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Biodiversity and ecosystem function: How experimental large herbivore loss influences savanna carbon dynamics

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Doctor of Philosophy
in
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by

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September 2021

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For my family: mom and dad, Colin and Quynh and Anh, Margaret and Nate, Timmy and Bee, and of course Mark (and Stevie).

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Abstract

Biodiversity and ecosystem function: How experimental large herbivore loss influences savanna carbon dynamics

by

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The Anthropocene is characterized by profound human-mediated effects on biodiversity loss and climate change. These two phenomena are traditionally studied as separate, yet parallel processes. However, today it is clear that wildlife, and large-bodied wildlife in particular, have significant impacts on carbon storage and cycling in ecosystems worldwide. It is increasingly necessary to incorporate large-bodied animals into characterizations of the carbon cycle to better predict the possible outcomes of their loss on climate. I first asked how large, wild herbivore species and their experimental loss influence a range of essential ecosystem processes with a global review and analysis. I focused on ecosystem carbon dynamics with a meta-analysis, parsing data on metrics of carbon storage or cycling from all published studies that use large-scale, large herbivore exclosure experiments. Using conclusions from this study, I narrowed my scope for empirical research on a single exclosure experiment located in Laikipia, Kenya: the Kenya Long-term Exclosure Experiment (KLEE). KLEE has been operating since 1995 in a savanna ecosystem where a robust community of large-bodied herbivores (and which share the landscape with domestic cattle) persists. I used classic soil science methods and developed new tools to monitor soil carbon storage and rates of cycling, in a range of experimental herbivore treatments simulating large herbivore loss and their spatial replacement with domestic cattle. My analyses explore the indirect effects of these large-bodied herbivores on ecosystem carbon, via their direct effects on savanna structure and

assembly. Understanding the outcomes of large herbivore losses on landscape scales like those simulated in KLEE will add necessary context-specific nuance to predictions of how continued large herbivore losses will impact carbon budgets globally.

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Chapter 1

Introduction

1.1 Background

The planet is experiencing a massive biodiversity crisis, largely due to humankind and its overuse of global landscapes, resources, and wildlife (Ripple et al. 2016; Young et al. 2016). Large-bodied species face the greatest risk because of their large energy requirements, large home ranges, relatively small population sizes, and more frequent conflict with humans through hunting or land-use change (Galetti Dirzo 2013; Dirzo et al. 2014). Such size-selective wildlife loss, which can result in great reductions in species' abundances and local extinctions worldwide, is now a hallmark of the Anthropocene. It is increasingly urgent to understand the cascading impacts of large wildlife losses on the essential ecosystem processes that underpin successful ecosystem functioning worldwide.

Large-bodied herbivores (here, >5kg), in particular, have distinct direct and indirect effects on ecosystem functions thanks to their role as primary consumers. While some large-bodied herbivore species have and are experiencing recent recoveries in population size (e.g. white-tailed deer, moose) because of factors like similarly-dramatic large predator loss worldwide, most populations of the world's largest herbivores are threatened by

overhunting, habitat loss, and/or displacement by livestock (Ripple et al. 2015). Their loss from or decline in ecosystems around the world can therefore have large yet context-dependent consequences. Large herbivores often act as ecosystem engineers, in part due to their proportionately large consumption of primary production, their export of plant-associated nutrients to other patches or even ecosystems as they move (e.g. Stears et al. 2018), their consumption and dispersal of an ecosystem's larger fruits and seeds, and even their trampling or destruction of ecosystems' structural components, including trees (Ripple et al. 2015).

The loss of any of these functions can result in outcomes ranging from lower biodiversity (Burns, Collins, and Smith 2009), to altered fire regimes (Holdo, Holt, and Fryxell 2009), to increased disease prevalence (Young et al. 2014), even to the ecosystem's succession to an alternative state (van Wieren and Bakker 2008). Because of their functional importance, it is crucial that ecologists seek to systematically understand the possible ecosystem-level outcomes of large herbivore loss where they are currently threatened.

In this dissertation, I approach this task from the top down, first by reviewing and analyzing data on the functional outcomes of experimental large herbivore exclusion ('loss') from large herbivore manipulations across the globe. I then develop a novel, reproduceable technology to monitor and assess ecosystem carbon exchange (in the context of large herbivore impacts) to promote systematic data collection practices. Last, I apply these theories and lessons to a savanna in central Kenya, where I explore the impacts of experimental large herbivore loss and their replacement with domestic cattle on carbon and nitrogen storage and cycling.

1.2 Chapter one:

Historically, the cascading ecological consequences of size-selective wildlife loss has focused on the trophic cascades caused by the declines and losses of large predators (Estes et al. 2011; Ripple et al. 2014). However, it is increasingly recognized that large herbivore loss can drive similarly strong cascading effects within and across ecosystems (Daskin and Pringle 2016; Osuri et al. 2016; Young et al. 2016). Many of these effects are direct and driven by the loss-induced reduction in primary production consumption, physical disturbance, and deposition of nutrients via metabolic waste (van Wieren 1998). The roles that large herbivores play in maintaining ecosystem structure and function may not be filled by smaller consumers (e.g. Pérez-Méndez 2016). The loss of large herbivores can also trigger cascading, hard-to-predict indirect effects on vital ecosystem functions.

However, the effects of large herbivores (and conversely, the effects of their loss) on ecosystem function are likely context dependent: varying from ecosystem to ecosystem and through time in both cyclical and stochastic ways. Not only that, "ecosystem functions" are ill-defined in the ecological literature and also not always identified and measured in comparable methods across studies. As such it is difficult to systematically assess the effects of large herbivores across different ecological and experimental contexts.

In my first chapter, a review and meta-analysis, I define ecosystem function to determine the effects of large herbivores (and their experimental loss via exclusion) on some vital ecosystem functions. I then pull from an extensive literature search of large-scale, large-bodied, wild herbivore exclosure experiments to systematically explore the effects of experimental large herbivore 'loss' on these functions. I use these studies to describe the current state of the science regarding such experiments worldwide, and identified which five functions were most studied; nutrient cycling, primary productivity, plant regeneration, ecosystem resilience and resistance, and carbon cycling. I and my coauthors

then qualitatively review the impacts of large herbivore 'loss' on each of these functions on a global level.

Also in Chapter 1, I use carbon cycling as a case study function, and pull data from all identified published studies that used exclosures to quantitatively assess the effects of large herbivore 'loss' on carbon dynamics. I synthesize these assessments into recommendations for the size, experimental setups, and distribution of large herbivore exclosure experiments, as well as for greater consistency in metrics for measuring a given function of interest. These recommendations are presented as suggested best practices for developing a global network of comparable large herbivore exclosure experiments, assessing the effects of large herbivores and their possible loss from ecosystems on essential functions.

