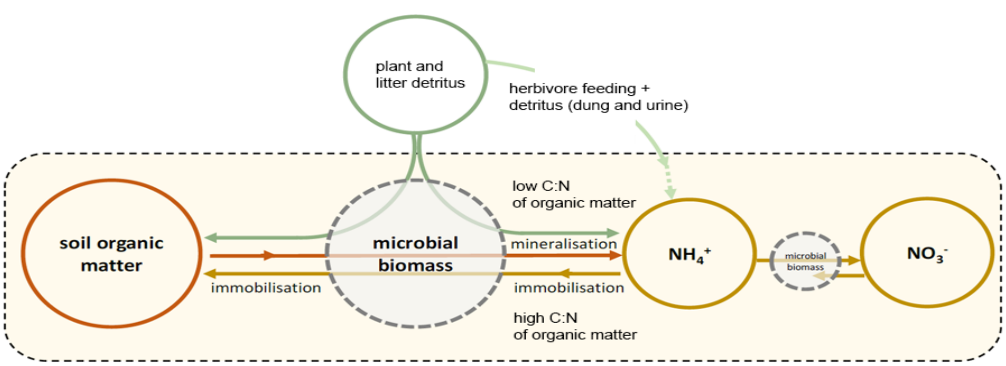

Figure 2.1: Schematic diagram illustrating the organic C:N conditions through which soil microbes may mineralise or immobilise nutrients in the soil.

In contrast to herbivores, fires indiscriminately consume massive amounts of biomass C and may convert soil organic matter to pyrogenic C compounds such as charcoal that are relatively resistant to decomposition (Harden et al., 2004; González-Pérez et al., 2004; Knicker, 2007). Short-term losses of C and other nutrients from fire can trigger long-term compensatory responses in the ratios of plant C:N by shifting the community towards high C:N plant composition (Pellegrini, 2015). Plant response-induced nutrient ratio changes to herbivory, fire or both may ultimately affect the storage of organic C in the soil as they also directly affect nutrient allocations in the soil (Giardana et al., 2004).

The competitive interplay between fire and herbivores (van Wilgen et al. 2005; Archibald and Hempson 2016; Bond and Keeley 2005) is important if we are to successfully manage rangelands for climate resilience. A particularly important question is how fire and herbivory interact to modulate feedbacks between C and N pools in plants and soil and ultimately altering their cycling in grassland ecosystems (Harris et al., 2007). There is usually tight coupling between soil C and N (Conant et al., 2005), hence any effect of herbivory or fire that alters soil mineral N may in turn cause changes in soil organic C sequestration. Fornara and Tilman (2012) found in their study that 27 years of constant N additions to prairie grasslands increased soil organic C sequestration in mineral soils *via* a potential mechanism of N-induced root mass increase. Other studies concur with this and suggest that high N availability affects belowground C allocation *via* changes in root and mycorrhizal exudation (Giardana et al., 2004) and in organic matter decomposition in litter and soils (Keeler et al., 2009). Mechanisms that reduce ecosystem losses of N such as microbial immobilisation, recalcitrant organic matter formation and small or inactive microbial populations may be less effective in N-rich systems in terms of increasing soil organic C sequestration (Conant et al., 2005), whereas in N-poor systems, losses in an already-N-limited ecosystem may negatively affect soil organic C sequestration rates. The soils in southern African grasslands and savannas have total soil N estimates ranging from 3060 to 4635 kg N ha^{-1} (Scholes and Walker, 1993; Woghiren, 2002), and based on the above, we expect relatively low soil organic C stocks per unit area. However, considering the extent of grasslands and savannas worldwide (*ca.* 40% of global terrestrial ecosystems; White, 1983), the cumulative sequestration potential is immense.

Given what we know about how fire and herbivory modify grasslands and savannas ecosystems, we hypothesised that selective grazing of relatively nutrient-rich (low C:N) plants by herbivores on low nutrient soils would provide high quality inputs from excreta but then shift vegetation towards high C:N ratios in plant species in the long-term, with subsequent low quality detrital inputs as litter. With these inputs being more resistant to microbial decomposition, they would therefore contribute to the stable soil organic C pool. Contrastingly, fire was expected to reduce soil N pools by volatilisation but to increase the stable soil organic C pool by addition of mostly recalcitrant pyrogenic organic C (charcoal, tar and soot) from biomass burning.

2.3 Methods and materials

2.3.1 Study sites

The study was conducted during the wet-warm growing season in the mesic montane grasslands and mesic savanna of South Africa. Grassland sampling was conducted in the experimental burning plots on the Brotherton burn trial at Cathedral Peak, uKhahlamba-Drakensberg Park (Fig 2.1). Savanna sampling was conducted in the herbivore exclosures in Long Term Ecological Research (LTER) sites at Nkuhlu and Satara, Kruger National Park (Fig 2.1). The description of the pedo-topo-climatic context, vegetation and common herbivore composition are given in Table 2.1.

Table 2.1. Pedo-topo-climatic context, vegetation, common herbivores and establishment dates of the Long-Term Ecological Research (LTER) sites: Mean annual precipitations (MAP); mean annual temperatures (MAT); soil types according to the World Reference Base (WRB); large stock unit (LSU).

	Grassland	Savanna	
	High altitude	Moist	
LTER site	Brotherton	Nkuhlu	Satara
Established	1980	2002	2006/7
MAP (mm)	1075	560	650
MAT (°C)	15	22	23
Altitude (m)	1890	240	266
Location	29.00°S	24.99°S	24.40°S

	29.00°E	31.77°E	31.78°E
Lithology[1]	Basalt overlying cave sandstone	Grey migmatite and gneiss of the Nelspruit Suite, Swazian Erathem	Olivine-rich basalts, subordinate alkali-basalts
Soil (WRB)[2]	Ferralsols and Arenosols	Eutric Leptosols	Pedorhodic and Peducutanic-lithic (luvisols or lixisols)
Vegetation type[3,5]	uKhahlamba Basalt Grassland (Gd7)	Granite Lowveld	Basalt sweetveld
Dominant vegetation species	*Bromus speciosus, Festuca costata, Pentaschistis tysonii, Themeda triandra*	*Vachellia nigrescens, V.grandiconurta, Combretum apiculatum*	*Sclerocarya birrea, V.nigrescens*
Common herbivores	*Alcelaphus buselaphus caama, Damaliscus pygargus phillipsi, Ourebia ourebi, Pelea capreolus, Redunca arundinum, Redunca fulvorufula, Tragelaphus oryx*	*Aepyceros melampus, Giraffa camelopardalis, Hippopotamus amphibious, Kobus ellipsiprymnus, Loxodonta* Afric*ana, Pedetes capensis, Syncerus caffer, Tragelaphus strepsiceros, Connochaetes taurinus, Equus quagga*	
Animal densities	Very low (55 ha LSU^{-1})[4]	High	High

[1]SOTER database (Engelen and Dijkshoorn, 2013); [2]IUSS Working Group (2015); [3]Mucina and Rutherford (2006); [4]Rowe-Rowe and Scotcher (1985), [5]Venter and Govender (2012).

2.3.2 Sampling design

At the grassland Brotherton experimental burn trial, sampling area was defined by the existing 25 x 25-m plots subjected to annual fire, biennial fire or no fire (Fig 2.2). To obtain pyric-herbivory treatments, plots were defined outside the trial, i.e in the fire-break accessible to herbivores and burnt annually, and in the generality of the park accessible to herbivores and burnt biennially as part of the fire management regime of the park. Thus, we had an unbalanced sampling design with five treatments since the herbivory treatment lacked a 'no-fire' component. Each of the three plots per treatment were randomly sampled in two areas to include heterogeneity within treatments where the resulting six replicates per treatment (n = 30) were related to plots.

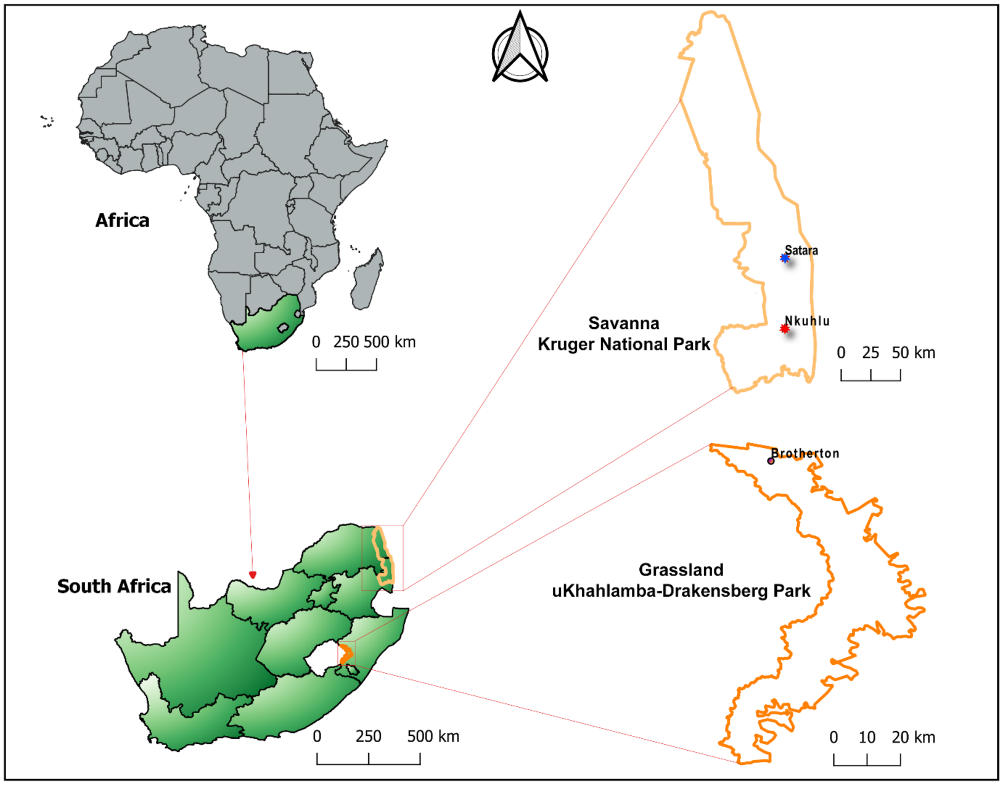

Figure 2.2: Location of study sites in savanna and grassland biomes of South Africa.

In the savanna sites, we used fence-line measurements between existing treatments in LTERs (Fig 2.3). At the Nkuhlu LTER site, we had a balanced sampling design with fire-herbivory treatments: herbivory - triennial fire (for this site fire frequency was classified as 3 to 5 years), triennial fire only, no disturbance and herbivory only. Sampling for soil and vegetation was semi-random. We constrained sampling to fence-lines, a similar soil type and between-canopy positions to reduce between-treatment variation. Once at the sampling site, ten points were randomly selected for sampling. At Satara, six full herbivore exclosures were selected and sampled inside and out from three fire-based treatments (annual, triennial, no fire). Due to the experimental design constraint of the LTER sites being setup decades ago without replicates, we had to alternatively use pseudo-replicates. These LTER sites cover spatially large areas and replicates were thus obtained from collecting multiple samples from different areas within the LTER sites. With this fence-line design, we acknowledge that our sampling plots are pseudoreplicated (sensu Hurlbert, 1984). However, we feel that the common difficulty in obtaining treatment replicates in long-term or natural experiments is widely acknowledged to be offset by the rich information gained from these systems (Davies and Gray, 2015). We also acknowledge that our replicates are not independent treatment replicates. However, considering the plots are directly adjacent either side of the fence with similar biotic and abiotic characteristics, we consider it reasonable to interpret changes due to management (including grazing, fire, animal density and stocking rate).

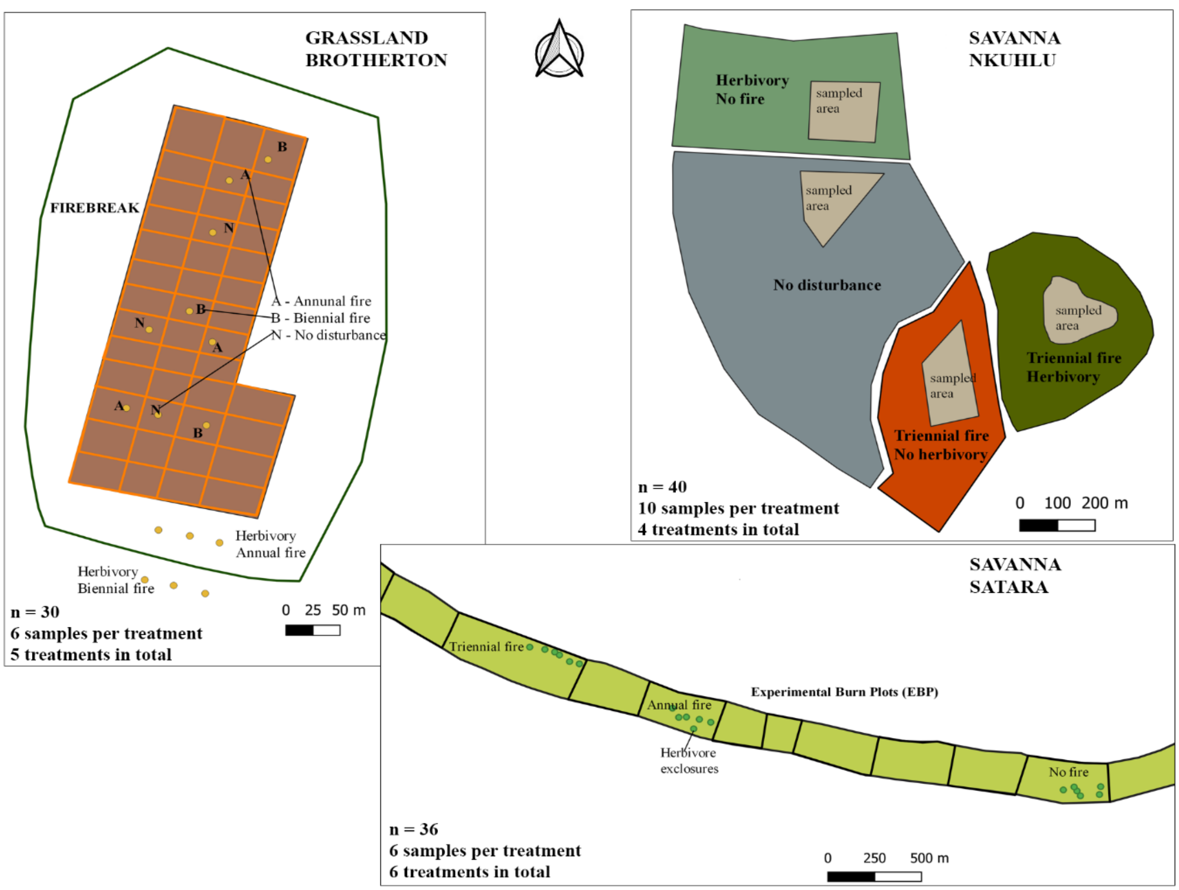

Figure 2.3: Sampling design showing selected treatments and sampling points from the three sites.

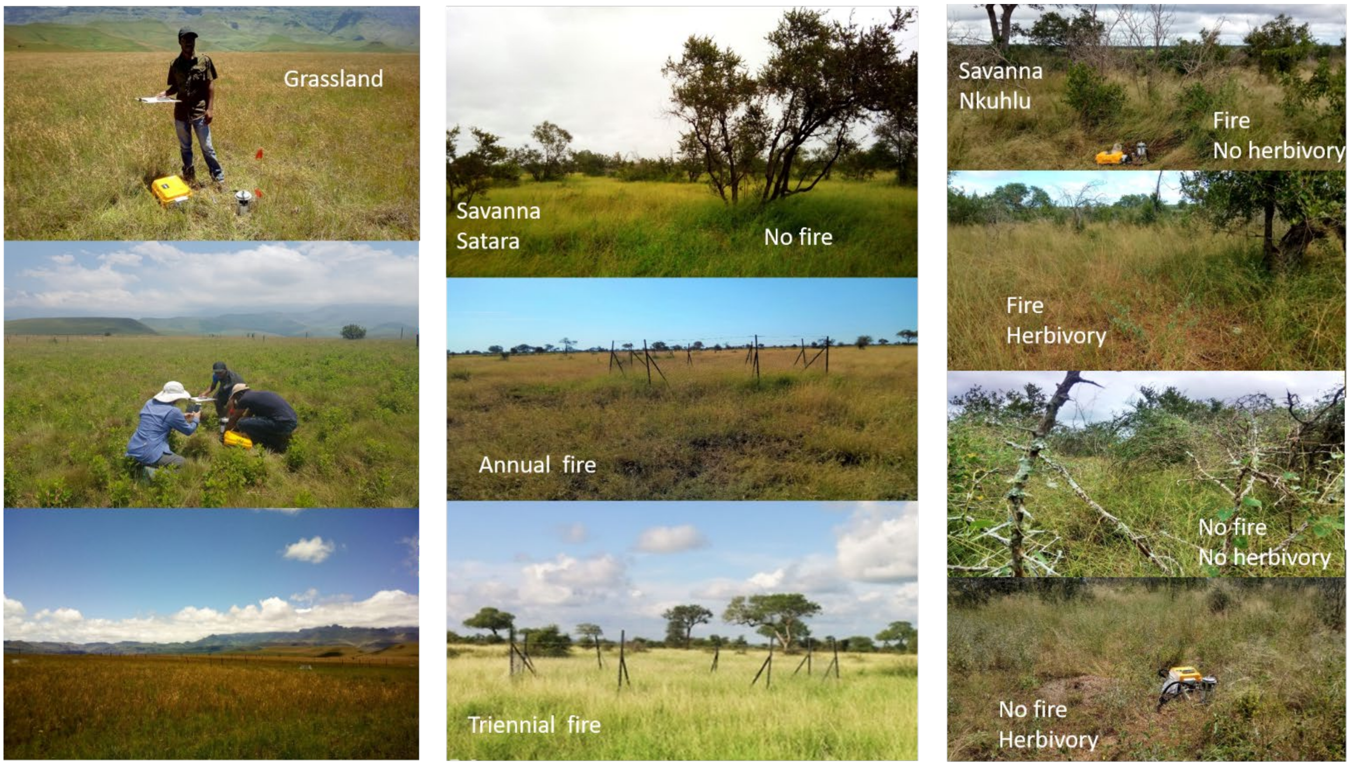

Figure 2.4 Landscape images showing the visual differences among the study sites.