1.3 Chapter two:

One of the essential ecosystem functions directly and indirectly altered by loss of large wildlife is the carbon cycle (Schmitz et al. 2014), reviewed in Chapter one. This understanding comes at a time when both biodiversity loss and climate change are occurring in parallel, and at unprecedented scales. This synchronicity underscores the need to identify where large wildlife loss impacts ecosystem carbon storage or carbon emissions to the atmosphere, using metrics that are comparable across ecosystems. For example, ecologists use carbon flux rates (the rate at which carbon dioxide, CO2, cycles between the soil and the atmosphere) to assess the balance of CO2 in an ecosystem, or whether it is a source or sink for atmospheric carbon (Houghton 2007).

Accurate and reliable data on how wildlife loss impacts carbon flux in a variety of ecosystems is necessary to predict how unprecedented wildlife losses will change carbon budgets on local to global scales. However, broad data collection across time and space

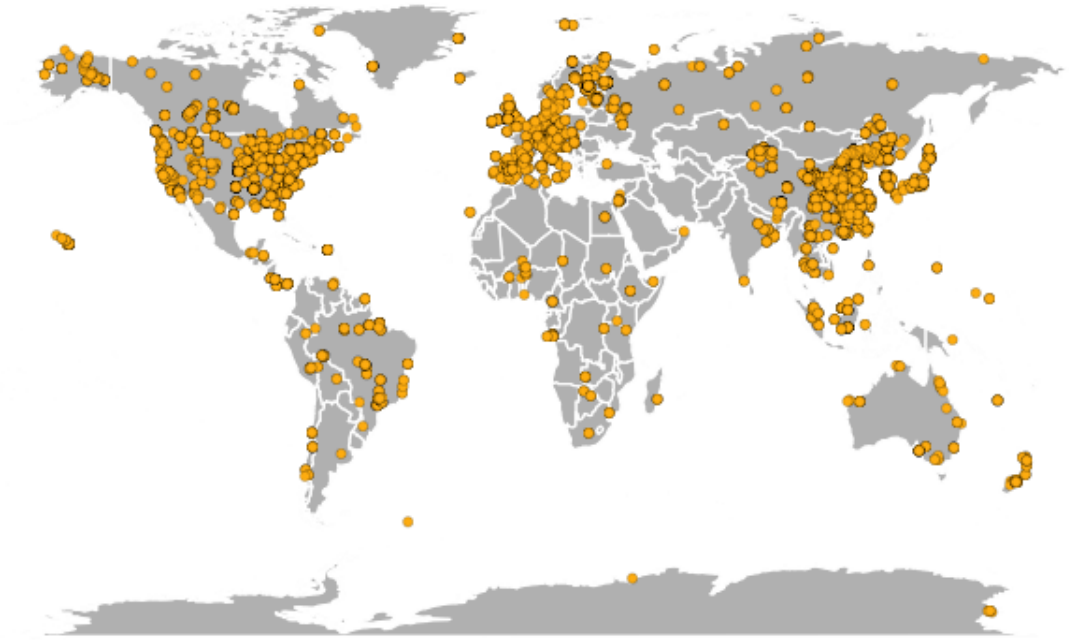

Figure 1.1: Global distribution of the locations of existing published datasets on soil carbon flux, collected using infrared-gas analyzers (IRGAs) and measured in the field. All data were taken between 1961 and 2017. Data from version 5.0 of "A Global Database of Soil Respiration Data", Jian et al. 2021.

requires significant funding (Hughes et al. 2017). In addition, ecosystems in the Global North that are already highly-studied continue to receive the most time, funding, and support (Martin, Blossey, and Ellis 2012), skewing the scales at which carbon flux is monitored. This results in regions where environmental change like wildlife loss is occurring rapidly, but that are nonetheless understudied (like tropical grasslands) (Martin, Blossey, and Ellis 2012; Hoekstra et al. 2005) (Fig. 1.1). In light of continuing wildlife loss, the costs of environmental monitoring, and geographic bias in research, it is important to innovate less expensive ways to make environmental monitoring more tenable worldwide.

In my second chapter, I explore a novel 'do-it-yourself' soil carbon flux sensor, developed in response to the need for an inexpensive, resilient, autonomous device that can monitor soil carbon flux across both space and time. By installing a network of

these chambers in an existing large-scale large herbivore exclosure experiment, to collect hourly carbon flux data across multiple large herbivore community contexts and seasonal shifts, we aim to enable deeper exploration of carbon cycling than previously possible. In developing this network of sensors we hope to demonstrate that there are accessible possibilities for collecting spatially-explicit yet large-scale datasets characterizing the effects of large herbivore loss on carbon cycling. Importantly, if similar devices and network approaches were adopted in other exclosure experiments, the data would be comparable, and researchers would be able to parse the ecological contexts responsible for differences in the response of ecosystem carbon dynamics to large herbivore 'loss'.

1.4 Chapter three:

Chapter one focuses on global-scale datasets from large herbivore exclosures, and the effects of large herbivore exclusion on ecosystem functions. Chapter two takes recommendations from chapter one regarding reproducibility and collecting comparable datasets, and applies them to the development of an inexpensive tool to collect data on carbon dynamics across space and time. Chapter three zeros in on ecosystem carbon cycling in one large herbivore exclosure experiment, in an ecosystem that maintains a relatively intact community of large-bodied herbivores: a savanna ecosystem in Laikipia, Kenya.

The Kenya Long-term Exclosure Experiment (KLEE) there has been exploring the experimental 'loss' of mega herbivores like elephants and giraffes, as well as wild meso-herbivores like zebra, for over 25 years (Fig. 1.2). Of the existing large herbivore exclosure experiments detailed in chapter one, it is larger and has been running longer than the great majority. Crucially, it also incorporates treatments with domestic cattle, reflective of not only the reality that this and many other grasslands are and have been mixed-use rangelands for centuries, but also that much modern wildlife loss occurs due to habitat

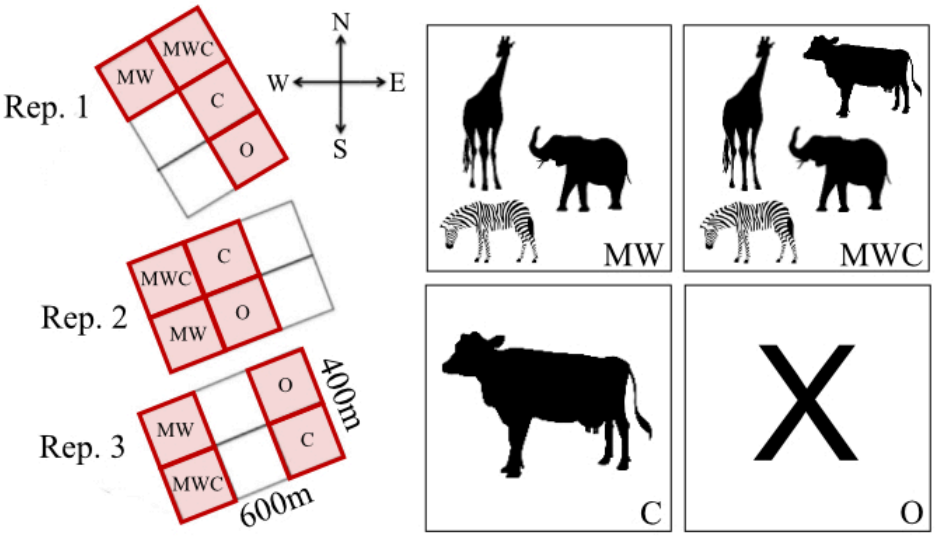

Figure 1.2: An aerial schematic diagram of the Kenya Long-term Exclosure Experiment, indicating its approximate extent over the landscape as well as the treatments studied in this chapter (highlighted in red, with pictorial explanations of the treatment labels, e.g. which herbivores are present in which plots, on the right).

loss, often to livestock production (Tilman et al. 1994; Young et al. 1998; Lamprey and Reid 2004; Reid 2012). It is therefore realistic and necessary to experimentally probe the effects of wild large herbivore loss, their coexistence with domestic herbivores, and their spatial replacement with domestic herbivores.

Lastly, KLEE is an ideal location to specifically study the effects of large herbivores and their reassembly on ecosystem carbon dynamics. Rangelands currently cover approximately half of the globe's ice-free land (Lund 2007). They also store between ten and 30 percent of its soil organic carbon content, making them an important pool of the global carbon cycle (Derner and Schuman 2007). The extent of this storage on a landscape scale is highly impacted by multiple system characteristics, including stocking densities, native wildlife abundance and diversity, soil fauna, soil type, floral community composition, and seasonality. Currently, we lack a general understanding of how these factors affect rangeland carbon storage and cycling (Derner and Schuman 2007). As such, KLEE

is a powerful representative rangeland experiment to explore ecosystem-level carbon dynamics in the context of large herbivore community reassembly and domestic herbivore presence, over time.

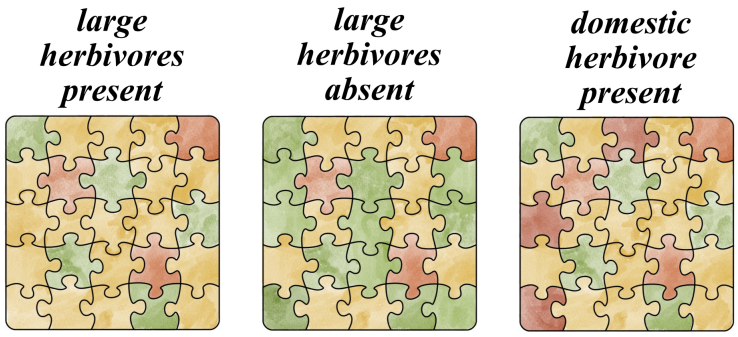

Figure 1.3: Stylized representation of differences in ecosystem re-assembly according to the presence or absence of wild and domestic herbivores. For example, previous studies in the KLEE have demonstrated that the exclusion of the largest herbivores results in proportionally total canopy cover (green) compared to open soil (yellow) or termite mounds (red); others have demonstrated that the presence of domestic cattle could result in greater numbers of termite mounds. The re-assembly of these ecologically distinct features depending on the composition of its herbivore community will likely have cascading consequences for the ecosystem's carbon cycle.

In this chapter I demonstrate the indirect effects of large herbivores and domestic cattle presence/absence on soil carbon pools and mineralization via changes to ecosystem assembly: the arrangement of key ecological features like tree canopies and termite mounds (Fig. 1.3). Large herbivores have direct impacts on ecosystem assembly and structure, and their indirect effects on functions like carbon cycling can be viewed (e.g. Sitters et al. 2020; Charles et al. 2021). I explore several metrics of carbon storage and mineralization rates in KLEE, focusing on differences across within-treatment landscape features and scaling these differences up using percent cover of each feature to calculate whole-treatment pools of carbon and microbial biomass.

1.5 Permissions and Attributions

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Chapter 2

Synthesizing the effects of large, wild herbivore exclusion on ecosystem function

2.1 Abstract

Wild large herbivores are declining worldwide. Despite extensive use of exclosure experiments to investigate herbivore impacts, there is little consensus on the effects of wild large herbivores on ecosystem function.

Of the ecosystem functions likely impacted, we reviewed the five most-studied in exclosure experiments: ecosystem resilience/resistance to disturbance, nutrient cycling, carbon cycling, plant regeneration, and primary productivity.

Experimental data on large wild herbivores' effects on ecosystem functions were predominately derived from temperate grasslands (50% grasslands, 75% temperate zones). Additionally, data were from experiments that may not be of adequate size (median size 400m^2 despite excluding all experiments below 25m^2) or duration (median duration 6

years) to capture ecosystem-scale responses to these low-density and wide-ranging taxa.

Wild herbivore removal frequently impacted ecosystem functions; for example, net carbon uptake increased by three times in some instances. However, the magnitude and direction of effects, even within a single function, were highly variable.

A focus on carbon cycling highlighted challenges in interpreting effects on a single function. While the effect of large herbivore exclusion on carbon cycling was slightly positive when its components (e.g. pools vs. fluxes of carbon) were aggregated, effects on individual components were variable and sometimes opposed.

Given modern declines in large wild herbivores, it is critical to understand their effects on ecosystem function. However, this synthesis highlights strong variability in direction, magnitude, and modifiers of these effects. Some variation is likely due to disparity in what components are used to describe a given function. For example, for the carbon cycle we identified eight distinctly meaningful components, which are not easily combined yet are potentially misrepresentative of the larger cycle when considered alone. However, much of the observed difference in responses likely reflects real ecological variability across complex systems.

To move towards a general predictive framework we must identify where variation in effect is due to methodological differences and where due to ecosystem context. Two critical steps forward are 1) additional quantitative synthetic analyses of large herbivores' effects on individual functions, and 2) improved, increased systematic exclosure research focusing on effects of large herbivores' exclusion on functions.

2.2 Introduction

Large-bodied wildlife are declining precipitously in distribution and abundance (Ceballos et al. 2015, Young et al. 2016), especially taxa of large mammalian herbivores

(Smith et al. 2018). The loss of these herbivores not only constitutes a critical loss of intrinsic biodiversity but is hypothesized to have broad impacts on ecosystem functions. However, quantitative syntheses of the impacts of biodiversity loss on ecosystem functions have focused nearly exclusively on studies of small or sessile organisms like invertebrates and plants (Hooper et al. 2012, Delgado-Baquerizo et al 2015, Soliveres et al. 2016). The lack of synthesis is surprising given that 1) large taxa are often suggested to have disproportionately influential roles on ecosystem function (Owen-Smith 1988, Pringle et al. 2010) (Fig. 1), and 2) multiple efforts have attempted to synthesize effects of excluding large, wild herbivores on producers (e.g. Gruner et al. 2008, Jia et al 2017) and smaller consumers (Foster et al. 2014, Daskin and Pringle 2016).