2.3.3 Soil sampling

Four soil cores (10-cm diameter and 20-cm deep) were augured, combined, homogenised and sub-sampled from each sampling point at each site. The samples were air-dried to constant weight, i.e for *ca.* 7-d following collection. Bulk density samples were taken using a 100-cm^3 Eijkelkamp bulk density kit (Eijkelkamp Soil and Water, Netherlands) and oven-dried at 105ºC to a constant weight and subsequently weighed.

2.3.4 Plant sampling

Foliage from herbaceous biomass were collected from around the soil cores for determination of plant C:N ratios and potential detritus inputs to the soil. Plant samples were air-dried immediately after collection and then subsequently oven-dried at 65°C for 5 d to a constant weight. Herbaceous biomass was determined using a disc pasture meter following the method Trollope and Potgieter (1986). Woody cover was obtained by the determining the amount of area covered by tree canopies using satellite imagery. Normalised Difference Vegetation Index (NDVI) data were measured using a handheld Trimble GreenSeeker (Trimble Inc., California, USA) by moving along the plot, following equidistant gridlines after which an average of all NDVI values was recorded by the GreenSeeker.

2.3.5 Soil physio-chemical analyses

Soil organic C and total N stocks within the top 20 cm of the soil (g C or N m^{-2} soil) were calculated as in FAO (2018), where soil C and N (g 100 g^{-1} of soil, i.e. % w/w) were multiplied by the fine fraction soil bulk density (cm^3) and soil sampling depth (cm). Soil particle size distributions were determined using a Malvern Mastersizer and Hydro 2000G (Malvern Instruments Ltd, Malvern, UK) on 1-mm sieved soils where organic matter had been removed by treating a 10-g subsample with *ca.* 200 mL 6% H_2O_2 (adjusted to pH 5 with 2 M sodium acetate). The proportion of the soil particles in each size class were determined and size classes were summed into categories representing clay, silt, and sand, according to the Wentworth grain size chart (Wentworth, 1933). Soil pH was determined using a pH meter after extraction in 1 M KCl buffer. Powdered subsamples of soil and plant samples were analysed for C and N content *via* Dumas combustion where approximately 2 mg of each sample was weighed into a tin capsule (5 x 9 mm, Säntis Analytical, Teufen, Switzerland) and thereafter combusted in a Flash 2000 organic elemental analyser. X-ray florescence was used to determine total elemental concentrations in soil and plant samples through analyses in a SPECTRO XEPOS X-Ray Fluorescence (XRF) analyzer (SPECTRO, AMATEK materials analysis division, Kleve, Germany).

2.3.6 Statistical analyses

All statistical analyses were computed in R 3.6.3 (R Core Team, 2019). Data were first tested for normality using the Shapiro-Wilk test and log-transformed if not conforming and tested for homogeneity of variances with Levene's test. Two-way ANOVA with fire and herbivory as factors and subsequent post-hoc Tukey Honest Significance Difference (HSD) pairwise comparisons were used to determine differences in soil organic C stocks, soil chemical (C, N, cations, metals, pH) and physical (particle size, bulk density) properties, herbaceous and woody biomass and NDVI among the treatments. The relationships between soil organic C and total soil N stocks, soil, plant and litter C:N ratios were explored *via* linear regression. Linear Mixed effects models estimated using Maximum Likelihood ratio, were used to determine other explanatory variables for soil organic C stocks. The variables used for the models as fixed factors were treatments, soil chemical

(C, N, cations, metals, pH) and physical (particle size, bulk density) properties, and vegetation characteristics (woody cover, herbaceous biomass, NDVI) and microbial biomass. Non-independent nested replicates were used as random factors. Models with the lowest Akaike Information Criterion (AIC) value and highest conditional and marginal R^2 values were selected.

2.4 Results

2.4.1 Soil organic C and total soil N stocks

In the grassland soil organic C stocks were affected by fire and herbivory where plots under biennial fires and annual fires had the highest mean stocks (Fig 2.5) and were lower where either biennial or annual fires were combined with herbivory. The presence of herbivory lowered the amount of soil organic C stocks by 16% in both fire treatments. The no disturbance plots were not different in mean soil organic C stocks to the fire and herbivory combined treatments. In the savanna sites, neither fire nor herbivory affected soil organic C stocks (Fig 2.5). Similarly, total soil N stocks in the grassland were higher with biennial and annual fires (Fig 2.6) and lowest when biennial and annual fires were combined with herbivory as well as with no disturbance. Again, treatments had no effect on total soil N stocks within the savanna.

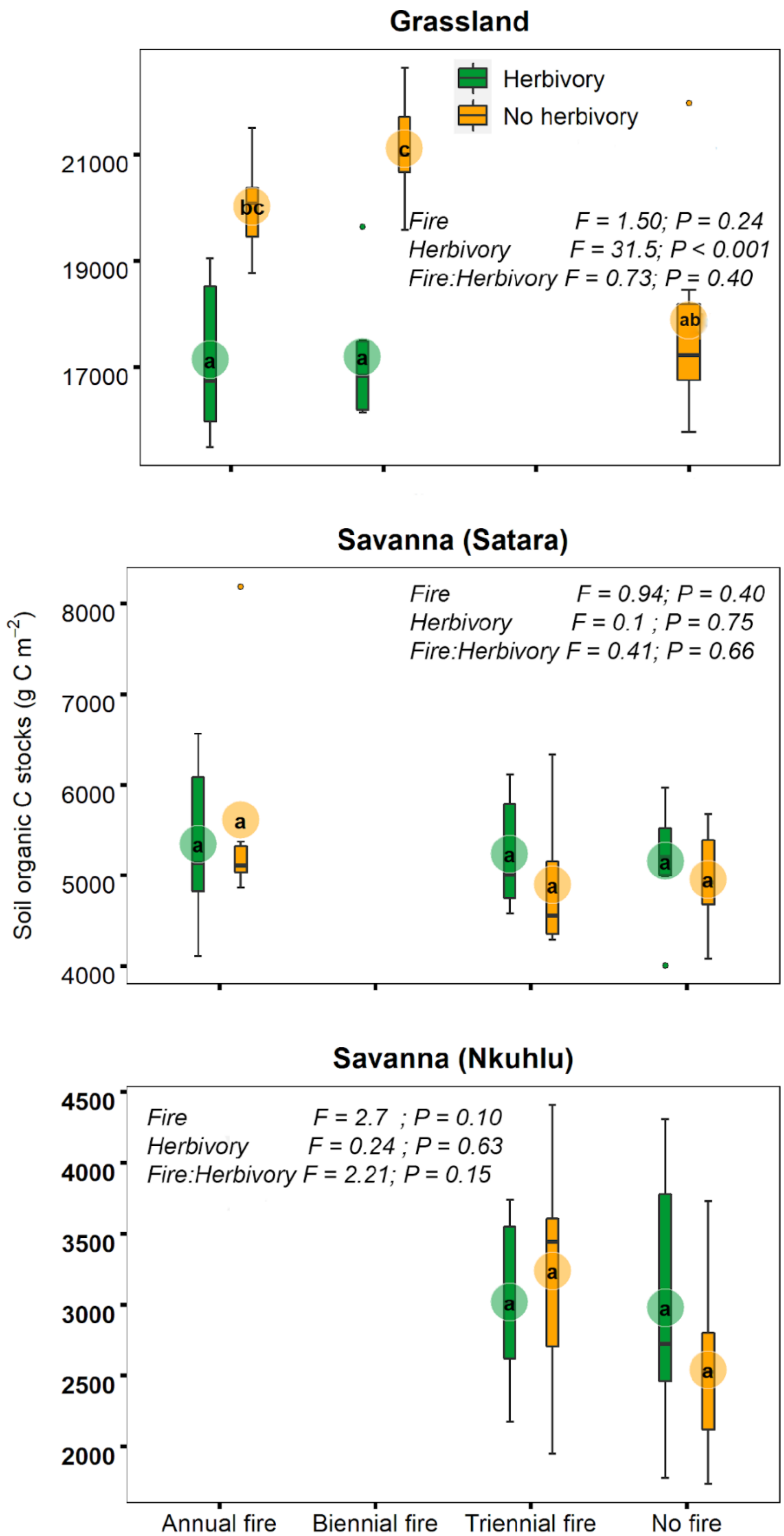

Figure 2.5: Comparison of soil organic C stocks in the grassland and savanna with various fire and herbivory treatments. Boxplots denote range of data (upper, median and lower quartiles) with means indicated by circles with letters. No common letter indicates significant differences at $P < 0.05$.

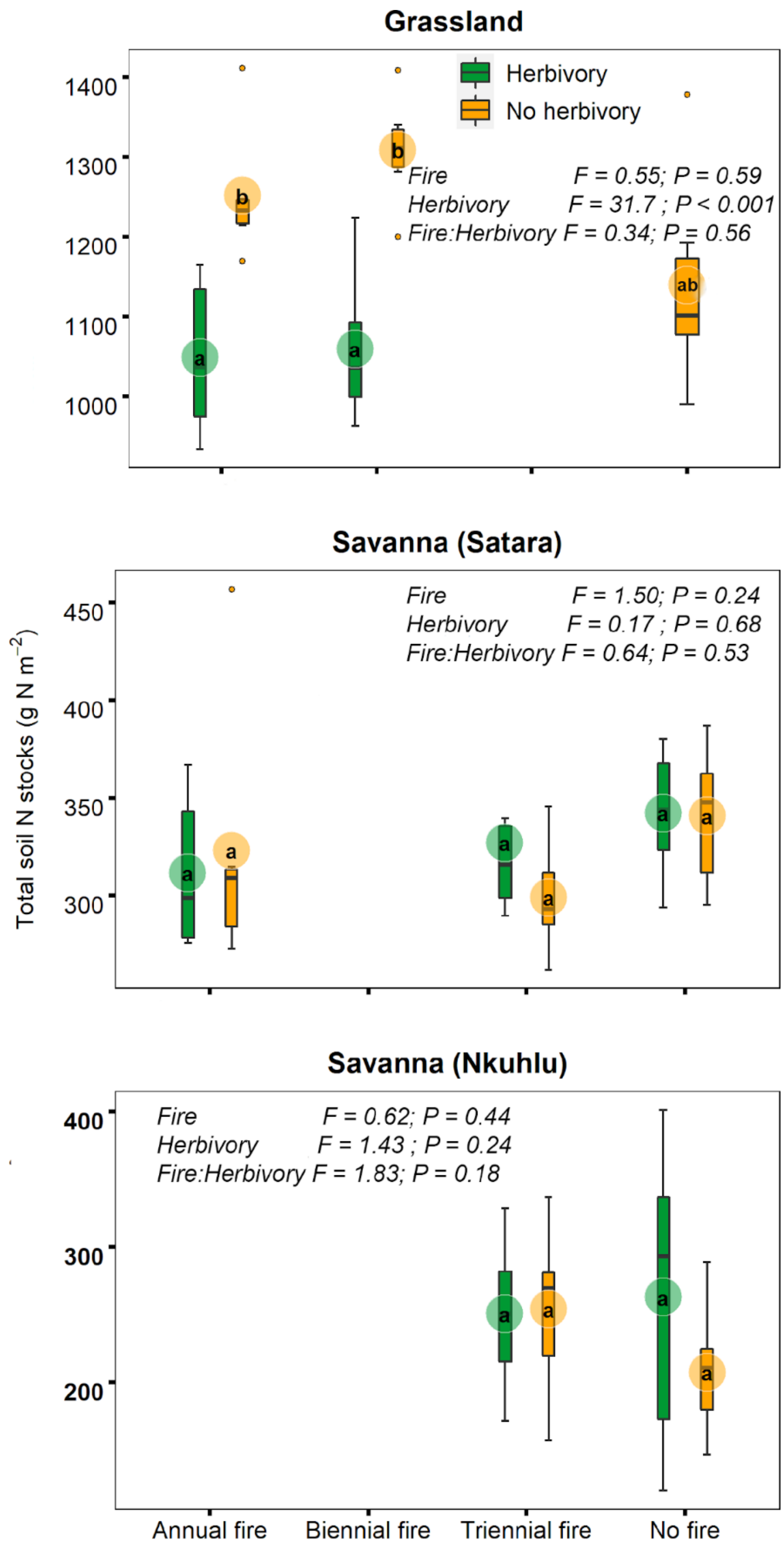

Figure 2.6 Comparison of total soil N stocks in the grassland and savanna with various fire and herbivory treatments. Boxplots denote range of data (upper, median and lower quartiles) with means indicated by circles with letters. No common letter indicates significant differences at P < 0.05.

2.4.2 Soil, foliar and litter C:N ratios

In the grassland, soil C:N did not vary overall (Fig 2.7). However, fire had a slight effect on soil C:N ($P = 0.041$). In one savanna site (Nkuhlu, Fig 2.7), soil C:N again did not vary across treatments while in Satara, plots with annual fire alone and with herbivory had significantly higher soil C:N ratios than no disturbance plots. Overall the Nkuhlu savanna site had the lowest soil C:N. Foliar C:N ratios in the grassland (Fig 2.8) were higher with biennial fires and herbivory compared to annual fire and herbivory or no disturbance. In the savanna (Satara), triennial fire with herbivory had significantly higher foliar C:N than other treatments. At Nkuhlu, triennial fire alone and no disturbance had higher foliar C:N ratios compared to other treatments. Litter C:N results were only available for the savanna. In Satara, no disturbance resulted in significantly higher litter C:N in comparison to the other treatments, while in Nkuhlu, herbivory alone resulted in relatively low litter C:N. Satara had more litter with high C:N ratios over 80:1 while in Nkuhlu the maximum litter C:N ratios were about 50:1. Litter had generally high C:N compared to foliar or soil samples.

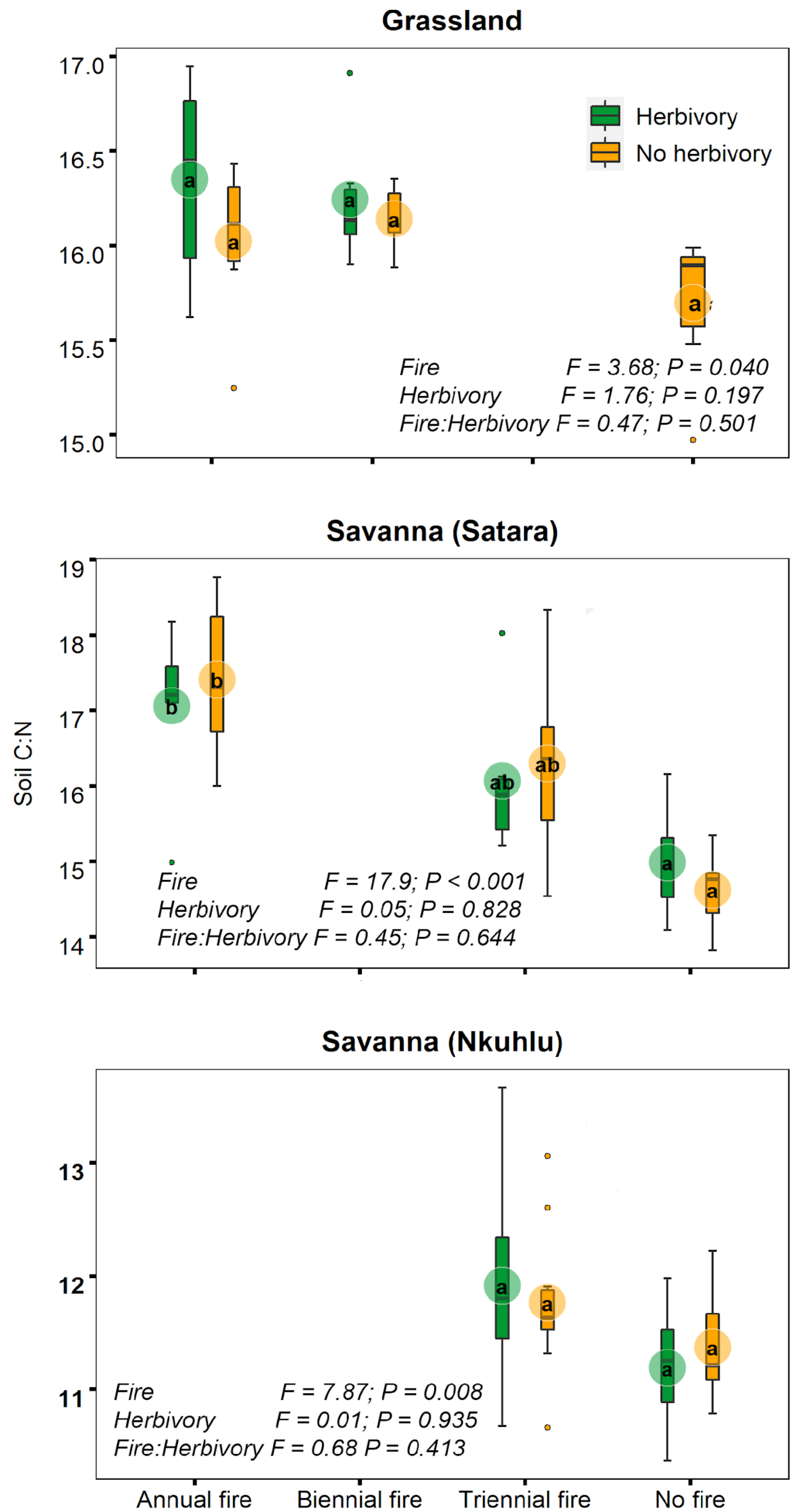

Figure 2.7: Comparison of foliar C:N in the grassland and savanna with various fire and herbivory treatments. Boxplots denote range of data (upper, median and lower quartiles) with means indicated by circles with letters. No common letter indicates significant differences at $P < 0.05$.