Yet, there is a rich body of theoretical and empirical literature on the effects of large wild herbivores (>5 kg; hereafter large herbivores) on ecosystem functions stretching back decades (e.g. McNaughton 1979). These species often fill functionally unique roles in ecosystems. For instance, their large body size allows for very high plant consumption (Clauss et al. 2013), large geographic ranges of movement, long-distance transport of nutrients via their waste (Wolf et al. 2013), and unique capability to physically modify habitats via soil compaction and cracking, erosion, and by breaking woody vegetation (Pringle 2008, Beck et al. 2010, van Klink et al. 2015, Long et al. 2017). The effects of large herbivores on both producers and consumers are often, but not always (see Jia et al 2018 and Koerner et al. 2018) mediated by environmental variables, for example ecosystem productivity (Daskin and Pringle 2016, Burkepile et al. 2017), which may mediate herbivore effects on several ecosystem functions (Fig. 2.1).

Here, we review the impacts of experimental removal of large herbivores on five commonly-studied ecosystem functions: ecosystem resilience/resistance, nutrient cycling, carbon cycling, plant regeneration, and primary productivity. Though distinct, these functions are often linked or synergistic (e.g. nutrient cycling influencing carbon cycling)

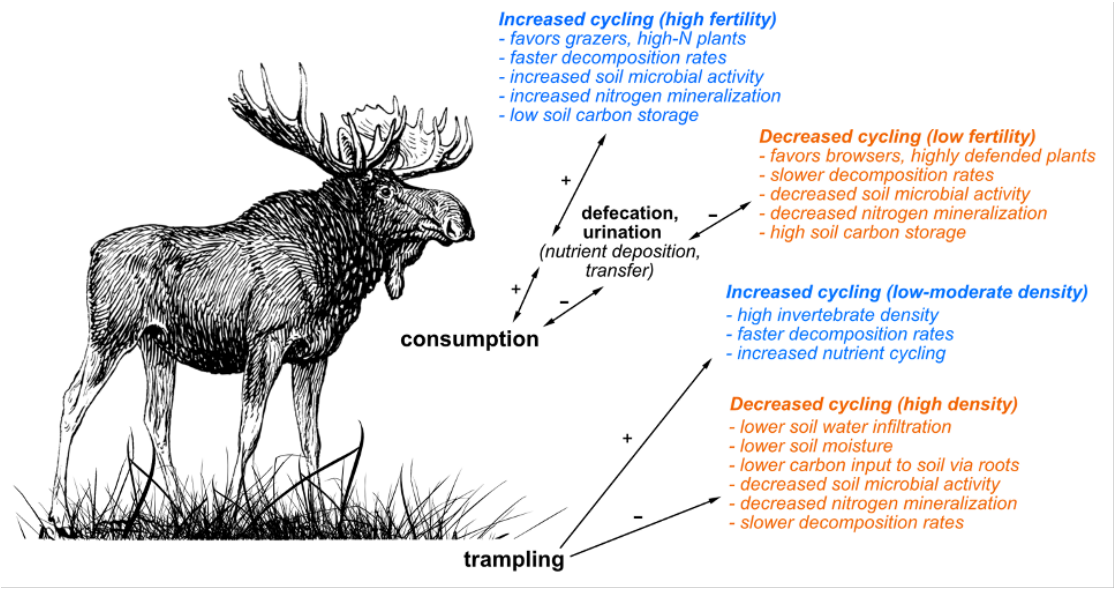

Figure 2.1: Hypothesized influences of large herbivores on an example ecosystem function: nutrient cycling. Direct effects of large herbivores (consumption, trampling) lead to highly context-dependent (ex: herbivore density, ecosystem fertility) indirect outcomes on nutrient cycling. Orange text denotes decelerating effects on nutrient cycling; blue denotes accelerating.

(Bennett, Peterson, and Gordon 2009). We focus this review exclusively on terrestrial exclosure experiments involving large wild herbivores, though we briefly discuss aquatic and domestic herbivores when discussing the importance of ecological context. We also present a meta-analysis on how large herbivores affect carbon cycling, which highlights different responses among the components of a single function.

2.3 Exclosure experiments: Distributions and biases

We identified 17 candidate ecosystem functions likely impacted by large herbivores (Appendix 1.1) and used standardized search procedures to identify 174 unique published experiments that 1) excluded large, native, wild herbivores from plots at least 25m^2 (to better capture indirect effects, and reduce likelihood of edge effects swamping treatment effects), and 2) collected data on ecosystem functional responses (Appendix 1.2, Fig.

A.1). While restricting this search to exclosure experiments has limitations (e.g. experimental artifacts, practical limits to size and duration of experiments (Diamond 1983)), these manipulations provide a controlled method to isolate the impacts of total removal of large herbivores on ecosystem function (Bakker et al. 2015). While natural experiments (e.g. observations of widespread herbivore loss or decline) are integral in detecting large-scale, long-term impacts of environmental perturbation on ecosystem functions like ecosystem resilience and resistance (Caves et al. 2013), they are difficult to replicate and often have confounding covariates (e.g. impacted sites often experience multiple human uses, climate change may confound temporal comparisons). Natural experiments also display more nuanced variation in herbivore density (e.g. decline rather than total removal), making comparisons between them more difficult.