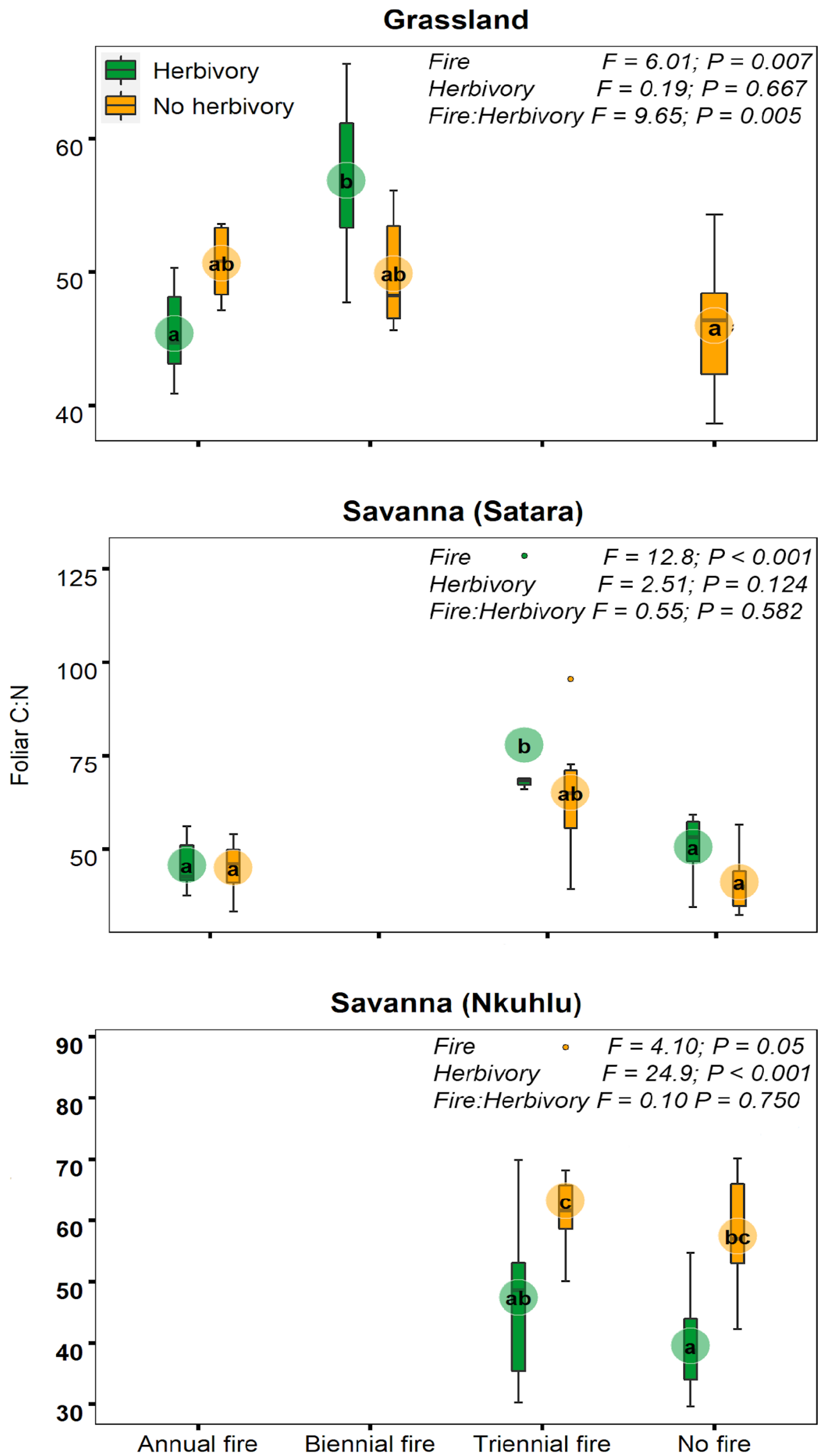

Figure 2.8: Comparison of foliar C:N in the grassland and savanna with various fire and herbivory treatments. Boxplots denote range of data (upper, median and lower quartiles) with means indicated by circles with letters. No common letter indicates significant differences at P < 0.05.

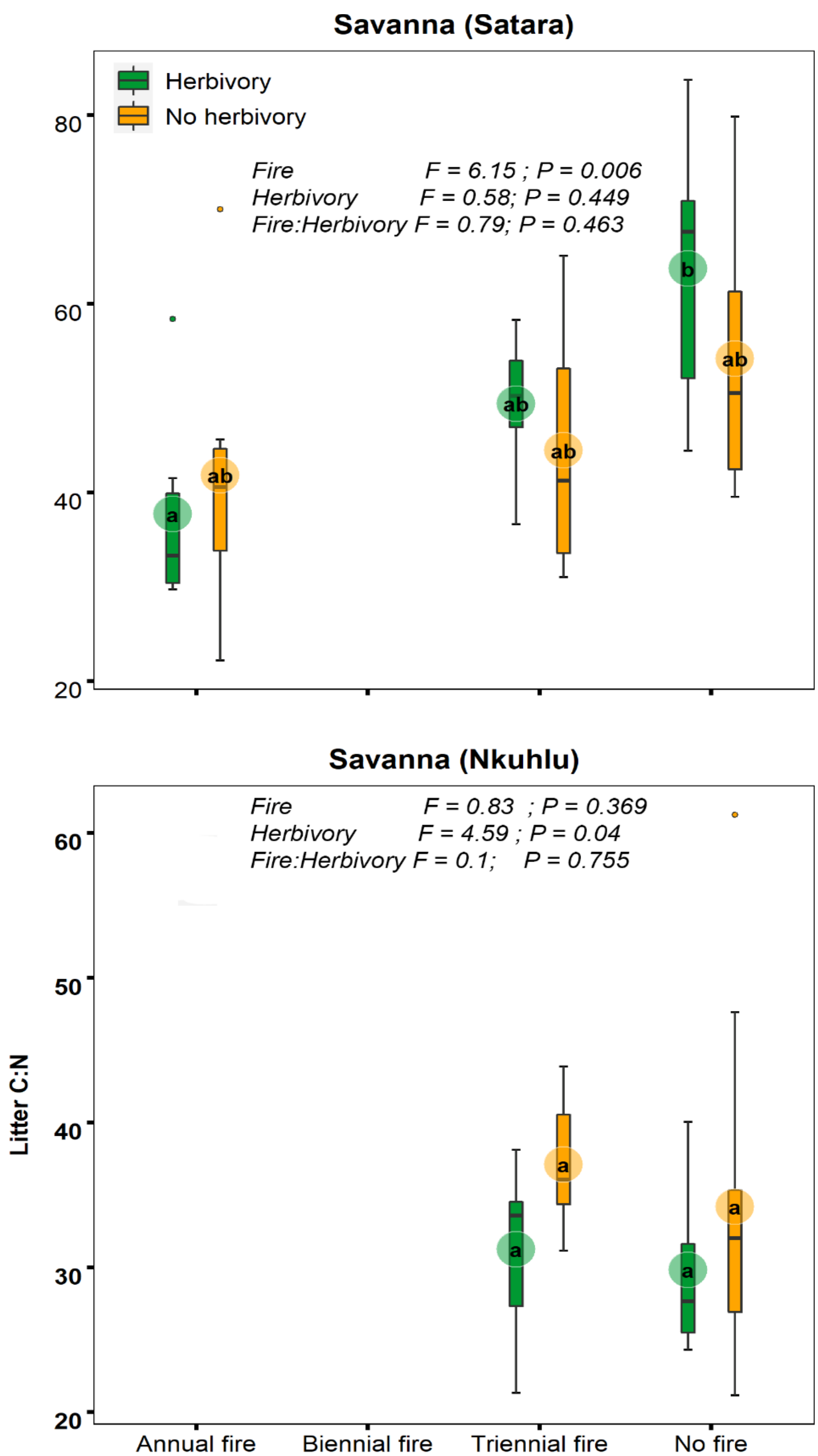

Figure 2.9: Comparison of litter C:N in the grassland and savanna with various fire and herbivory treatments. Boxplots denote range of data (upper, median and lower quartiles) with means indicated by circles with letters. No common letter indicates significant differences at P < 0.05.

Linear regression between soil organic C and total soil N stocks (Fig 2.10) showed strong positive relationships in the grassland and one savanna site (Satara) where relatively higher N and C concentrations in the grassland were again evident. Soil organic C stocks increased with increasing total soil N stocks. Within the grassland biennial fire, biennial fire with herbivory and no disturbance treatments exhibited the strongest relationships. In the savanna (Satara), annual fires and triennial fires had the strongest relationships. There were weak relationships between the two variables in the other savanna site (Nkuhlu). They were overall weak and insignificant linear relationships ($R^2 < 0.1$) between soil organic C stocks and soil, foliar or litter C:N ratios. Linear mixed effect models were fitted for soil organic C stocks with other soil physio-chemical properties and environmental variables to incorporate variation from random and fixed effects. In the grassland, soil organic C stocks were predicted by treatments, total soil N stocks, herbaceous biomass and bulk density (Table 2.2). The model's total explanatory power was substantial (conditional $R^2 = 0.96$). Within this model: the effect of total soil N stocks was positively very large and significant. The effect of herbaceous biomass was positive and can be considered tiny and significant. The effect of bulk density was positive and considered very small and significant.

In the savanna (Satara, Table 2.2), the fitted linear mixed effect model predicted soil organic C stocks with total soil N stocks, soil K and herbaceous biomass. The total explanatory power was substantial (conditional $R^2 = 0.87$ for both fixed and random effects) and the part related to the fixed effects alone (marginal $R^2 = 0.87$). Within this model the effect of total soil N was positive and considered as large and significant. The effect of soil K was negative and considered as very small while the effect of herbaceous biomass was negative and considered small and significant. In the other savanna site, very little differences were observed as shown in the ANOVA results for either soil organic N stocks, total soil N or herbaceous biomass. The fitted model for soil organic C stocks was only predicted by NDVI which had a small and significant effect, which explained 28 % of the variation (conditional $R^2 = 0.28$) and fixed effects alone explained 17 % of the variation (marginal $R^2 = 0.17$).

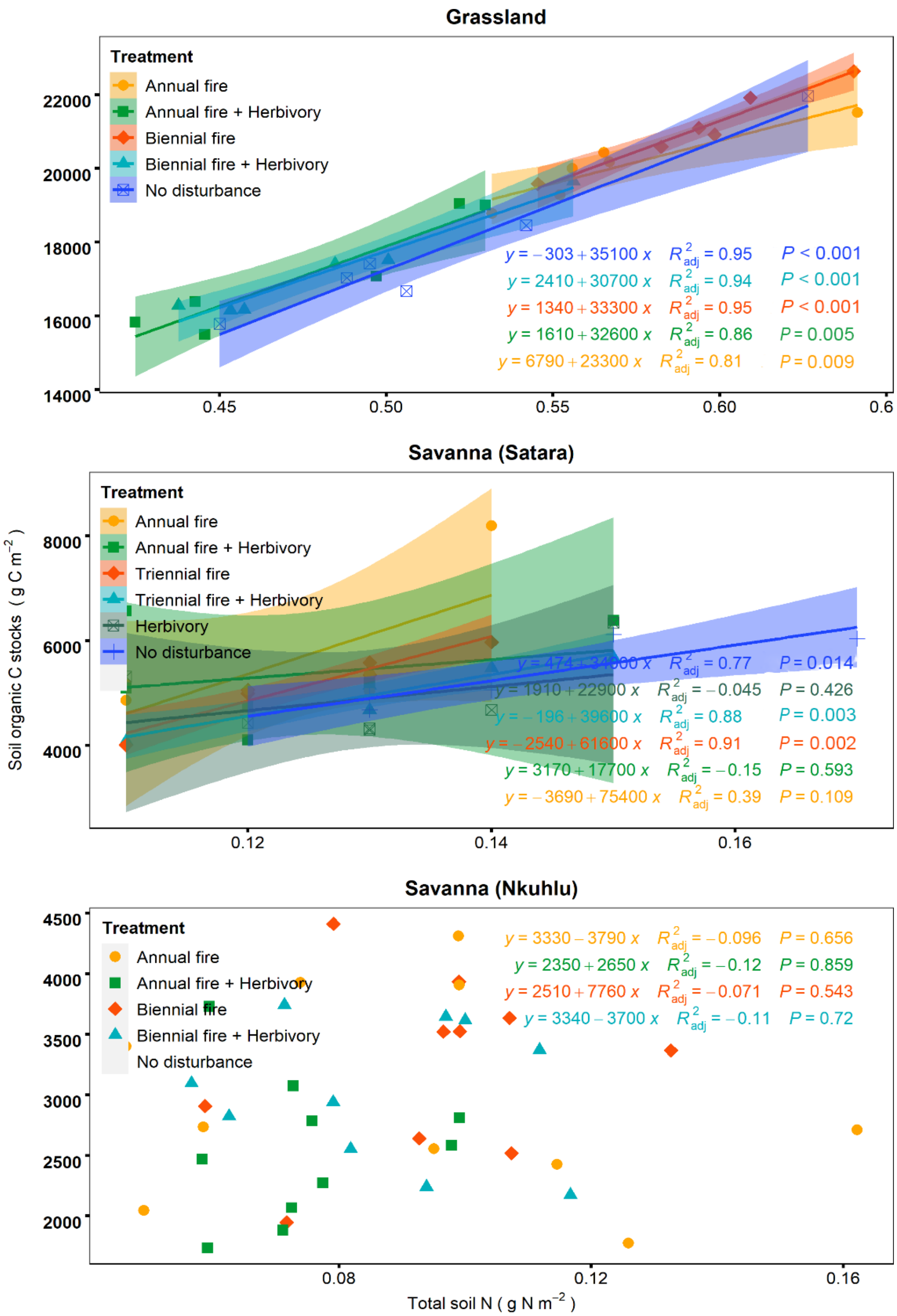

Figure 2.10: Relationship between soil total organic N and C stocks in the grassland and savanna with various fire and herbivory treatments. Models were fitted *via* linear regression and individual model and adjusted R^2 of each treatment are presented. Confidence intervals for model fit lines were computed for the top two graphs.

Table 2.2: Results of the linear mixed effect models for soil organic C from the grassland and savanna with various fire and herbivory treatments. Significant model parameters, coefficient estimates, and the significance (p-values) of the parameters, conditional (entire model) R^2 values are presented.

Parameter	Beta Coefficient	p-value	Model R^2
Grassland			
Treatment	138.62	0.040	0.96
Total soil N	14.62	< 0.001	
Bulk density	133.77	0.03	
Herbaceous biomass	- 0.41	0.040	
Microbial biomass	0.80	0.024	
Savanna (Satara)			
Total soil N	15.7	< 0.001	0.87
Soil K	-1203.2	0.016	
Herbaceous biomass	-2.1	< 0.001	
Savanna (Nkuhlu)			
NDVI	-4688	0.004	0.28

2.5 Discussion

Many studies have reported a fire-induced mechanism for soil organic C sequestration *via* formation of different recalcitrant pyrogenic C compounds, such as charcoal, that are relatively resistant to microbial decomposition (González-Pérez et al., 2004; Knicker, 2007). Our results from the grassland support this mechanism where we observed relatively higher soil organic C stocks with annual and biennial burning compared to herbivory or no disturbance. Another mechanism that may drive soil C stocks is fire-induced microbial death (Dooley and Treseder, 2012; Köster et al., 2014) and it is possible that the resultant reduction in microbial biomass reduced decomposition of soil organic matter, thereby allowing it to accumulate into the soil organic C pool. The low soil organic C stocks with fire plus herbivory may at face value, indicate that the increased disturbance pressure resulted in less detritus inputs to the soil. Herbivores, especially grazers are known to compete with fire for herbaceous fuel (van Wilgen et al., 2005) thereby exacerbating disturbance. However, in our grassland site, herbivore numbers are naturally low while being within the carrying capacity of the area and the burning regime outside the burn trial is consistent. We speculate that this may have led to less organic matter inputs overall, as exemplified by the no disturbance treatments which were similar in soil organic C stock levels. However, although no disturbance and fire with herbivory treatments had similar organic C stocks, the mechanisms could reasonably be different for example, in no disturbance most C stays aboveground and has less opportunity to enter the soil pool, while for fire and herbivory, volatilisation of N and excess consumption of aboveground biomass by herbivores and fire could limit how much C enters the soil pool. Other studies have observed contrasting effects of herbivory particularly grazers, on soil organic C stocks ranging from very low (Golluscio et al., 2009) to very high (Pei et al., 2008).

Results from the savanna indicate that the time which the different areas are subjected to different treatments is more important in causing changes in soil organic C stocks. For example, the Satara savanna experimental burning trial was established in 1954 (Biggs et al., 2003) while the herbivore exclusion plots are much more recent having been setup in 2006/7. In support of the time phenomenon, Deng et al (2016) found that it took *ca.* 30 years for new soil organic C to incorporate with old soil organic C within the top 20 cm of soil with change in land-use, the same depth that the soil samples of this study were taken. Other studies in the Satara savanna site found that frequently repeated fires increased grazing intensity (Enslin et al., 2000; Jacobs and Biggs, 2001, Biggs et al., 2003; Smit and Archibald, 2018), and based on this, we expect more variation in soil organic C stocks to be exhibited from the influence of herbivory and fire with increased site age.

We found soil organic C stocks to be predicted by several soil physio-chemical properties and environmental variables which mainly included treatments, total soil N stocks, herbaceous biomass, microbial biomass and bulk density. Total soil N stocks were an especially strong predictor where soil organic C stocks generally increased with increasing soil total N, thus indicating the strong link between soil N and soil organic C sequestration. In a comparable meta-analysis study by McSherry and Ritchie (2013), 85% of the variation in soil organic C stocks were explained by several variables which included soil texture, grazing intensity, herbaceous biomass composition, precipitation, study duration and soil sampling depth. However, one savanna site (Nkuhlu) lacked many strong predictors of soil organic C stocks possibly due to the low soil N which could have potentially limited the formation of soil organic C and thus reducing the effects of fire and herbivory. The microbial C-use efficiency is a concept that supports this, where the ratio of microbial growth to total assimilation of C as it is used for their biomass production or respired as CO_2 depends on the N quantities in the soil (Rousk and Soares, 2019; Blagodastkaya et al., 2014). Our hypothesis predicted that herbivory would change foliar and litter C:N ratios thereby driving soil organic C stocks *via* the quality of organic matter inputs. This was based on the known intrinsic linkage between microbial organic matter decomposition, soil C:N ratios and soil organic C turnover (Roberston and Groffman, 2007). Microbes are known to decompose more organic matter when it is rich in N i.e organic matter with a low C:N ratio (Aanderud et al., 2018) and in turn regulate the formation of soil organic C stocks (Rousk and Soares, 2019). However, in our study we found that soil N was the most important driver of soil organic C stocks. This concurs with other studies (Giardana et al., 2004; Keeler et al., 2009; Boddey et al., 2010; Luo et al., 2014; Deng et al., 2016) where increases in soil N positively affected the sequestration of soil organic C.

We compared the effects of fire and herbivory on the total soil N stocks and contrary to our hypothesis, the opposite was observed where the plots subjected to fire had a consistently higher total soil N as opposed to the expected lower soil N due to increased N volatilization. Some studies support these findings and have shown that fires can increase soil N following the first few years after burning (Certini, 2005; James et al., 2018). This increase in soil N is due to the release of ammonium and nitrates following the stimulation of nitrification processes by heat from fire (Wan et al., 2001). Hart et al. (2005) also observed that some nitrifying bacteria increased by as much as 10 times the levels of those in unburnt areas, while surviving fungi became more functionally diverse, thus increasing soil nitrate levels. The increase in pH often caused by ash addition to soils has also been shown to improve recovery of soils post-fire i.e increased aboveground vegetation and rhizospheric processes, thereby lessening the negative effects of fire on soil nutrients (Pietri and Brooks, 2008; Sanchez, 2019). The main aspect of note here is the facilitation of favourable conditions for N accumulation in the soil following a relatively short period since burning, which our sites had.

Herbaceous biomass was one of the main predictors of soil organic C stocks in two sites albeit with a small effect. In the grassland and one savanna site, the effects of herbaceous biomass were small, and soil organic C stocks increased with decreasing biomass. The treatments without either herbivory or fire had the highest standing biomass, however that did not translate to higher soil organic C stocks. Even though the treatment plots without either herbivory or fire had more biomass, the absence of these two disturbances meant very low standing herbaceous biomass to litter turnover. Herbivores for instance, can enhance herbaceous species richness and biomass in rangelands (Hickman et al., 2004) and facilitate organic matter accumulation *via* trampling and feeding on vegetation (Jacobs and Naiman, 2008). Van Wilgen et al. (2000) found that probability of fire increased with increasing grass biomass, and the resultant post-fire herbaceous flush then attracts grazers, which has been termed pyric-herbivory. This interaction of disturbances potentially increases nutrient cycling and turn-over rates. It is therefore likely that the low disturbance in the plots without herbivory or fire resulted in most of the organic matter being locked in standing above-ground biomass such as grasses, shrubs and trees, hence the low organic matter turnover rates.

2.6 Conclusions

Overall, fire alone increased both soil organic C and total soil N stocks, while either the combination of fire with herbivory or no disturbance, resulted in relatively lower C stocks in the grassland. The savanna appeared to be relatively less responsive to the effects of fire and herbivory, and may suggest a resistance to disturbance-induced change. Total soil N stocks were an especially strong predictor of soil organic C stocks, indicating a close coupling between soil organic C and soil N. Overall fire and herbivory drove soil C stocks mainly *via* effects on soil N. This relationship was clear in grasslands but less evident in the savanna, possibly due to some threshold low level of soil N in our sites. The unique fire and herbivory management histories of the sites likely contribute to these varying results. It is therefore important to include site history in future soil organic C sequestration research in order to obtain a full outlook of the impacts of fire and herbivory.

Chapter 3

Pyric-herbivory with moderate fire frequency reduces fluxes of greenhouse gases compared to high or low disturbance in grassland and savanna soils

3.1 Abstract

The assessment of soil greenhouse gas emissions is critical in mitigating global emissions considering that soils are a large C reservoir and significant sinks and sources of the principal greenhouse gases, carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). We examined soil CO_2, CH_4 and N_2O fluxes from a grassland and savanna within Long-Term Ecological Research sites, using a combination of herbivory and fire treatments distinguished by fire frequency. Herbivory was expected to reduce all gas fluxes *via* lowering soil N accumulation and increasing soil organic C stocks. Fire was expected to reduce CO_2 and N_2O fluxes *via* addition of stable pyrogenic compounds and N volatilisation respectively, while increasing CH_4 fluxes due to fire-induced death of methanotrophic microbes. In the grassland, herbivory plus annual fires resulted in the highest CO_2 fluxes (2.28 ± 0.72 µmol m^{-2} s^{-1}) and no disturbance treatments had the lowest fluxes (1.28 ± 0.33 µmol m^{-2} s^{-1}; $P < 0.001$). Soil CO_2 fluxes did not differ in either of the savanna sites ($P > 0.05$). Treatments affected root respiration and had no effect on microbial respiration. Generally, lower frequency fires (biennial or triennial) resulted in increased CH_4 uptake (-1.84 ± 0.44 µg m^{-2} h^{-1}) while high frequency fires (annual), resulted in CH_4 emissions (1.38 ± 2.84 µg m^{-2} h^{-1}). We estimate that the grassland and savanna soils we sampled can consume *ca.* 0.47% per m^2 of the CH_4 released by wetlands and extrapolation to global levels will greatly depend on spatial-temporal scales and management practices. It is evident that the higher disturbance level brought about by high frequency fire exacerbated by herbivory led to higher emissions of both soil CO_2 and CH_4 overall. Although tropical grasslands and savannas are thought contribute about 16% to the global terrestrial N_2O emissions, our results indicate a very low contribution from South African grasslands and savannas even under frequent burning. Fluxes of CO_2 constituted 99% of overall fluxes in all sites, and we therefore suggest that soil emission reduction strategies in African grasslands and savannas consider soil processes that predominantly cause CO_2 loss. Additionally, adoption of low frequency fire regimes and moderate stocking rates of wild and domestic ungulates can curb greenhouse gas emissions in native grasslands, savannas and working rangelands.

3.2 Introduction

The increase in global greenhouse gas emissions within a relatively short period since the 1950s (Ciais et al., 2013), has prompted intensive research focused on mitigating emissions from various sources, including soils. Soils contain the largest terrestrial C and N pools, unequally distributed within the uppermost metre of the soil where *ca.* 1500 Pg of total C and *ca.* 136 Pg of total N are stored (Kutsch et al., 2009; Schaufler et al., 2010). Soils can either be sinks or sources of greenhouse gases and this can be influenced by land use (Lubbers et al., 2013; Oertal et al., 2016; Paustian et al., 2016). Anthropogenic activities such as uncapped agricultural expansion, unregulated burning and unplanned movement of wild and domestic ungulates can increase losses of C and N from soils as greenhouse gases (Lal, 2010; Onti and Schulte, 2012; Sahoo et al., 2019). Large areas of grasslands and savannas are used as rangelands, and these are under mounting threat of conversion to agricultural and bushlands (Rashford et al., 2012). Although grasslands and savannas sequester less C per unit area than forests (Du Toit and Du Preez, 1995; Mills and Fey, 2003; Brandt et al., 2018), their wide coverage of the earth's land surface (White et al., 2000) make them important sinks of C. These biomes are therefore pivotal in mitigating rising greenhouse gas concentrations *via* their large potential in sequestering C in their soils (Toma et al., 2013). Moreover, grassland and savanna soils allocate as much as 87% of their biomass belowground (Lee et al., 2010) compared to forests which allocate *ca.* 29% and C stored in belowground biomass is more stable and less susceptible to depletion than aboveground C (Paustian et al., 2016; Conant et al., 2017). The greenhouse gas sink potential of grassland and savanna soils (due in part to their wide coverage) is thus large enough to compensate for most of the greenhouse gases emitted from anthropogenic sources such as agriculture (Schulze et al., 2009). Therefore, more concise local and regional soil greenhouse gas emissions data are required for extensive global and localised C budgets, especially in Africa given that soil degassing contributes 35% CO_2, 47% CH_4 and 53% N_2O to the total global emissions (IPCC, 2007). In this regard, soil greenhouse gas emissions are integral in global change research and natural land management (Oertal et al., 2016).

Several biotic processes (autotrophic and heterotrophic respiration, microbial activity) and chemical decay processes occur within the uppermost soil rhizosphere resulting in the production of soil greenhouse gases (Chapuis-Lardy et al., 2007; Lubbers et al., 2013). The biotic processes that produce soil greenhouse gases are proximally driven within the direct environment by local climates and soil type, wherein substrate availability (mineral N and labile C) is particularly important (Lubbers et al., 2013). Microbial activity is dependent on substrate availability, and the quality (C:N ratio) and quantity of the substrate determine whether microbes mineralize or immobilize mineral nutrients when they decompose soil organic matter (Fig 2.1: Chapter 2).

Besides climatic and edaphic factors, fire and herbivory are principal drivers of ecosystem function within grasslands and savannas (Pingree and DeLuca, 2017) and unlike climate and soil, these ecological disturbances can be managed. Thus, we examined how fire and herbivory affected soil greenhouse emissions at the local scale by making use of ecological research sites with a combination of herbivory and different fire frequencies. Fire and herbivory are prominent features of grasslands and savannas, which if not adequately managed can result in soil greenhouse gas losses *via* herbaceous biomass reduction erosion (FAO and ITPS, 2015). Grasslands and savannas play host to an assemblage of different-sized herbivores with varying dietary preferences (Plas et al., 2016). Ecological theory predicts that herbivores increase plant diversity in high productivity areas and decreases it in low productivity areas (Bakker et al., 2006; Hillebrand et al., 2007) and they can maintain local-scale plant diversity by selectively feeding on dominant plants (Borer et al., 2014). Dominant higher nutrient quality (low C:N) plant species may be selectively consumed by herbivores, thereby altering the abundance and composition of plant species (Pastor et al., 1993; Harrison and Bardgett, 2004). Accordingly, the rates of litter decomposition are driven mainly by plant species with the highest nutrient availability (Wang et al., 2018) and such plants are highly palatable to herbivores (Cornelissen et al., 1999). As such, grazing would result in low quality (high C:N) detritus inputs to the soil that reduce decomposition rates (Pastor et al., 1993; Moretto et al., 2001), which would translate to reduced soil greenhouse gas emissions from soil organic matter decomposition.

On the other hand, fire affects plant communities very often positively, by triggering changes in soil nutrient cycling when immediate nutrient mineralisation occurs during burning (Bowman et al., 2016; Kelly and Brotons, 2017). This in turn may increase plant productivity due also in part due to the resultant reduced plant competition (Scott et al., 2013). The frequency and intensity of fires is key in influencing plant species composition (Bond and Keeley, 2005), where vegetation homogenisation can occur with high fire frequency (Gill et al., 2014) or conversely increased plant diversity results with low fire frequency (Bowman et al., 2016). Fire-altered organic matter (charcoal/biochar, soot, tar), also known as pyrogenic C, is a major product of burning in ecosystems (Pingree and DeLuca, 2017) and is highly recalcitrant due to its high C and low N concentrations (Preston and Schmidt, 2006; Bostick et al., 2018). As such, the persistence of pyrogenic C in soils has been estimated to range from hundreds to thousands of years (Gavin et al., 2003; Laird et al., 2008; Liang et al., 2008).

In grassland and savanna ecosystems among others, herbivores and fire interact closely (pyric-herbivory) where herbivores depend on fire by feeding on both burnt and unburnt patches and occurrence of fires is depended upon removal of fuel by herbivores (Fuhlendorf and Engel, 2001; Fuhlendorf et al., 2008). Determining the effects of pyric-herbivory rather than independently decoupling the effects of herbivory and fire (Fuhlendorf et al., 2008) can provide a better understanding of these two disturbances given that they interact with other disturbances at multiple and broad scales (Archibald et al., 2005; Waldram et al., 2007). Furthermore, rangelands have historically been shaped by fire and herbivory (van Etten, 2010; Hempson et al., 2015; Bernardi et al., 2019) and as such an in-depth understanding of their interplay is critical to the management and conservation of grassland and savanna ecosystems for climate change resilience.

Based on this, we hypothesised that herbivory would reduce emissions of all soil greenhouse gases when herbivores selectively grazed nutritious herbaceous material, leaving out the poor-quality plant material which when eventually incorporated into the soil, would cause microbial immobilization of soil nutrients. We further hypothesised that fire would reduce soil greenhouse gas emissions by increasing additions of recalcitrant pyrogenic C compounds that are more resistant to microbial decomposition. Volatilization of N and death of methanotrophic bacteria including other microbes during conflagration would further reduce emissions of all soil greenhouse gases.

3.3 Materials and methods

3.3.1 Site characteristics

The study was conducted during the wet growing season (late 2018 to early 2019) of the mesic montane grasslands and mesic savanna of South Africa. Grassland sampling was conducted in the experimental burning plots on the Brotherton burn trial at Cathedral Peak, uKhahlamba-Drakensberg Park, South Africa (Fig 2.2; Chapter 2). Savanna sampling was conducted in the herbivore exclosures in Long Term Ecological Research (LTER) sites at Nkuhlu and Satara, Kruger National Park (Fig 2.2; Chapter 2). The pedo-topo-climatic context, vegetation and herbivore compositions are described in Table 2.1; Chapter 2.

3.3.2 Sampling design

At the grassland Brotherton experimental burn trial, sampling area was defined by the existing 25 x 25-m plots subjected to annual fire, biennial fire or no fire (Fig 2.3; Chapter 2). To obtain pyric-herbivory treatments, plots were defined outside the trial, i.e in the fire-break accessible to herbivores and burnt annually, and in the generality of the park accessible to herbivores and burnt biennially as part of the fire management regime of the park. Thus, we had an unbalanced sampling design with five treatments since the herbivory treatment lacked a 'no-fire' component. Each of the three plots per treatment were randomly sampled in two areas to include heterogeneity within treatments where the resulting six replicates per treatment (n = 30) were related to plots. In the savanna sites, we used fence-line measurements between existing treatments in LTERs (Fig 2.3; Chapter 2). At the Nkuhlu LTER site, we had a balanced sampling design with fire-herbivory treatments: herbivory - triennial fire (for this site fire frequency was classified as 3 to 5 years), triennial fire only, no disturbance and herbivory only. Again sampling (soil, vegetation, gas flux) was semi-random. We constrained sampling to fence-lines, a similar soil type and between-canopy positions to reduce between-treatment variation as well as to allow for the logistics of moving quickly between sites during gas sampling, which was only done between 10h00 and 14h00. Once at the sampling site, ten points were randomly selected for sampling. At Satara, six full herbivore exclosures were selected and sampled inside and out from three fire-based treatments (annual, triennial, no fire).

3.3.3 Soil greenhouse flux determination

Soil CO_2 fluxes were measured using a dynamic closed soil respiration chamber equipped with an infrared gas analyser (LI-COR 8100A Automated Soil CO_2 System, LI-COR Biosciences Ltd). The flux measurements were taken using PVC collars (0.11-m diameter; 0.09-m height) inserted into the soil at least 24-hours prior to sampling. Volumetric differences caused by collar-soil surface offsets were accounted for in the chamber volume calculations. Repeat measurements were taken for 5-d where each measurement was applied for 90 s, after which a flux rate measured in µmol m^{-2} s^{-1} was recorded. Soil temperature and moisture measurements were obtained using a temperature probe (Soil Temperature Thermistor probe) and moisture probe (GS1 soil moisture probe) inserted adjacent to the soil collar during flux measurements and these were used to in the final flux calculation.