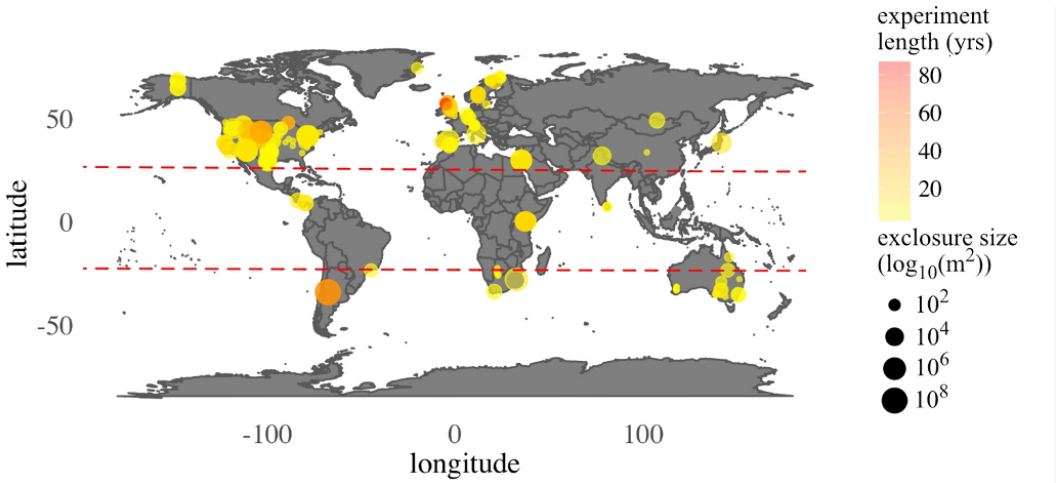

Figure 2.2: Map of 117 distinct, published functional responses to large herbivore removal with experimental exclosures. Point color (yellow-red) indicates duration of exclosure experiment at time response data were collected; point size indicates exclosure size. Note a single exclosure site could have multiple responses published from it (e.g. more than one function measured; a single function measured at experimentally distinct times, or in ecologically distinct locations within the experiment). Points with high opacity thus indicate a site from which multiple responses were published, opacity increasing with the number of unique responses.

Plot sizes in exclosure experiments in our synthesis ranged from $25m^2$ to $128km^2$

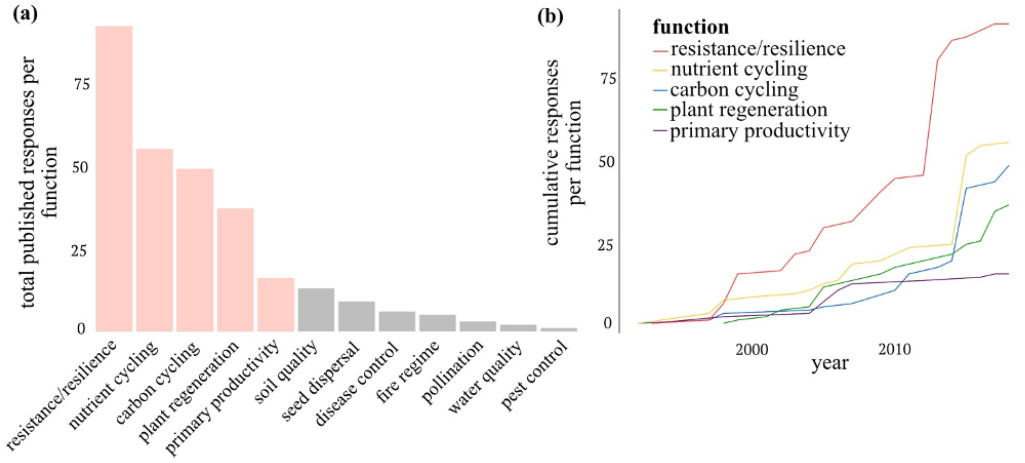

Figure 2.3: a) Total number of published responses per function. The top 5 most commonly-studied functions under these parameters, marked in red, comprise 87% of published efforts and are the focus of this review. b) Cumulative number of functional responses of the five most- studied ecosystem functions, over time, demonstrating trends in the academic study of ecosystem functional response to experimental large herbivore loss.

(median size 400m^2; Appendix 1.2). Duration of exclosure ranged from <1 year to 85 years (median 6 years). We found 12 of the 17 a priori identified functions had been explicitly studied with exclosure experiments, totaling 107 unique publications and 288 individual functional responses from 174 unique experimental sites (Fig. 2.2). The great majority (86%) of functional responses to large herbivore exclusion experiments concentrated on just five functions: 1) ecosystem resistance/resilience, 2) nutrient cycling, 3) carbon cycling, 4) plant regeneration, and 5) primary productivity, and we limited our review to these (Fig. 2.3a, b). Research was heavily concentrated in temperate biomes (approximately 75%; Figs. 2.2, 2.4) and grasslands (approximately 51%; Fig. 2.5) (Appendix 1.2), despite evidence that size-selective defaunation is most pervasive in the tropics (Fritz, Bininda-Edmonds, and Purvis 2009).

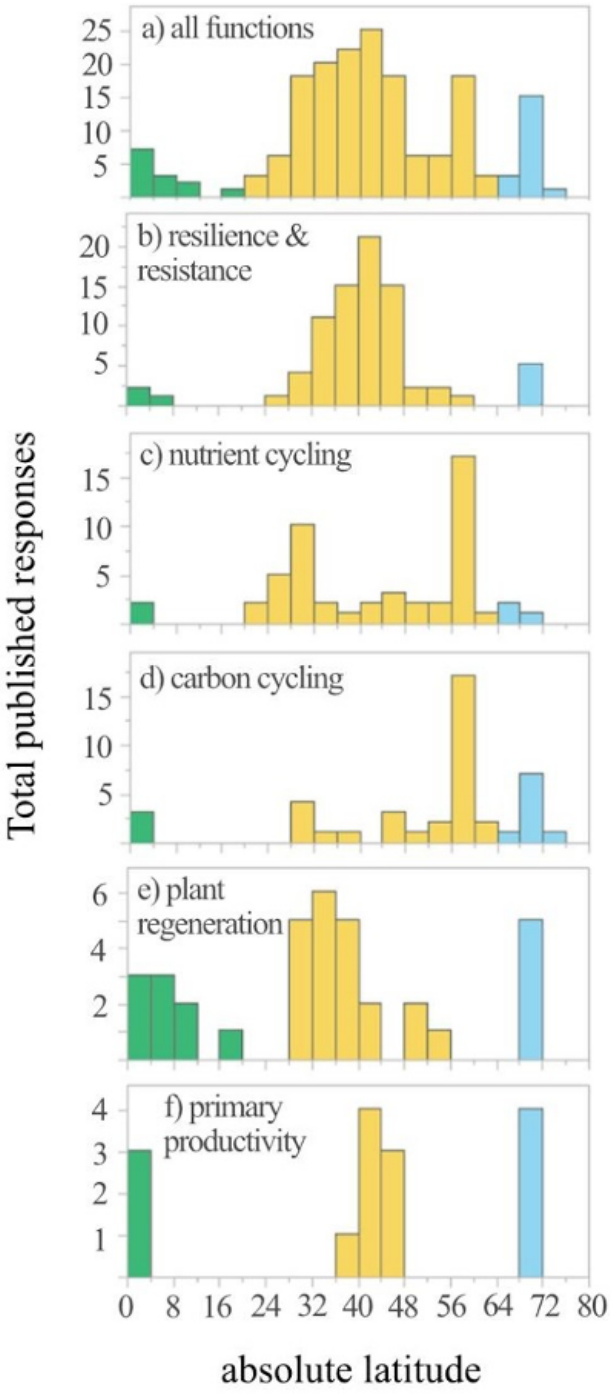

Figure 2.4: Total number of responses to large herbivore exclosure, arranged by absolute latitude, with green indicating that data are from the tropics, yellow from temperate zones, and blue from the arctic.