Soil core samples were collected from the field from which 300-g soil microcosms were subsampled for *ex situ* laboratory gas flux measurements. Field soil samples were homogenized, and air dried for 7-d (Chapter 2: Materials and Methods). The laboratory soil CO_2 flux measurements represented the microbial portion of soil respiration minus root respiration (roots killed during soil air drying) in comparison to the field CO_2 fluxes that included all soil respiration sources. Soil microcosm pots were set up in a phytotron chamber maintained at 25°C in the dark at 60% relative humidity and constant air circulation. Soil subsamples were first rewetted to 100% saturation and subsequently monitored and maintained at 40% field capacity prior to taking CO_2 flux measurements. Initial monitoring measurements were first taken until the soil CO_2 flux measurement fluctuations caused by a soil respiration flush due to soil rewetting levelled off. Repeated flux measurements were subsequently taken for 5 d. Final flux CO_2 flux measurements were adjusted based on measured soil temperature and moisture in the data processing within the Soil Flux software.

Soil trace gases (CH_4 and N_2O) were measured on a single occasion at each field site. We used static closed-chambers with a volume of 1711 cm^3 that were placed on top of PVC collars inserted into the ground as described above. Gas volumes for flux calculations were adjusted to account for both collar offsets and chamber-collar overlaps. Gas samples (20-mL) were collected *via* a syringe inserted into a rubber septum on each chamber and immediately transferred into 12-mL evacuated *via*ls, at four-time intervals: t_0, t_{15}, t_{30} and t_{45} minutes to obtain flux rates. Gas samples were analysed for concentrations of N_2O and CH_4 with a gas chromatograph (Bruker, Scion 456-GC Bruker Daltonics, Chemical Analysis, NL) equipped with an electron capture detector for N_2O and flame ionization detector for CH_4. Gaseous flux rates over the 45-minute period were calculated using the slope of gas concentrations (in ppm) over time following the method by Beetz et al. (2013):

$$Flux = \frac{273.15}{T}\frac{V}{A}\frac{\Delta c}{\Delta t}$$

where the calculated flux (μg m^{-2} s^{-1}) was determined by T**,** the mean temperature inside the chamber in Kelvin, V the total volume of the chamber (including collar offset) in m^3, A the area of the collar (0.01 m^2), and $\frac{\Delta c}{\Delta t}$ the concentration change in the chamber headspace over time (mg kg^{-1} h^{-1}). Soil physical and chemical characteristics, foliar and litter nutrient contents were analysed *via* mass spectrometry and x-ray fluorescence (Chapter 2: Materials and methods). Soil microbial biomass was obtained *via* chloroform fumigation extraction (Chapter 2: Materials and methods). Besides fire and herbivory treatments, soil temperature and moisture, soil physical and chemical characteristics, foliar and litter nutrient contents, soil microbial biomass and vegetation (herbaceous and woody) biomass were considered as drivers of soil gas flux.

3.3.4 Statistical analyses

All statistical analyses were computed in R 3.6.3 (R Core Team, 2019). Data were first tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests respectively. Differences in soil CO_2 fluxes from the field and lab measurements were examined using Factorial Repeated Measures ANOVA where treatments, day of measurement and sample number were used to determine within and between subject effects *[formula: flux = treatment * day + (day|sample)]*. Data were further tested for sphericity [Mauchly's test] and extreme outlier assumptions. Two-way ANOVA and Tukey Honestly Significant Difference (HSD) post-hoc tests were applied to distinguish differences among the treatments for the three dependent variables (CO_2, CH_4, N_2O fluxes). Mean values are presented ± standard de*via*tion. To determine other explanatory variables for fluxes, Linear Mixed effect models using the Maximum Likelihood ratio were used, where Akaike Information criterion (AIC), Bayesian Information Criterion (BIC) and R^2 (conditional and marginal) values were used to select the most parsimonious models. The fixed effect variables considered for the mixed models were treatments, soil temperature and moisture, soil organic C stocks, total soil N, soil macronutrients (P, K, Ca, Mg), soil texture, soil pH, bulk density, herbaceous and woody biomass, microbial biomass. Non-independent nested replicates were used as random factors. All relevant explanatory variables were first correlated (*via* Pearson bivariate correlation) with flux values to determine any significant relationships to inform selection of variables for linear mixed effect models.

3.4 Results

3.4.1 Soil CO_2 fluxes

The relationships between soil CO_2 fluxes and soil temperature and moisture in the field were weak in all the sites ($R^2 < 0.12$; $P > 0.05$). Slight positive and negative correlations were observed with soil CO_2 and soil temperature and moisture respectively, most notably in Nkuhlu when there was a singular occurrence of precipitation preceding one of the measurement days. In the grassland, field CO_2 fluxes which included root and soil microbial respiration sources were affected by treatments over the measurement period (Fig 3.1). The boxplots in Fig 3.1 represent the mean CO_2 fluxes of all 5-d. Tukey-HSD pairwise comparisons showed that annual fires plus herbivory produced the highest mean fluxes whereas the lowest fluxes were from the no disturbance treatments. In the savanna sites, treatments did not affect field soil CO_2 fluxes over time (Fig 3.1) but day of measurement had a significant effect on fluxes (in Nkuhlu). Grassland *ex-situ* (soil microbes) fluxes representing soil microbial respiration were not affected by treatments (Fig 3.1) but were significantly lower than field fluxes ($P < 0.001$). *Ex-situ* fluxes were also not affected by treatments in both savanna sites. In both savanna sites, field fluxes were significantly higher than the *ex-situ* fluxes ($P < 0.001$).

Fluxes from the field in the grassland were initially high but converged towards the end of the 5-d measurement period (Fig 3.2). In one savanna site (Satara), fluxes were constant *in situ* over the measurement period and but fluctuated in the *ex-situ* measurements (Fig 3.2). In the second savanna site (Nkuhlu), field fluxes were initially high in the first measurement but decreased drastically on the second measurement, then eventually tapered off to initial levels by the end of measurements on day five. The trend in fluxes for the second savanna site (Nkuhlu) corresponded with the local precipitation where there was a single day of precipitation after the second measurement, thereby raising the subsequent fluxes. In the grassland and other savanna site, no precipitation occurred during the measurement days.

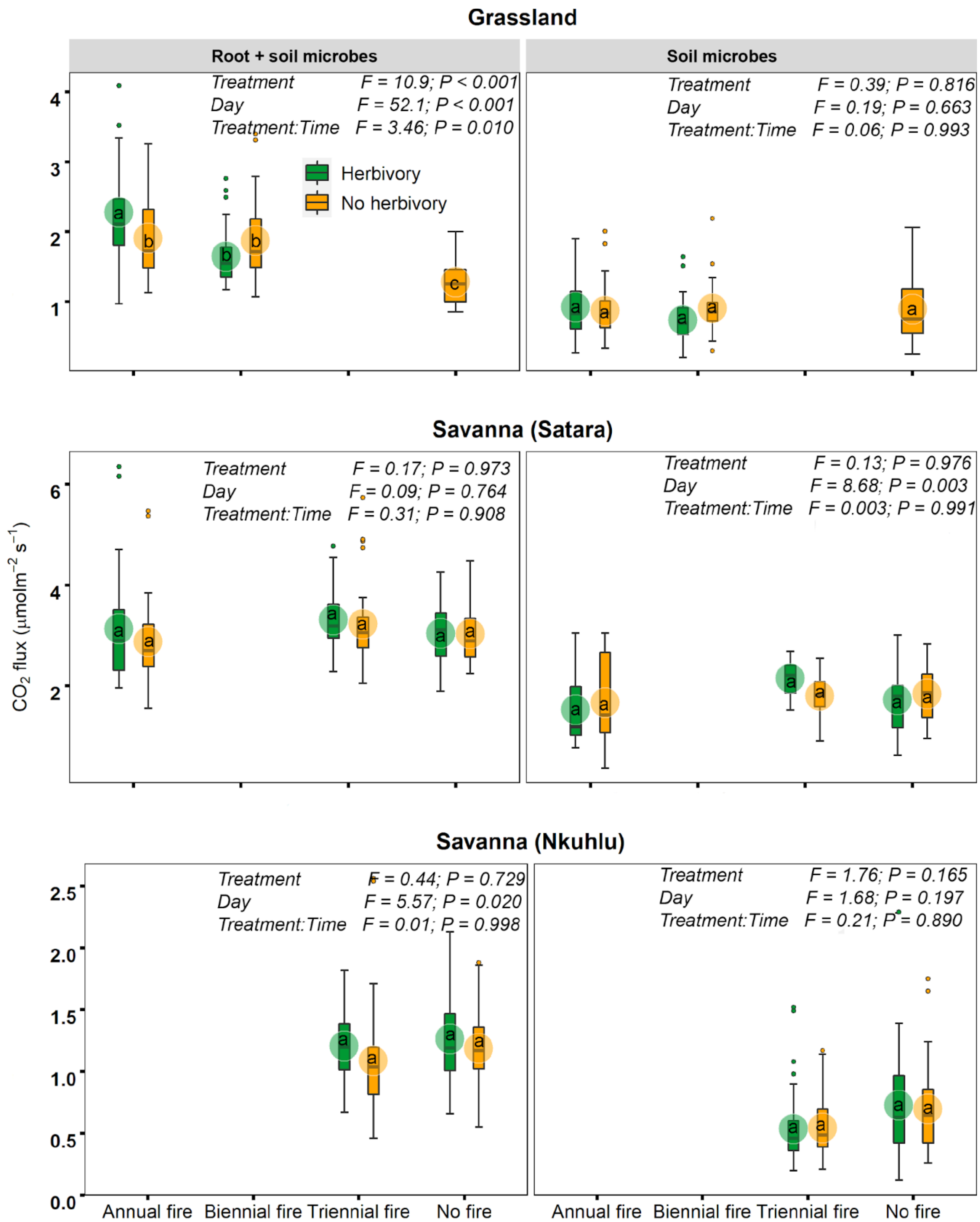

Figure 3.1: Average root + soil microbial (Field) and soil microbial (Lab) CO_2 fluxes based on a 5-d measurement period in the grassland and savanna. Text shows results of factorial repeated measures ANOVAs for soil CO_2 fluxes with treatments and days of measurements as factors. Boxplots denote range of data (upper, median and lower quartiles) with means of the 5-d indicated by circles with letters. Different letters indicate significant differences at P < 0.05.

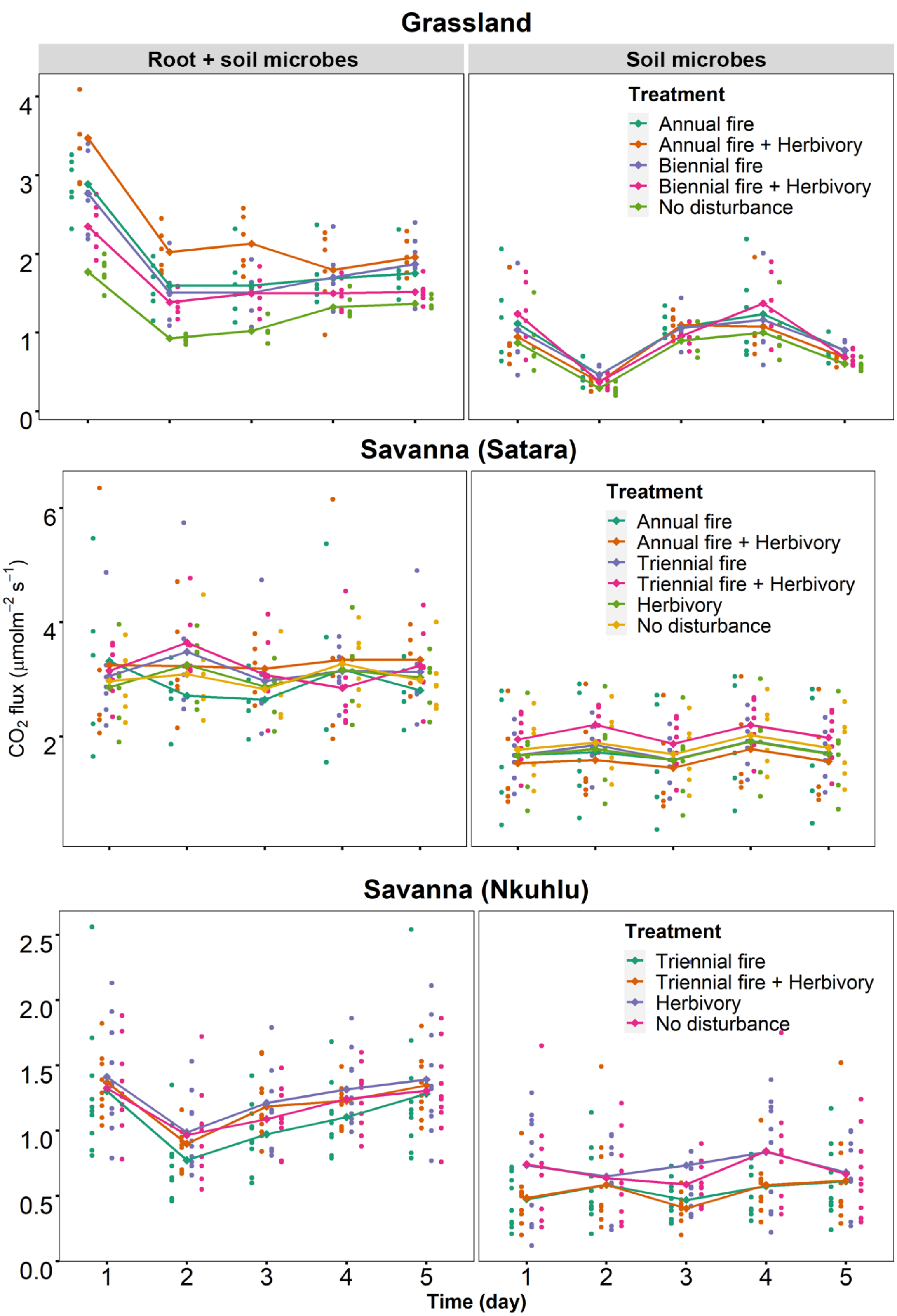

Figure 3.2: Root + soil microbial and soil microbial CO_2 fluxes over a 5-d period in the grassland and savanna. Line nodes represent the mean CO_2 flux per treatment and day.

3.4.2 Soil CH_4 fluxes

Methane fluxes from the grassland were affected by herbivory and fire treatments (Fig 3.5), where the highest CH_4 emission occurred with herbivory and annual fires, and the highest CH_4 uptake with herbivory and biennial fire. All other treatments from the grassland had negative CH_4 fluxes (uptake). In the savanna (Satara), only fire affected CH_4 fluxes (Fig 3.5) with the highest emission occurring with annual fire and herbivory and highest uptake with triennial fire plus herbivory. In Nkuhlu, the interaction of herbivory and fire affected fluxes. Triennial fire plots had the highest uptake of CH_4. Generally, lower frequency fires (biennial or triennial) resulted in increased CH_4 uptake while the effect of high frequency fires (annual) resulted in CH_4 emissions.

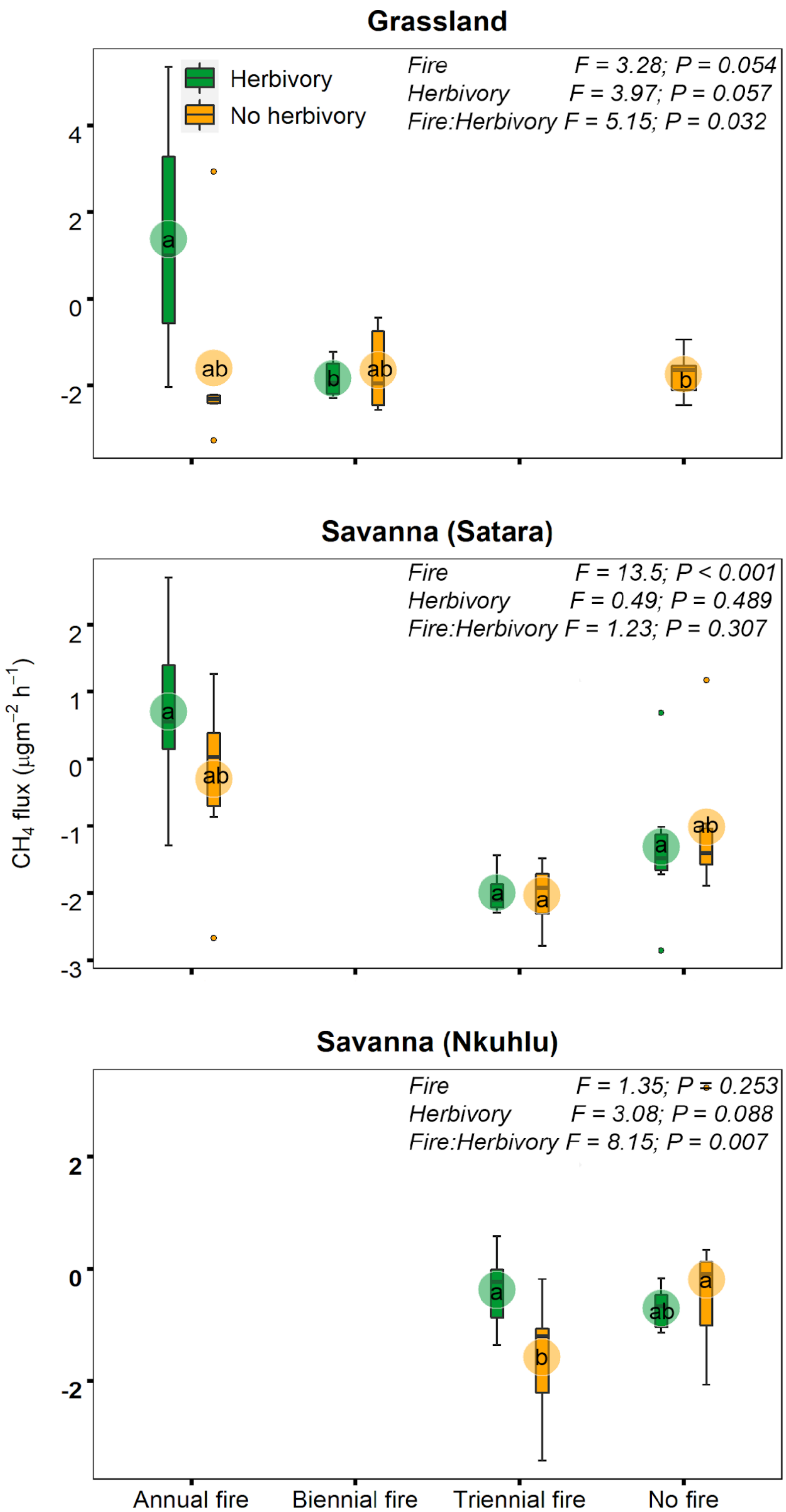

Figure 3.3: Comparison of field soil CH_4 fluxes in the grassland (Brotherton) and savanna (Satara and Nkuhlu). Boxplots denote range of data (upper, median and lower quartiles) with means indicated by circles with letters. Different letters indicate significant differences at P < 0.05.

3.4.3 Soil N_2O fluxes

Nitrous oxide fluxes only varied in the savanna (Satara) where they were affected by fire (Fig 3.6). Fluxes were highest from no disturbance treatments, while there was minimal uptake of N_2O within other treatments e.g from triennial fire only treatments. Although N_2O fluxes did not differ in the other savanna site (Nkuhlu) and grassland, net positive fluxes were obtained from all treatments. Overall, N_2O fluxes were small to negligible in all sites, with some data points near zero.

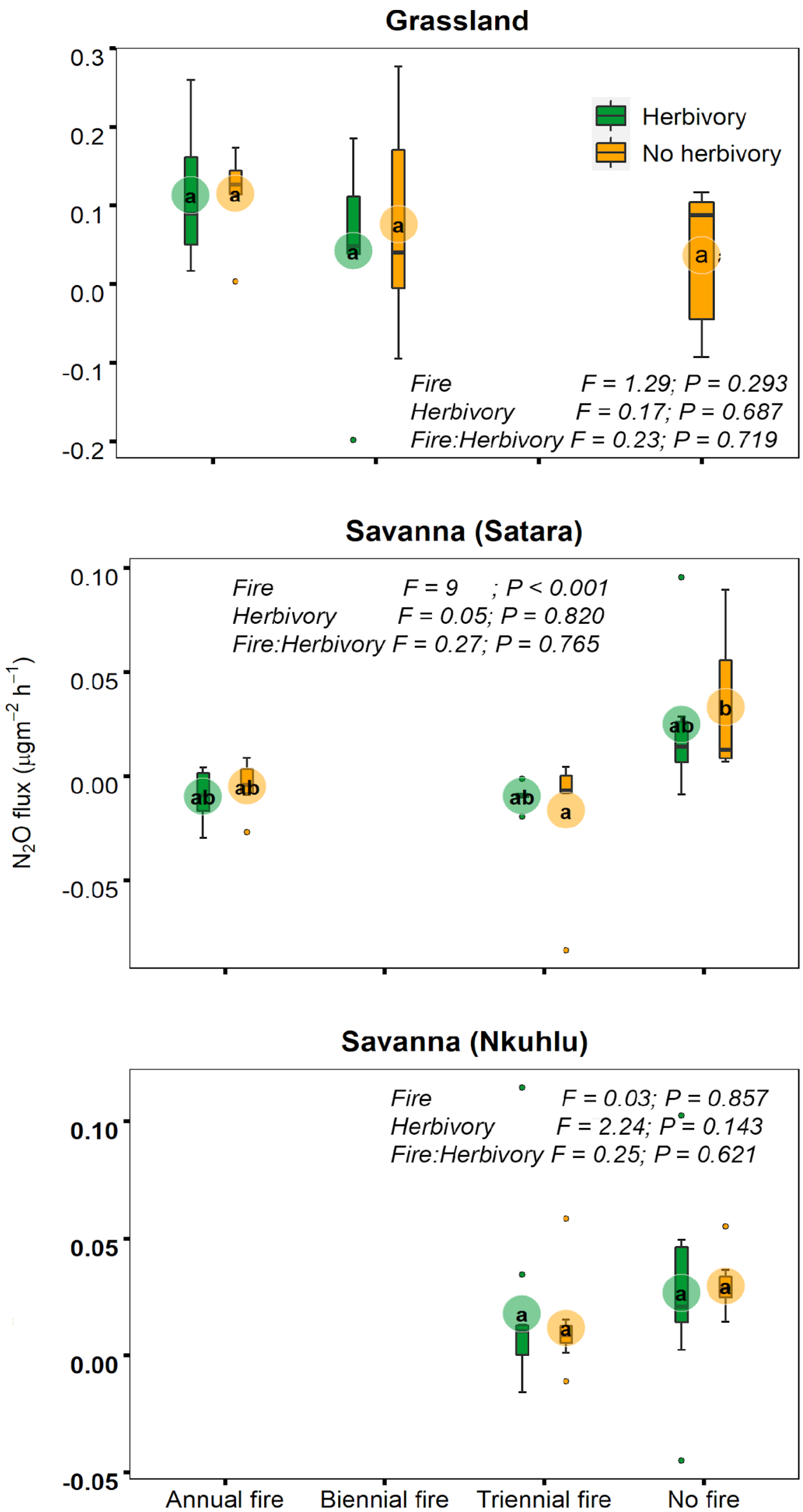

Figure 3.4: Comparison of field soil N_2O fluxes in the grassland (Brotherton) and savanna (Satara and Nkuhlu). Boxplots denote range of data (upper, median and lower quartiles) with means indicated by circles with letters. Different letters indicate significant differences at P < 0.05.

In the grassland, we fitted linear mixed effect models (Table 3.1) that predicted that field CO_2 fluxes depended on treatments (fire and herbivory), soil organic C stocks, total soil N and herbaceous biomass. The final parsimonious model was obtained after reducing the estimator variables to avoid overparameterization, by comparing AIC values with the null model (37.2 vs 22.6) that included all relevant variables for CO_2 fluxes. The difference in explanatory power between the null model and final model was significant ($P < 0.05$). In the model (Table 3.1), treatment levels had varying effects on CO_2 fluxes; annual fire plus herbivory had a very large and positive effect, biennial fire had a medium negative effect, biennial fire plus herbivory a large negative effect and no disturbance had a very large negative effect. Soil organic C stocks had a very large negative effect while total soil N also had a very large but positive effect on CO_2 fluxes. Herbaceous biomass had a medium positive effect on CO_2 fluxes. The grassland model accounted for 87 % of the variation in grassland CO_2 fluxes. In the savanna, none of the measured variables predicted CO_2 fluxes. The linear mixed effect model (Table 3.1) for the grassland indicated that CH_4 fluxes were predicted by treatment where annual fire and annual fire plus herbivory had very large positive effects while biennial fire plus herbivory had a medium negative effect). Microbial biomass had a small negative effect on CH_4 fluxes while clay content had a very large positive effect. This model accounted for 49% of the variation in CH_4 fluxes. In the savanna, CH_4 fluxes were predicted by treatment where the effect of annual fire was large and herbaceous biomass was small in one site, accounting for 42% of the variation. Models to predict N_2O fluxes were limited by few predictors for example in the grassland, N_2O fluxes were predicted only by soil pH (Table 3.1). Overall, soil CO_2 fluxes contributed 99% of all fluxes when compared under similar units of measurement.

Table 3.1: Linear mixed effect model results showing the significant explanatory variables of soil greenhouse fluxes (CO_2, CH_4, N_2O). Parameters, beta estimates for effect size, model conditional R^2 and p-value of estimates are shown.

Variables Gas fluxes	Parameter	Beta Coefficients	P-value	Model R^2
Grassland				
CO_2	Treatment	1.67	< 0.001	0.87
	Soil organic C	- 0.0004	0.003	
	Total soil N	0.007	< 0.001	
	Herbaceous biomass	0.003	0.018	
CH_4	Treatment	- 0.05	0.040	0.49
	Microbial biomass	- 0.02	0.040	
	Clay content	0.03	0.031	
N_2O	Soil pH	- 0.38	0.021	0.16
Savanna (Satara)				
CO_2	*Model not significant*			
CH_4	Treatment	2.16	< 0.001	0.42
	Herbaceous biomass	4.13	0.007	
N_2O	Treatment	0.03	< 0.031	0.45
Savanna (Nkuhlu)				
CO_2	*Model not significant*			
CH_4	Treatment	- 0.87	0.003	0.13
N_2O	*Model not significant*			

3.5 Discussion

There are two main mechanisms by which fire can reduce soil CO_2 fluxes, firstly by adding more pyrogenic C compounds that are resistant to microbial decomposition and secondly by reducing plant cover thereby reducing root respiration (Kim, 2013). These widely accepted mechanisms were derived from the northern hemisphere soils and with the different soils and climates in some of the more ancient landscapes in the southern hemisphere including Africa where there are mostly poor well drained soils, fire maybe required for nutrient cycling. As such, neither of these mechanisms were supported by our data here. Soil CO_2 flux findings in the grassland showed the opposite effect in which unburnt areas had lower fluxes than burnt areas with high frequency annual fires increasing fluxes considerably, despite the reduced plant biomass. When root respiration was completely excluded in the *ex-situ* laboratory flux measurements, no differences were observed indicating a similar level of microbial respiration in grassland and savanna sites. This highlights the importance of root respiration in overall soil CO_2 emissions. Many studies however, also present similar findings to our grassland results where the prevailing trend is that low frequency fire lowers soil CO_2 fluxes compared to high frequency fire (Burke et al., 1997; Knapp et al., 1998; Pinto et al., 2002, Andersson et al., 2004; Michelsen et al., 2004; Bremer and Ham, 2010; Voncina et al., 2013). The vegetation flush in the subsequent season following a fire increases root respiration leading to the increase soil CO_2 fluxes due to high frequency fire. Some studies report no differences between burn treatments (Castaldi et al., 2010) as was the case in our savanna sites. A study by Zepp et al. (1996) conducted in the same savanna site found that fluxes only rose sharply after burning but would revert to pre-burn levels after a few days, which would agree with our results.

Data from our and other studies suggests that soil CO_2 emissions are widely variable depending on ecosystem and local conditions and the interaction of factors that cause these variations are not simple to discern. The great variability in soil CO_2 fluxes is an aspect which has been shown to complicate measurements and modelling of soil respiration due to its simultaneous response to different drivers such as soil temperature, moisture and vegetation productivity at differing spatio-temporal scales (Webster et al., 2008). In our study, there were no discernible relationships between soil CO_2 fluxes and soil temperature and moisture levels, even though strong relationships have been widely reported (e.g Tang et al., 2003; Tang and Baldocchi, 2005; Makhado and Scholes, 2011). An increase in soil temperature would theoretically result in higher soil respiration and emissions from increased microbial metabolism *via* a positive feedback response mechanism while soil moisture controls microbial activity and all related processes (Oertel et al., 2016). However, the lack of relationships from our study may be because we sampled within one season and a relatively narrow period, limiting temporal variation and therefore seasonal or higher temporal resolution data can help to account for seasonality-induced temperature and moisture variations.

Contrary to what we predicted, herbivory did not reduce soil greenhouse fluxes, nor did it increase them. In our case, herbivory had a consistent neutral effect on fluxes except when in combination with fire. Herbivores are known to have positive, negative, or neutral effects on soil CO_2 emissions depending on habitat type, grazing or browsing pressures and landscape scales (Stritar et al., 2010). The neutrality of herbivory as demonstrated in this study can be explained by the interplay of processes involved within above and belowground ecosystems which as a result, can cause herbivory to have indirect or sometimes idiosyncratic effects such as increased rhizodeposition and production of plant secondary metabolites to resist herbivory (Bardgett and Wardle, 2003). Herbivores in grasslands have also been shown to stimulate soil microbial communities *via* addition of fresh dung and urine thereby enhancing nutrient cycling (Harrison and Bardgett, 2008). In our overall hypothesis, we indeed expected herbivory to add nutrient rich excreta to the soil in the short term, but our study measured the long-term effects which were neutral. Other factors were more important in determining soil CO_2 fluxes and the linear mixed models in the grassland predicted significant effects from soil organic C, total soil N and herbaceous biomass. Even though herbivory did not lead to higher soil organic C stocks and low soil N (Chapter 2: Figs 2.5 and 2.6), as predicted, the mechanism of higher soil organic C stocks and low soil N reducing soil CO_2 fluxes and vice-versa was proven true. This effect is due to the strong linkage between soil C:N stoichiometry and soil microbial activity where low C:N increases soil microbial activity and thus higher soil CO_2 fluxes while high C:N has the opposite effect (Maier, 2015; Winkler et al., 2019). Herbaceous biomass contributed to the root respiration component, that is higher aboveground biomass being linked to higher field soil CO_2 fluxes presumably due to higher belowground biomass. Our evidence thus suggests that reduced burning frequency combined with herbivory can potentially reduce soil CO_2 emissions. Other studies support this (Bremer and Han, 2010) and report more soil organic C sequestered instead of being lost as CO_2 with lower fire frequencies, and this is important in the reduction of soil CO_2 emissions overall.

Methane was mostly taken up in both grassland and savanna soils. High frequency annual fires plus herbivory however, increased CH_4 emissions compared to biennial fires or no disturbance, in support of our hypothesis that fire-induced death of methanotrophic bacteria would increase CH_4

emissions. Indeed, the low microbial biomass with high frequency fires affected CH_4 uptake negatively as reported in the linear mixed effect model. It is evident that the higher disturbance level brought about by high frequency fire exacerbated by herbivory led to higher emissions of both soil CO_2 and CH_4 overall. The frequency of fire drives two main mechanisms for soil CH_4 efflux, (1) abundance of methanotrophic bacteria that utilise CH_4 and that can be killed by high frequency fires (Zhao et al., 2015) and (2) the removal of the litter layer and the barrier effect it provides, thereby improving gas diffusivity and thus enhanced CH_4 loss (Sullivan et al., 2011; Fest, 2013). The impact of burning is a function of not only frequency but also intensity (50℃ to 121℃ for microbial mortality; Knicker, 2007). Although we had no measure of fire intensity, we assume that the intensity was sufficient for death-inducing heat exchange down the soil profile, again as supported by the reduced microbial biomass with high frequency fires.

In our study, the linear mixed effect model predicting CH_4 fluxes showed that a coarse soil texture favoured CH_4 uptake which is explained by the importance of adequate soil pore space for optimum gas diffusivity. In general, our CH_4 fluxes were in the midrange of values from other studies in grasslands which have obtained varying scales of fluxes. For example, Skiba et al. (2009) found insignificant CH_4 fluxes (less than 9.5 E^{-4} µmol m^{-2} h^{-1}) while other studies in different ecosystems have reported net CH_4 sinks comparable to our study e.g. (-2.36 µmol m^{-2} h^{-1} from steppes; Liu et al., 2009) and (-2.75 µmol m^{-2} h^{-1} from tropical forests; Dalal and Allen, 2008). Increased grazing intensity has been reported to reduce soil CH_4 uptake by 36.5% under heavy versus light grazing (Tang et al., 2018), but herbivory did not have strong effects in our study. Although we did not measure grazing intensity in our study, the presence of herbivores did not have any effect on CH_4 fluxes. An empirical model from thirty-year data (1981 to 2010) predicted a slight increase in global CH_4 uptake in dry and warm temperate grasslands due to increasing temperatures while it predicted decreased uptake in dry tropical grasslands due to increases in precipitation (Yu et al., 2017). The sites we used provide a potential sink to neutralise CH_4 emitted by wetlands, which are the largest terrestrial source of CH_4 (508 µmol m^{-2} h^{-1}; Cao et al., 1998). Using our results, we estimate that the grassland and savanna soils we sampled can consume *ca.* 0.47% per m^2 of the CH_4 released by wetlands, representing an immense potential for greenhouse gas emission mitigation. The variation in levels of CH_4 removed from the atmosphere will greatly depend on spatial-temporal scales and management practices (Sanchez et al., 2017; Wanyama et al., 2019).

Although tropical grasslands and savannas are thought contribute about 16% to the global terrestrial N_2O emissions (IPCC, 2001), our results indicate a very low contribution from South African grasslands and savannas even under frequent burning. Other savanna studies also reported overall low N_2O emissions (0 to 1.58 mg m^{-2} day^{-1}; Castaldi et al., 2004) where variations in fluxes were driven by fine soil particle size and volumetric water content. Rees et al. (2006) found higher N_2O fluxes (42 µg m^{-2} h^{-1}) that were strongly linked with rainfall in a southern African savanna. In addition to the effect of rainfall, they found that local differences in soil organic matter and drainage also influenced fluxes. We found N_2O fluxes to be affected by soil pH and this is in turn mediated by soil organic matter and water retention capacities among other factors. We did not find strong effects of herbivory and fire on N_2O fluxes in our sites except at one savanna site where there was a significant reduction in N_2O fluxes with triennial fire relative to no fire. However, due to the low temporal resolution, we can only make the general observation that fluxes of N2O were low. These low N_2O fluxes can be attributed to the low soil N contents of the sites. Other studies have reported an influence of grazing on soil N_2O emissions where cattle-grazed pastures emitted the most (4.07 to 4.89 µmol m^{-2} h^{-1}) followed by sheep-grazed pastures (1.63 to 2.44 µmol m^{-2} h^{-1}) and the least from non-grazed ones (0.41 to 0.82 µmol m^{-2} h^{-1}; Saggar et al., 2007). Native grasslands and savannas appear to be lower emitters of N_2O compared to rangelands where livestock graze, probably due to higher stocking rates per unit area and higher deposition of excreta that increases the soil N pool (Soussana et al., 2010). Possibly, maintaining grassland and savannas in their native state by e.g re-wilding, could have beneficial implications for global change.