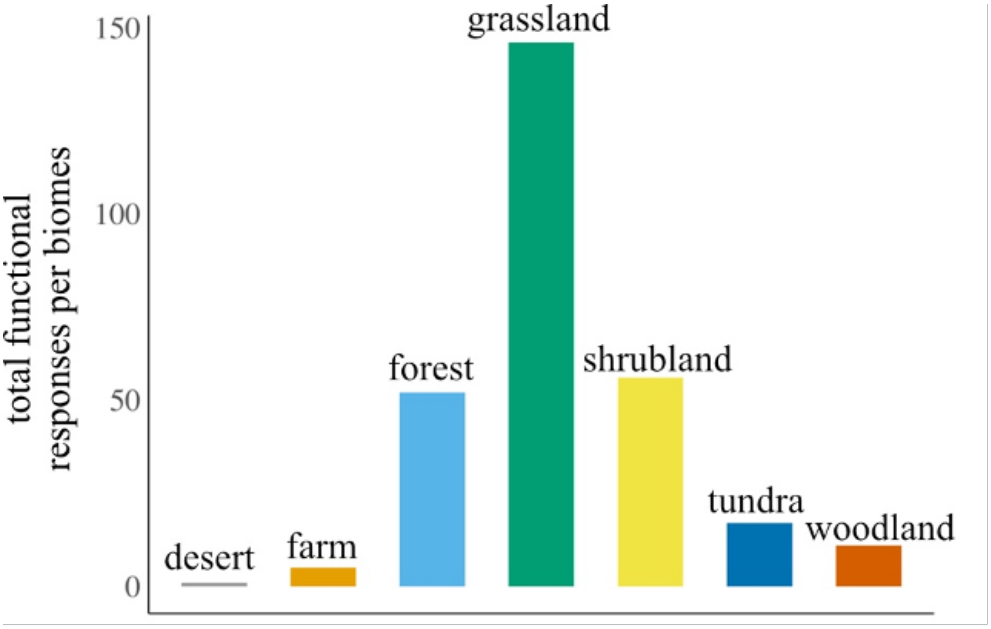

Figure 2.5: Total number of responses to large herbivore exclosure, by biome. Biomes provided in publications were binned into broad categories (e.g. savanna, prairie in 'grassland') to demonstrate general patterns in the locations of studies examining large herbivore exclosure on ecosystem function.

2.4 Insights from well-studied ecosystem functions

2.4.1 Nutrient cycling and translocation

Large herbivores often cause strong changes in nutrient cycling, although the magnitude and even direction of effect is typically understood to vary across contexts. Generally, large herbivores are thought to accelerate nutrient cycling in highly productive ecosystems with long histories of herbivory, and with low to moderate grazing intensities (McNaughton et al 1997, de Mazancourt et al 1998). Although there are many pathways involved, the main mechanism is via the conversion of large quantities of aboveground biomass into labile waste products (Tracy and Frank 1998). Large herbivores also shift plant allocation of nutrients to roots, increasing soil microbial activity and in turn soil nitrogen mineralization (Ruess and McNaughton 1987). In contrast, large herbivores in

low-productivity systems or those with historically low population densities often decelerate nutrient cycling (Bardgett and Wardle 2003) via selective foraging for nutrient-rich plants, which subsequently shifts communities toward species that decompose slowly (e.g. Harrison and Bardgett 2004).

However, in contrast to this general theory, many studies have found contradictory effects. In some cases, increased productivity simply does not result in accelerated nutrient cycling (e.g. Cherif and Loreau 2013, Stark et al. 2015). In other cases, effects are more associated with location- and time-specific variation (e.g. Wardle 2002, Stark et al 2002); for example, summer grazing by reindeer increases tundra nutrient cycling rates from fecal nutrient deposition, while winter grazing results in the opposite when these nutrients leach from the system (Stark and Grellman 2002, Stark et al. 2007). This inconsistency in effect may be because secondary mechanisms (soil compaction, temperature, trampling, litter chemistry, lateral nutrient transport, among others) override the general mechanisms detailed above. Unfortunately, there is currently no theory to integrate these highly disparate results into a predictive framework. This gap has prompted a call to revisit the generalizations about productivity mediating herbivore effects on nutrient cycling and conduct more rigorous synthesis to help identify other moderators (Sitters and Venerink 2015).

An important caveat in interpreting these results is that work from exclosure experiments is, logistically, almost exclusively focused on nutrient cycling within a system, ignoring lateral transfer of nutrients between systems or across space within a system. However, the important effects of lateral nutrient transfer are well documented and may often overpower the effects of herbivory on nutrient cycling within systems (e.g. Stark et al 2015, Leroux and Schmitz 2015). Moose and hippopotamus, for example, move substantial quantities of nutrients between terrestrial and aquatic ecosystems, increasing nutrient availability and subsidizing consumers in recipient systems (Stears et al 2018);

similarly, rhinoceroses maintain nutrient (and secondarily, structural) heterogeneity via the lateral transfer of nutrients across a single savanna system (Veldhuis et al. 2017). Though exclosure experiments are generally inappropriate to study these landscape-scale effects of herbivores on nutrient cycling, recent synthesis nonetheless suggests that effects of such transfer likely vary across characteristics of both nutrient donor and recipient ecosystems and the herbivore species involved (Subalusky and Post 2019).

2.4.2 Ecosystem resilience and resistance

Resilience is often defined as an ecosystem's capacity to return toward its previous state following a disturbance, while resistance generally refers to an ecosystem's ability to maintain its integrity in the face of that disturbance (Mitchell et al. 2000). Exclosure experiments have addressed the resilience/resistance of microbial community dynamics (Hodel et al. 2014, Rudgers et al. 2016), exotic species invasions (Seabloom et al. 2009, Ender et al. 2017), nutrient dynamics (Bakker et al. 2009), and chemical or physical defense (Young et al. 2003). For example, removal of large herbivores often results in dramatic reductions in plant defenses, making them less resistant to future herbivory (Young and Okello 1998, Ward and Young 2002, Palmer et al. 2008). Large herbivore exclusion can also lead to increases in exotic plants (Seabloom et al. 2009, Ender et al. 2017) suggesting that wild herbivores help ecosystems resist exotic plant invasions.