3.6 Conclusions

The findings of our study suggest that soil greenhouse gas emissions are more affected by fire than herbivory, with high frequency fires generally increasing CO_2 fluxes, reducing CH_4 uptake and having minimal effects on N_2O emissions. In contrast, low frequency fires tended to lower CO_2 emissions and increase soil CH_4 uptake. We note that our soil gas fluxes were obtained from a snapshot survey as compared to long-term soil gas flux surveys. However, gas flux data were collected over several days from multiple replicates to obtain an average, and thus they can represent typical conditions during the season and period data were collected. We can cautiously, thus conclude that adoption of low frequency fire regimes and moderate stocking rates of wild and domestic ungulates can curb greenhouse gas emissions in native grasslands, savannas and working rangelands. Future soil greenhouse gas emission research should consider long-term greenhouse gas flux surveys to obtain an all-encompassing outlook on multi-seasonal gas flux trends and therefore adequately inform management practices. In addition, future research could determine whether maintaining grassland and savannas in their native state or re-wilding rangelands could lower CO_2 emissions and thus have beneficial implications for global change. Overall, our results support the maintenance of the native state of grasslands, savannas and working rangelands to reduce and maintain low soil greenhouse gas emissions.

Chapter 4

General Discussion and Synthesis.

4.1 Importance of conserving and maintaining native grasslands and savannas

We explored how fire and herbivory, two of the main ecosystem drivers in African grasslands and savannas, mediated the sequestration of soil organic C and the emission of soil greenhouse gases. This synthesis chapter draws on all results from this thesis and discusses them in the broader context of global change. The results we obtained highlight the potential of African grasslands and savannas to sequester more stable soil organic C while reducing soil greenhouse gas emissions in presence of disturbances. The need to lower atmospheric greenhouse gas concentrations presents an immense challenge for this century (Amundson and Biardeau, 2018) leading to the idea that the conservation of natural ecosystems can accelerate soil organic C sequestration compared to restoration of agriculturally degraded lands due to the longer successional times with more degraded systems (Yang et al, 2019). This impetus has given rise to potentially controversial initiatives such as the French "4 per mille" (www.4p1000.org) which advocates for an increase of 0.4% a year in the C sequestered in soils in order to curtail annual atmospheric CO_2 emissions. Another initiative is the Bonn Challenge (www.bonnchallenge.org) which promotes the restoration of deforested ecosystems by planting trees. While this may be beneficial for legitimately degraded former forest ecosystems, it threatens the biodiversity and ecosystem function of African grassy biomes if they are inappropriately targeted by trees-for-carbon projects whose proponents use data were historical grasslands and savannas have been erroneously classified as deforested (Bond et al., 2019). Loss of these biomes would mean the loss of some of the world's last remaining and most charismatic megafauna, such as zebra, lions, elephants, and rhinoceros. Loss of ecosystem function in grassy biomes could even lead to the loss of their largest C pool, i.e. soil organic C. Due to the momentum driven by the high funding of these initiatives, some of the last remaining untransformed rangelands in Africa, that have evolved with fire and herbivory are under threat of conversion to plantations, which several studies have shown to be ineffective in reducing atmospheric CO_2 levels (Baldocchi and Penuelas, 2019; Lewis et al., 2019) and in many cases have resulted in alien species invasions. Griscom et al. (2017) estimated that in Africa, optimal grazing intensity and improved fire management in rangelands can potentially reduce emissions by 1 to 2 Tg CO_2e yr^{-1}. Considering that the social cost of C sequestration is estimated as US $ 100 per Mg

CO_2e, initiatives aimed at conserving natural ecosystems, versus restoration or conversion, should thus be targeted.

4.2 Comparison of local soil organic C sequestration and soil greenhouse gas emissions to regional and global data

Here we synthesise results of soil organic C stocks and soil greenhouse gas emissions in tandem with fire and herbivory as main ecosystem disturbances. Using a tropical montane grassland and a tropical savanna ecosystem from Southern Africa, we selected sites that had perennial histories of burning, herbivory and their combinations. Scharlemann et al., 2014 (Fig 4.1) highlighted the above and belowground C stocks in different global ecosystems, particularly focusing on the tropical biomes from where our sites were located. Here it is evident that the tropical montane and dry ecosystems where our grassland and savanna sites were selected store *ca.* 81% of their organic C belowground compared to e.g tropical rain forests where *ca.* 50 % to 70% of the organic C is stored in aboveground biomass. In South Africa, grasslands and savannas constitute *ca.* 57 % of the land area out of a total of 7 ecosystems. As such, the conservation of these native grasslands and savannas may present a significant potential for soil organic C sequestration in South Africa.

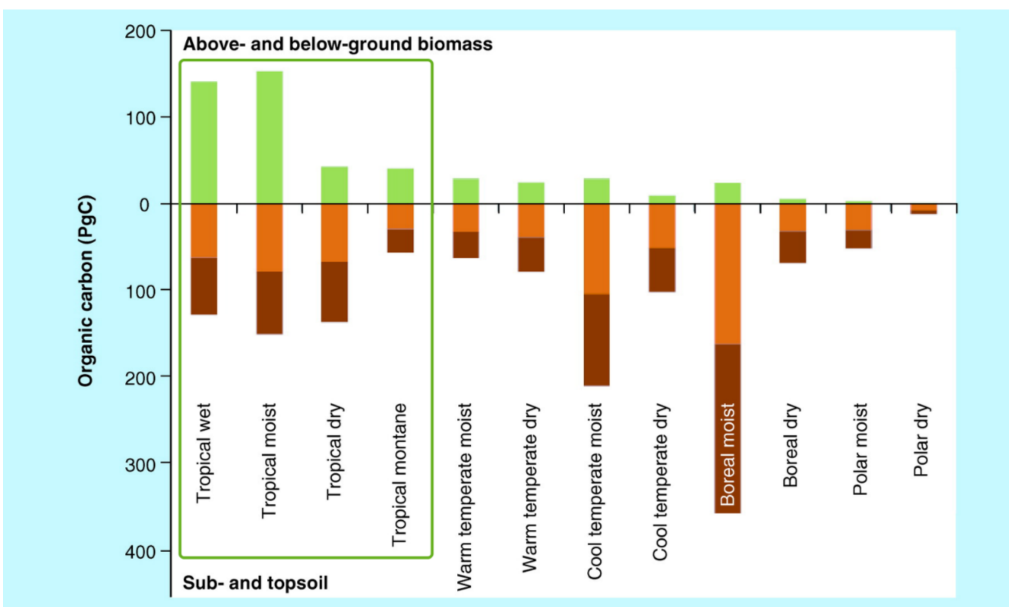

Figure 4.1: Organic C stocks in above (green) and belowground (topsoil-orange; subsoil-brown) biomass in different global ecosystems. The relevant data to this study is demarcated by a green rectangle, (Scharlemann et al., 2014).

After converting emissions to CO_2 equivalents (CO_2e) representing Global Warming Potentials (GWP), we found that the exclusive grassland site emitted less than the savanna (Fig 4.3), bearing in mind that these are pooled figures across common sites and treatments to give a broader perspective. There was net uptake of CH_4 in both biomes, further increasing the soil organic C pool. Tropical grasslands and savannas are thought contribute about 16% to the global terrestrial N_2O emissions however, our results indicate a very low contribution from South African grasslands and savannas. A review by Oertel et al. (2016; Fig 4.1) reports the majority of global GWP contributions from CO_2 emissions compared to CH_4 and N_2O emissions from sub-tropical, temperate and mediterranean grasslands.

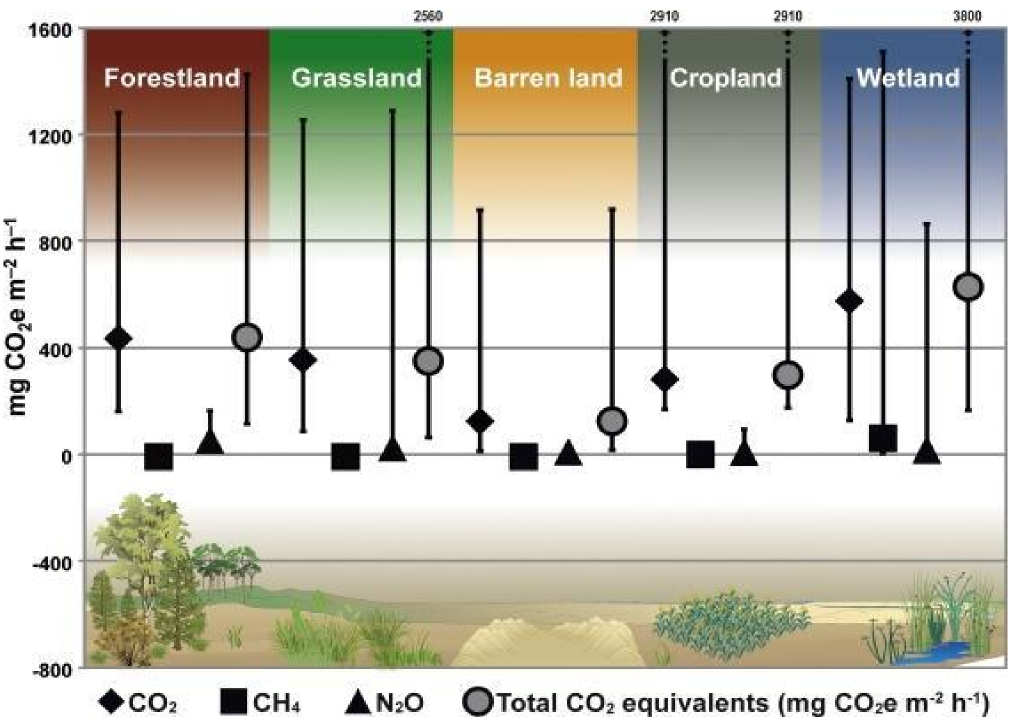

Figure 4.2: Comparison of GWP from different global landuse systems from Oertel et al. (2016).

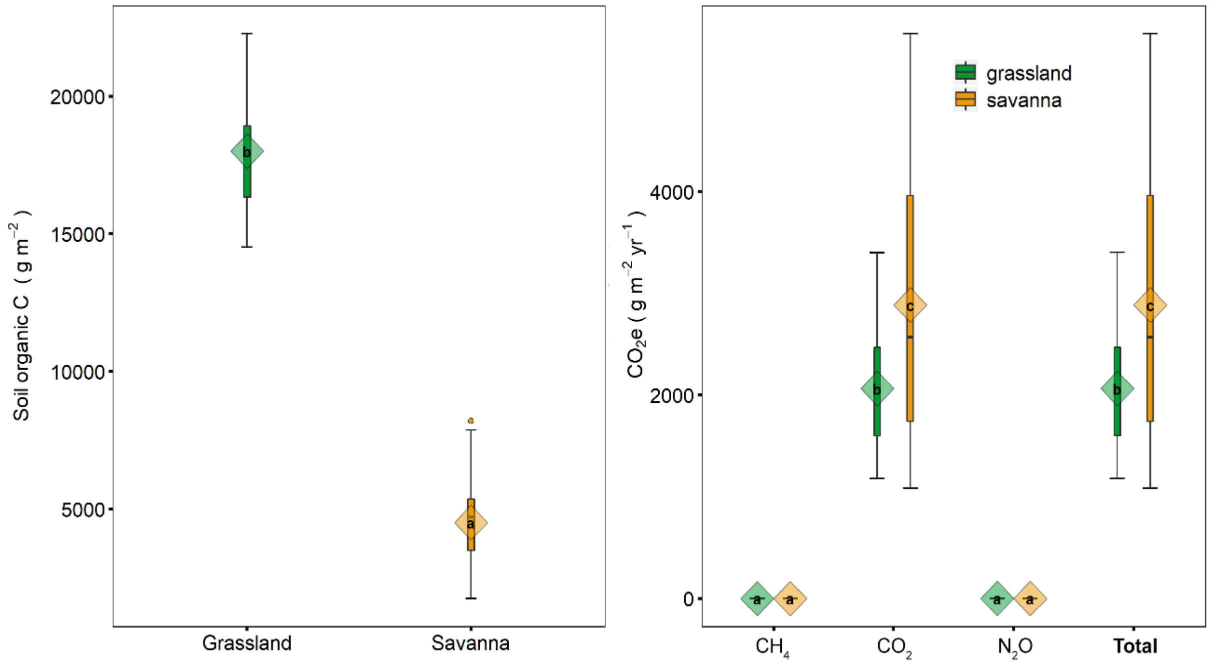

Figure 4.3: Pooled mean CO_2e and soil organic C stocks from the grassland and savanna sites. Boxplots illustrate data ranges with means depicted by diamonds with letters representing comparisons at $P < 0.05$.

Our findings (Fig 4.3) similarly indicate that CO_2 emissions contribute 99% to the total pooled GWP compared to CH_4 and N_2O emissions from the South African grassland and savanna sites that we sampled. However, the emissions from our sites, even after pooling across treatments are well below the global levels when compared under similar units of measurement. We further compared the soil organic C pools with other estimates from South Africa in the Soil Carbon Database (Schutte et al., 2019). Soil organic C stocks in the topsoil under natural vegetation had medians between 3000 to 10000 g C m^{-2} with a few areas with stocks >10000 g C m^{-2}. Our soil organic C stocks from the grassland were above the majority estimates and represent the few areas with greater soil organic C stocks. The savanna soil organic C stocks were within the range of the national estimates from the soil organic C maps. The data from this study is therefore important to the further enhancement of the South African Soil Carbon Database.

4.3 Localised soil C balance

We formulated a localised net soil organic C balance for the grassland and savanna sites (Fig 4.4) based on a template from FAO (2004) and the MEMS v 1.0 model (Roberston et al., 2019) using pooled measurements from both sites to summarise the soil organic C sequestration and soil CO_2e emissions per land-cover type. We expressed our CO_2 data in g m^{-2} yr^{-1} while soil organic C data was expressed in g C m^{-2} for comparability. Our soil organic C stock estimates were obtained at the top 20 cm of soil and the total organic C was presented. As highlighted by the MEMS model, it is important to further fractionate the total soil organic C into different pools based on stability. Future research on soil organic C sequestration can provide further detail and precision in data by factoring in soil organic matter fractionation.

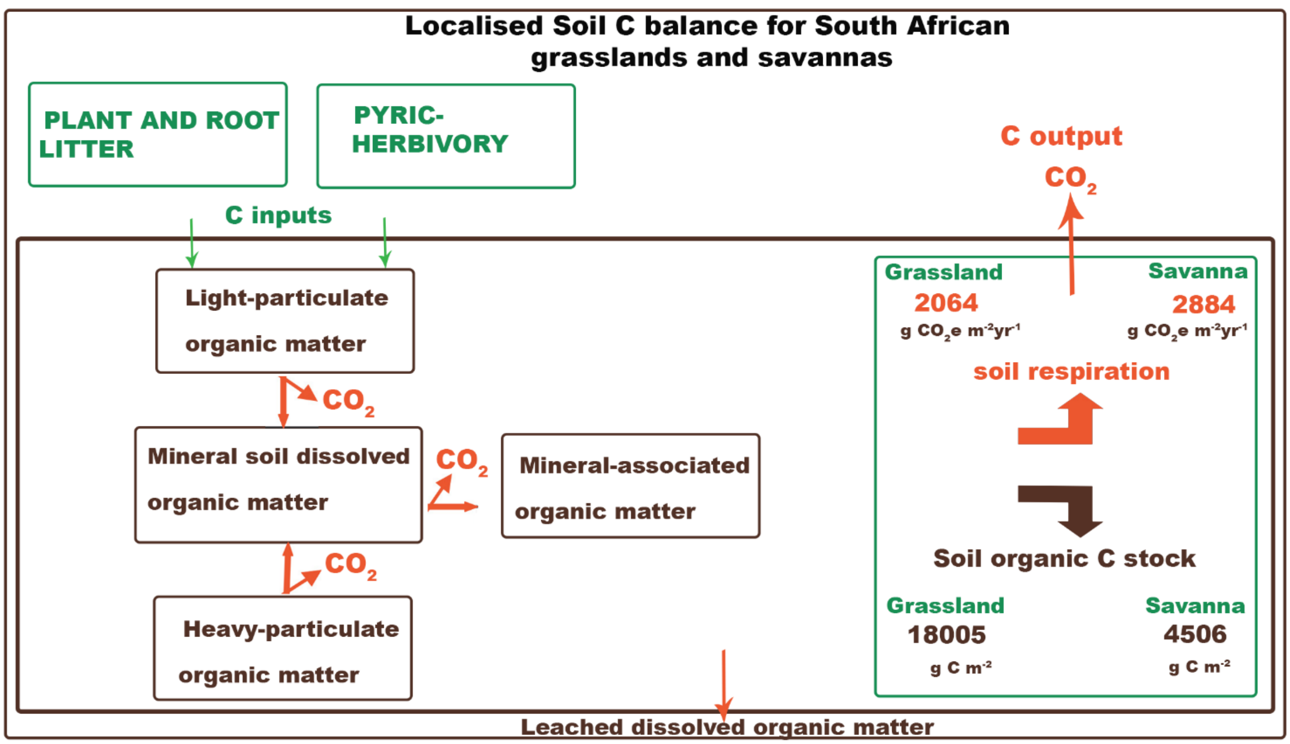

Figure 4.4: Soil C balance schematic model showing the different soil organic matter fractions as informed by the MEMS model. Soil respiration (in CO_2e) and soil organic C storage data are derived from pooled figures from this study.

Using the **EX-ANTE CARBON-BALANCE TOOL (EX-ACT)** developed by FAO (2017), we estimated the amount of emissions emitted per ha per year (tCO_2e $ha^{-1}yr^{-1}$) from a theoretical 70 ha grassland plot managed with or without fire, which is similar to total size of the savanna Long-Term Ecological Research sites sampled in this study. Four fire regimes were used and each yielded different estimates as shown in Table 4.1 below. Fig 4.5 shows the relative contributions of each soil greenhouse gas to the total theoretical soil greenhouse gas emissions. The results from the tool are a slight underestimate when only considering emissions under fire management. Inclusion of herbivory is provided in the tool but is more focused on livestock instead of wild ungulates. Nevertheless, the estimates are consistent with our results were no disturbance and moderate frequency fire produces the least emissions compared to high frequency fires. This further supports our recommendation for rangeland management to employ moderate fire frequency regimes to significantly curtail soil greenhouse emissions.

Table 4.1: Estimates of total soil emissions from a theoretical grassland area of 70 ha as calculated by the EX-ACT tool. Total emissions figures represent the total of all greenhouse gases (CO_2, CH_4, N_2O) per year.

Fire management	**Total emissions (tCO_2e ha^{-1} yr^{-1})**
No fire	963
Annual fire	1433
Biennial fire	1198
Triennial fire	1119

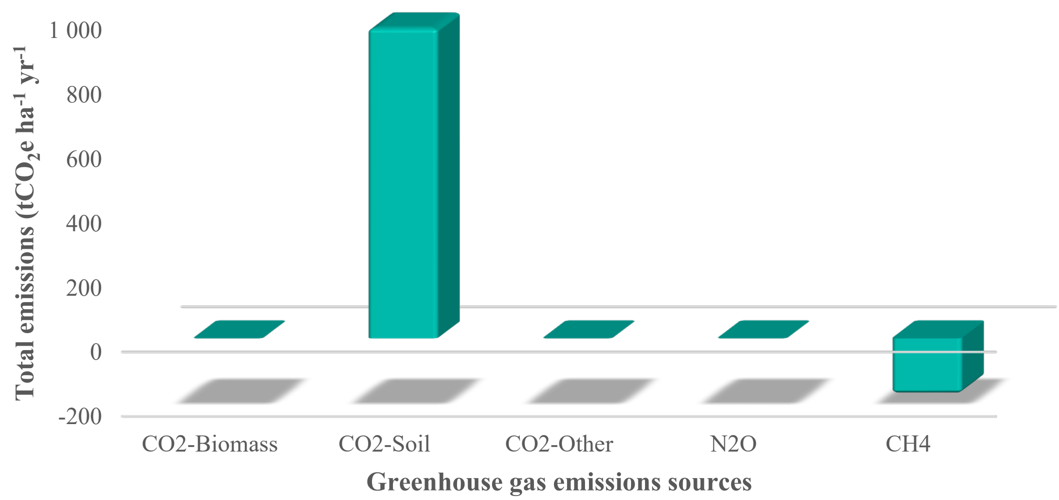

Figure 4.5: Proportional contributions of each soil greenhouse gas source to the total theoretical emissions (EX-ACT tool; FAO, 2017).

4. 4 Conclusions

We thus conclude that South African grasslands, in particular and savannas have a significant potential for soil organic C storage and reduction of soil greenhouse gas emissions, based on their proportional land area coverage and comparative global figures. The extent to which there is potential for increased organic C storage in soils, however, depends on prior landuse and management choices (particularly fire) and changes in those choices in the future. The significance of this potential is however, also dependent on time, as it takes *ca.* 30 years or more for new soil organic C to incorporate with old soil organic C within the top 20 cm of soil with change in land-use. Due to the inherent low nutrient status of African grasslands and savannas coupled with erosion of dissolved C, it may take even longer than the estimated 30 years for changes aboveground to reflect within belowground pools. However, their relative size and proportional contribution to C sequestration compared to other biomes and their contribution to livelihoods across their range makes the conservation of these ecosystems very critical. This study will thus add to the body of research in support of conserving the only remaining intact grassy biomes in Africa for climate change mitigation and resilience.

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Appendix

5.1 CH_4 and N_2O flux static closed-chambers

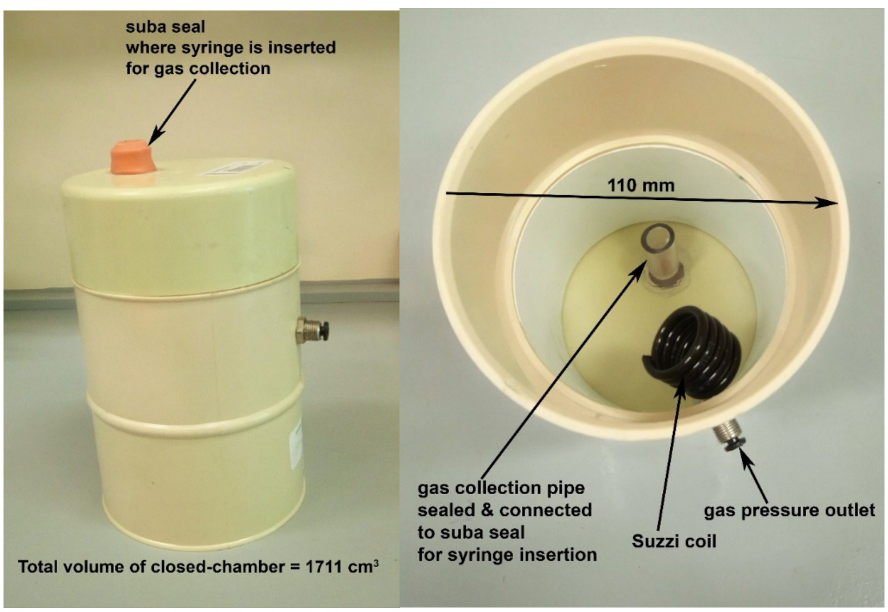

Figure 5.1: Illustration of the static closed-chamber used to collect CH_4 and N_2O gases.

5.2 Collars for ex-situ soil microbial respiration measurement

Figure 5.2: Illustration of the *ex-situ* soil microbial respiration measurement collars closed at the bottom end.

Table 5.1: Grassland descriptive statistics showing mean and standard de*via*tion and One-way ANOVA results for the other soil and environmental variables measured. One-way ANOVA results in bold are significant at P < 0.05.

Variable	**Treatment**	**Mean**	**Standard Deviation**	**One-way ANOVA (df = 4)**
Soil temperature (°C)	Annual fire	53.33	5.82	F = 2.49; P > 0.05
	Annual fire + Herbivory	54.57	5.23	
	Biennial fire	51.06	7.43	
	Biennial fire + Herbivory	60.76	3.98	
	No disturbance	56	5.08	
Soil moisture (%)	Annual fire	12.65	6.02	F = 1.23; P > 0.05
	Annual fire + Herbivory	13.65	6.61	
	Biennial fire	15.88	6.31	
	Biennial fire + Herbivory	20.13	7.41	
	No disturbance	19.88	10.82	
Soil P (g/100g)	Annual fire	0.06	0.01	F = 0.98; P > 0.05
	Annual fire + Herbivory	0.07	0.004	
	Biennial fire	0.06	0.003	
	Biennial fire + Herbivory	0.06	0.01	
	No disturbance	0.06	0.01	
Soil K (g/100g)	Annual fire	0.20	0.01	F = 1.20; P > 0.05
	Annual fire + Herbivory	0.21	0.01	
	Biennial fire	0.19	0.01	
	Biennial fire + Herbivory	0.19	0.01	
	No disturbance	0.20	0.02	

Soil Ca (g/100g)	Annual fire	0.09	0.01	**F = 4.15; P = 0.010**
	Annual fire + Herbivory	0.1	0.01	
	Biennial fire	0.09	0.01	
	Biennial fire + Herbivory	0.07	0.01	
	No disturbance	0.09	0.01	
Herbaceous biomass (g m^{-2})	Annual fire	224.57	8.74	**F = 48.74; P < 0.001**
	Annual fire + Herbivory	62.70	23.31	
	Biennial fire	296.73	71.78	
	Biennial fire + Herbivory	326.90	25.85	
	No disturbance	300.85	25.54	
Microbial biomass	Annual fire	70	28.67	F = 0.69; P > 0.05
	Annual fire + Herbivory	76.27	30.23	
	Biennial fire	70.43	53.67	
	Biennial fire + Herbivory	46.77	23.79	
	No disturbance	53.36	41.84	
pH	Annual fire	4.37	0.05	F = 0.59; P > 0.05
	Annual fire + Herbivory	4.383	0.09	
	Biennial fire	4.417	0.8	
	Biennial fire + Herbivory	4.417	0.08	
	No disturbance	4.383	0.041	
Clay content (%)	Annual fire	22.11	3.83	F = 0.45; P > 0.05
	Annual fire + Herbivory	22.12	1.29	
	Biennial fire	20.61	3.61	
	Biennial fire + Herbivory	21.36	1.49	
	No disturbance	22.58	2.94	
Silt content (%)	Annual fire	39.38	5.69	F = 1.54; P > 0.05
	Annual fire + Herbivory	36.59	3.86	
	Biennial fire	34.73	5.67	
	Biennial fire + Herbivory	36.11	4.83	
	No disturbance	41.29	5.88	
Sand content (%)	Annual fire	38.51	3.27	**F = 3.79; P = 0.015**
	Annual fire + Herbivory	41.28	4.18	
	Biennial fire	44.65	5.82	
	Biennial fire + Herbivory	42.53	3.98	
	No disturbance	36.12	3.32	
NDVI	Annual fire	0.41	0.04	F = 2.29; P > 0.05
	Annual fire + Herbivory	0.45	0.03	
	Biennial fire	0.38	0.02	
	Biennial fire + Herbivory	0.41	0.07	
	No disturbance	0.41	0.03	
Bulk density (g cm^{-3})	Annual fire	0.55	0.01	**F = 11.83; P < 0.001**
	Annual fire + Herbivory	0.65	0.02	
	Biennial fire	0.57	0.03	
	Biennial fire + Herbivory	0.67	0.05	
	No disturbance	0.63	0.05	

Table 5.2: Savanna (Satara) descriptive statistics showing mean and standard deviation and One-way ANOVA results for the other soil and environmental variables measured. One-way ANOVA results in bold are significant at $P < 0.05$.

Variable	Treatment	Mean	Standard Deviation	One-way ANOVA (df = 5)
Soil temperature	Annual fire	51.42	4.18	**F = 11.48; P < 0.001**
	Annual fire + herbivory	48.79	6.80	
	Herbivory	37.52	4.46	
	No herbivory	38.79	5.02	
	Triennial fire	50.04	2.57	
	Triennial fire + herbivory	49.47	1.82	
Soil moisture	Annual fire	26.47	3.55	**F = 2.66; P = 0.042**
	Annual fire + herbivory	30.20	4.12	
	Herbivory	28.08	2.08	
	No herbivory	25.13	4.54	
	Triennial fire	23.60	3.48	
	Triennial fire + herbivory	26.75	2.12	
Soil P	Annual fire	0.23	0.04	**F = 4.05; P = 0.006**
	Annual fire + herbivory	0.22	0.04	
	Herbivory	0.20	0.03	
	No herbivory	0.21	0.04	
	Triennial fire	0.17	0.03	
	Triennial fire + herbivory	0.16	0.03	
Soil K	Annual fire	1.45	0.06	**F = 4.82; P = 0.002**
	Annual fire + herbivory	1.42	0.03	
	Herbivory	1.58	0.11	
	No herbivory	1.56	0.05	
	Triennial fire	1.60	0.11	
	Triennial fire + herbivory	1.61	0.12	
Soil Ca	Annual fire	1.06	0.08	**F = 14.70; P < 0.001**
	Annual fire + herbivory	1.04	0.08	
	Herbivory	1.28	0.13	
	No herbivory	1.28	0.16	
	Triennial fire	0.80	0.17	
	Triennial fire + herbivory	0.79	0.15	
Clay content	Annual fire	14.13	1.66	F = 0.59; P > 0.05
	Annual fire + herbivory	15.13	2.12	
	Herbivory	13.61	2.978	
	No herbivory	14.56	2.67	
	Triennial fire	13.16	2.78	
	Triennial fire + herbivory	14.95	2.308	
Silt content	Annual fire	38.63	3.87	F = 0.82; P > 0.05
	Annual fire + herbivory	40.50	4.12	
	Herbivory	36.02	6.28	
	No herbivory	36.87	3.87	
	Triennial fire	38.09	7.17	
	Triennial fire + herbivory	40.72	4.49	

Sand content	Annual fire	47.22	5.33	F = 0.71; P > 0.05
	Annual fire + herbivory	44.35	5.14	
	Herbivory	50.37	9.04	
	No herbivory	48.55	5.82	
	Triennial fire	48.74	9.78	
	Triennial fire + herbivory	44.31	6.62	
NDVI	Annual fire	0.35	0.06	**F = 36.22; P < 0.001**
	Annual fire + herbivory	0.41	0.06	
	herbivory	0.64	0.03	
	No herbivory	0.57	0.02	
	Triennial fire	0.43	0.03	
	Triennial fire + herbivory	0.47	0.05	
pH	Annual fire	6.43	0.15	**F = 6.91; P < 0.001**
	Annual fire + herbivory	6.45	0.12	
	Herbivory	6.21	0.14	
	No herbivory	6.25	0.27	
	Triennial fire	6.73	0.21	
	Triennial fire + herbivory	6.65	0.21	
Herbaceous biomass	Annual fire	124.83	130.64	**F = 5.01; P = 0.002**
	Annual fire + herbivory	167.09	131.49	
	Herbivory	359.12	71.91	
	No herbivory	381.19	90.59	
	Triennial fire	255.17	156.27	
	Triennial fire + herbivory	305.33	66.61	
Bulk density	Annual fire	1.30	0.16	**F = 3.16; P = 0.021**
	Annual fire + herbivory	1.26	0.16	
	Herbivory	1.36	0.05	
	No herbivory	1.31	0.04	
	Triennial fire	1.15	0.13	
	Triennial fire + herbivory	1.16	0.04	

Table 5.3: Savanna (Nkuhlu) descriptive statistics showing mean and standard de*via*tion and One-way ANOVA results for the other soil and environmental variables measured. One-way ANOVA results in bold are significant at P < 0.05.

Variable	**Treatment**	**Mean**	**Standard De*via*tion**	**One-way ANOVA (df = 3)**
Soil temperature	Herbivory	39.24	2.98	**F = 82.88; P < 0.001**
	No disturbance	31.94	1.96	
	Triennial fire	23.56	1.62	
	Triennial fire + Herbivory	27.63	2.51	
Soil moisture	Herbivory	0.86	1.08	F = 0.38; P > 0.05
	No disturbance	1.54	1.71	
	Triennial fire	1.03	1.06	
	Triennial fire + Herbivory	1.03	1.94	

Soil P	Herbivory	0.05	0.007	**F = 5.76; P = 0.003**
	No disturbance	0.05	0.007	
	Triennial fire	0.05	0.02	
	Triennial fire + Herbivory	0.04	0.006	
Soil K	Herbivory	2.59	0.11	F = 2.05; P > 0.05
	No disturbance	2.42	0.23	
	Triennial fire	2.25	0.28	
	Triennial fire + Herbivory	2.18	0.71	
Soil Ca	Herbivory	1.05	0.08	**F = 6.97; P = 0.001**
	No disturbance	1.05	0.11	
	Triennial fire	0.57	0.36	
	Triennial fire + Herbivory	0.73	0.41	
Woody cover (%)	Herbivory	0.61	0.08	**F = 25.42; P < 0.001**
	No disturbance	0.81	0.07	
	Triennial fire	0.86	0.11	
	Triennial fire + Herbivory	0.578	0.09	
Herbaceous biomass	Herbivory	203.53	48.84	**F = 7.34; P = 0.001**
	No disturbance	332.02	40.22	
	Triennial fire	235.66	96.95	
	Triennial fire + Herbivory	212.28	74.67	
Microbial biomass	Herbivory	12.87	3.32	**F = 3.87; P = 0.017**
	No disturbance	9.23	3.92	
	Triennial fire	14.56	3.83	
	Triennial fire + Herbivory	12.87	3.32	
Clay content	Herbivory	2.79	0.58	**F = 15.23; P < 0.001**
	No disturbance	1.45	0.39	
	Triennial fire	2.66	0.51	
	Triennial fire + Herbivory	3.41	1.01	
Silt content	Herbivory	13.25	2.74	**F = 10.91; P < 0.001**
	No disturbance	8.31	1.67	
	Triennial fire	13.43	2.52	
	Triennial fire + Herbivory	14.32	3.23	
Sand content	Herbivory	83.95	3.31	**F = 11.81; P < 0.001**
	No disturbance	90.23	2.06	
	Triennial fire	83.89	3.02	
	Triennial fire + Herbivory	82.25	4.21	
pH	Herbivory	5.54	0.25	**F = 4.22; P = 0.012**
	No disturbance	5.59	0.47	
	Triennial fire	6.21	0.6	
	Triennial fire + Herbivory	5.81	0.37	
NDVI	Herbivory	0.52	0.05	**F = 13.57; P < 0.001**
	No disturbance	0.55	0.04	
	Triennial fire	0.42	0.03	
	Triennial fire + Herbivory	0.51	0.05	
Bulk density	Herbivory	1.41	0.17	F = 0.83; P > 0.05
	No disturbance	1.39	0.12	
	Triennial fire	1.34	0.17	
	Triennial fire + Herbivory	1.45	0.12	