The concept of resilience/resistance may be best captured by how herbivores impact plant communities or ecosystem processes after a disturbance such as fire or drought (Porensky et al. 2013). Unfortunately, due to the experimental difficulty, exclosure experiments are not often combined with other disturbances or conducted on temporal scales long enough to test questions of resilience or resistance. However, observational data combined with what experimental data do exist suggest that herbivores and fire

act synergistically to influence resilience and resistance of plant communities, especially the transition between grass- and woody-dominated communities. In African savannas, fire and large herbivores together suppress woody vegetation growth and facilitate grasses (Augustine and McNaughton 2004, Staver et al. 2009). Large herbivores also keep woody individuals small, and more likely to be killed by fire (Midgley, Lawes, and Chamaillé-Jammes 2010). Both mechanisms suggest a strong link between large herbivores and savanna resilience. Indeed, large herbivore removal allows woody plants to grow tall enough to resist the effects of fire (Staver and Bond 2014). Elephants, the largest herbivores, may be one of the only forces that can facilitate the resilience of grass-dominated ecosystems after woody plants establish (Dublin et al. 1990, Skarpe et al. 2004, Pringle et al. 2015).

In mesic grasslands of North America, fire frequency appears to be the primary driver of ecosystem resistance, with frequent fires suppressing establishment of woody vegetation (Briggs et al. 2005). Therefore bison (and non-wild livestock) may in fact hasten woody vegetation expansion, as grazing removes fuel loads and subsequently lowers fire intensity and grass competition. However, these dynamics were only captured with decades-long fire manipulations. Thus, addressing how herbivores affect resilience/resistance to disturbances will be more difficult to capture at the temporal scales of most experiments (Fig. 2.6).

2.4.3 Plant regeneration

Large herbivores can strongly impact many components of plant regeneration (germination, recruitment, survival, etc.) (Kurten 2013) through a wide range of mechanisms, ranging from direct consumption to indirect effects of competition or facilitation. They can increase seed germination and emergence, for example by suppressing small con-

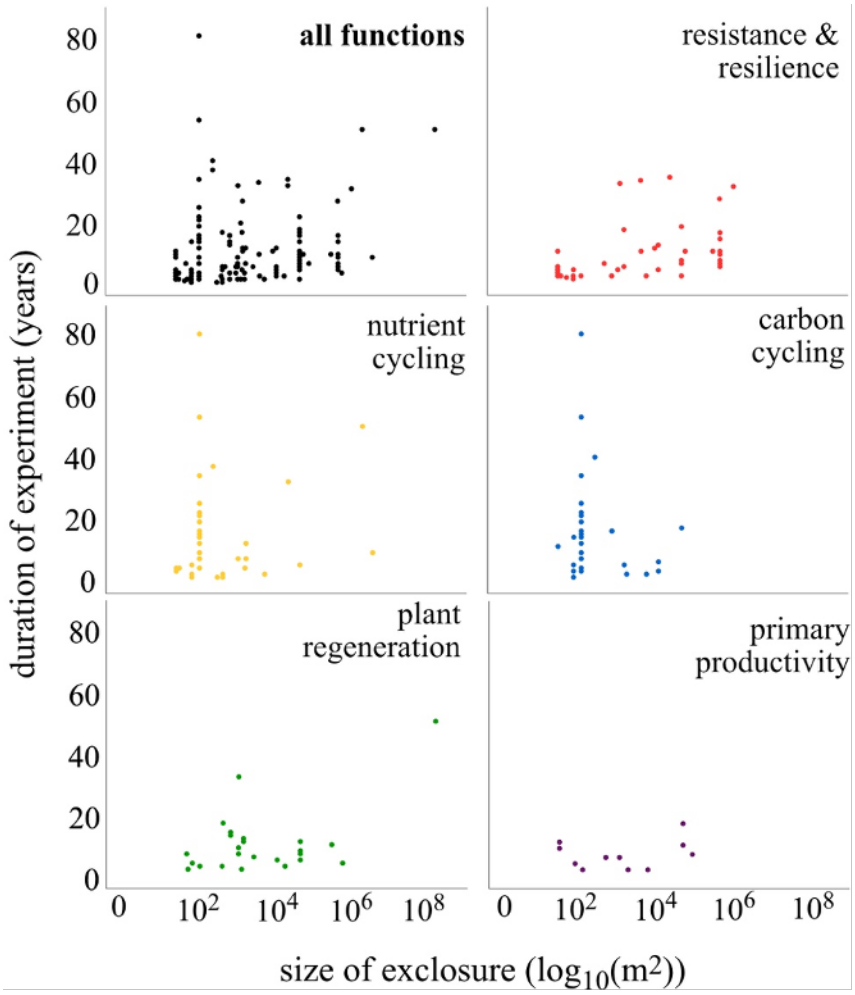

Figure 2.6: Distribution of elapsed duration and size of physical exclosures used to measure the responses of the top-five most studied ecosystem functions in the literature. The clustered spread of these experiments, both in total and separated by function, indicates both a size and time bias in these data: data frequently come from smaller and shorter-duration exclosure sites upon publication. Separated by function, these trends are generally retained with some variation across functions.

sumers that prey on seeds (Goheen et al. 2010, Maclean et al. 2011). However, dispersal-dependent components like seedling community composition (Kurten et al. 2015) and seedling diversity (Granados et al. 2018) vary due to differences in predominant dispersal method in a given ecosystem (biotic or abiotic). As with other functions, effects on plant regeneration are often contextually dependent on the identity and ecology of the herbivores in question. For instance, herbivores selectively consuming palatable species

suppress their regeneration, favoring dominance of unpalatable species. However, if the herbivores are migratory, seasonally-intense herbivory may favor regrowth of palatable species and result in their dominance (Augustine and McNaughton 1998). Herbivore body size also influences plant regeneration; very large herbivores (elephant, wildebeest) and smaller large herbivores (impala, warthog) can have equally-strong, but sometimes contrasting effects on plant species dominance, productivity, and seedling survival, and thus on community richness (Burkepile et al. 2017). Notably, herbivore density, migration patterns (Augustine and McNaughton 1998), and range size (Granados et al. 2018) can cause effects on plant regeneration to be spatially and temporally irregular.

Effects of large herbivores on plant regeneration also vary at different plant life stages. For example, when large herbivores are excluded, flowering and fruiting success can increase dramatically as these parts are no longer consumed (Young and Augustine 2007, Wilkerson et al. 2013, Pringle et al. 2014). However, as previously mentioned, when small mammal populations increase in large mammal exclosures due to competitive release, they can cause significant increases in seed and seedling predation (Goheen et al. 2004, Goheen et al. 2010, MacLean et al. 2011). The net effect of these opposing forces depends in part on the size and functional role of the large herbivores involved.

For example, excluding only elephants in a Kenyan savanna had weak positive effects on community-level shrub density, despite their strong negative effects on adult shrub survivorship and reproduction. One possible explanation is that in the absence of elephants, rodents' increased seed predation led to less shrub recruitment. However, when other large herbivores were also excluded, shrub density increased dramatically, despite even greater rodent seed predation, apparently due to increased fruit production and reproductive output of shrubs in the absence of those herbivores who specifically impact the fruits, and thus reproductive output, of mature plants (Pringle et al. 2014). Another example of potentially-opposing effects is preferential browsing of palatable species

by large herbivores, which can decrease regeneration via direct consumption of plant material, but also increase it via mechanisms like increased nutrient input or beneficial migration-based herbivory regimes (Augustine and McNaughton 1998). Once again, the observed variability in responses is likely driven by variation in ecosystem properties like soil fertility (Olff and Ritchie 1998) and ecosystem productivity (Burkepile et al. 2017).

2.4.4 Primary productivity

The activity of large herbivores (as consumers, disturbance agents, and fertilizers) can serve as major drivers of primary productivity (Milchunas and Lauenroth 1993, Bardgett and Wardle 2003). Although most exclosure research has focused on grass- and grass/shrub-dominated landscapes, even within this context effects are extremely variable: the effects of herbivores on primary productivity can vary from positive (e.g. McNaughton 1983, Charles et al. 2017) to negative (e.g. Pastor et al. 1993, Ritchie et al. 1998), depending on the ecosystem in question. As with nutrient cycling, large herbivores broadly promote primary productivity when soil nutrients and moisture are abundant, grazing intensity is light to intermediate, and herbivores and plants share long evolutionary histories. In contrast, they often have neutral or negative effects when soil resources are low, grazing intensities are high, and evolutionary histories between herbivores and plants are short (Milchunas and Lauenroth 1993).

While most studies have addressed the effects of herbivores on aboveground productivity, focus has increasingly expanded to include belowground productivity. Large herbivores can have positive (Frank et al. 2002), neutral (McNaughton et al. 1997) or negative effects (Archer and Tieszen 1983) on belowground productivity. In some cases, herbivores drive above- and belowground productivity in the same direction, while opposing effects occur in other systems. For example, grazers in northern India increase aboveground

primary productivity but reduce it belowground (Bagchi and Ritchie 2010), while in Yellowstone National Park ungulates stimulate increases in both above and belowground productivity (Frank et al. 2002). As with other functions, analysis incorporating both systematic context (e.g. soil and vegetation community properties, number and type of large herbivores) and the components of the function that were measured (e.g. above or belowground productivity) is crucial to understand observed variation.

Despite obvious differences among herbivore types, and potential for interactions among these species, effects of herbivore identity and composition on primary productivity have received relatively little attention. A noteworthy exception is the work of Charles et al. (2017), who addressed the individual, additive and interactive effects of co-occurring wild herbivores (and livestock) on ecosystem function with large-scale, size-selective exclosures. In this system, aboveground primary productivity did not differ between plots with both large herbivores and mega-herbivores (giraffe and elephants) and plots with only large herbivores. However, the addition of domestic cattle to the large herbivores-only communities enhanced aboveground primary productivity, though this effect was reduced when mega-herbivores were also present. Typical herbivore exclusion experiments may not pick up these nuanced effects as they rarely address the different functional roles of herbivores.

2.4.5 Carbon cycling: A case study

As noted for other functions, the effects of large herbivores on carbon cycling varies enormously across systems. This variation is hypothesized to be driven both by ecosystem properties like productivity (Piñeiro et al. 2010), grazing intensity (Olofsson et al. 2001), and spatial heterogeneity (Vowles et al. 2017), as well as experimental properties like plot size and duration (Marburg et al. 2013). Yet much of the observed variability is likely

also due to the challenges associated with measuring a function. What is often putatively considered a single function (e.g. carbon cycling) often truly consists of multiple, loosely related, sometimes even opposing components (e.g. measurements of carbon fluxes vs. pools). Variability in selection of components to measure a given function can result in an inability to generalize results across systems (Dale and Beyeler 2001). To better understand the sources of variability in responses, we conducted a quantitative analysis for carbon cycling, an important function for which management is of high interest due to climate change, and for which recent study has made apparent the consequential role of large herbivores (Schmitz et al. 2014).

The carbon cycle is an integrated system that refers to both pools (storage) and fluxes (cycling between pools) of carbon. Large herbivores directly impact carbon pools and fluxes through plant consumption, trampling plants and soil, removing woody vegetation like trees, and depositing waste products (Asner and Levick 2012, Tanentzap and Coomes 2012, Heggenes et al. 2017). Large herbivores also impact carbon storage and flux indirectly. For example, plants under moderate herbivory may reallocate carbon belowground to their roots, increasing belowground carbon storage despite aboveground biomass decreasing (Ritchie et al. 1998). Reindeer exclusion in the Arctic can decrease soil carbon dioxide flux (lessening emissions to the atmosphere) because of lower soil temperatures, while weakening soil's impacts as a methane sink (another, more potent carbon-based greenhouse gas) due to increased coverage of methane-producing lichens and bryophytes (Cahoon et al. 2012, Köster et al. 2017). While all effects of large herbivores should be considered effects on carbon cycling, individual components should neither be conflated nor considered representative of the cycle on their own.

To better understand the effects of herbivores on the carbon cycle, and the extent to which component selection risks conflating incomparable effects, we conducted a meta-analysis (detailed in Appendix 1.2). We began by considering the entire suite of com-

ponents that describe the carbon cycle, identifying 121 individual responses to large herbivore exclusion that represented an aspect of the carbon cycle. Overall, we revealed a slightly positive net effect of large herbivore exclusion on the 'carbon cycle' when all components were pooled (Fig. 2.7a). However, as discussed above, this result should be interpreted thoughtfully as it aggregates the multiple, inherently correlated components (both pools and fluxes) of the carbon cycle (Falkowski et al. 2000).

Therefore, stronger and more mechanistically meaningful responses would be expected for individual components. We identified eight components of carbon cycling reflected by the group of 121 responses and binned each response into one. Several of these components contained multiple, correlated metrics with which they were measured in the field (e.g. methane and carbon dioxide flux binned within carbon flux). Analyzing these distinct components revealed that some showed clear directionality while others remained highly variable. This is important, as the differences in response between components underscores how dissimilarities in study focus (e.g. which component is being measured) within a single function can precipitate different interpretations of the effects of large herbivore exclusion. For example, while carbon stored in soil increases in response to large herbivore exclusion (Fig. 2.7c), the response of soil carbon flux is highly variable and trends negative (Fig. 2.7b). Interpreting these contrasting results requires attention to what effect direction means for each component: herbivore exclusion seems to increase carbon storage significantly but can decrease or increase carbon emissions. Assessing the impacts of large herbivore exclusion on one component to represent the carbon cycle writ large may therefore result in management recommendations of limited value. For example, large herbivore exclusion results in higher aboveground biomass globally, a critically important pool of carbon. However, suggesting large herbivore removal to increase carbon sequestration (Tanentzap and Coomes 2012) overlooks potentially important and conflicting responses from other components of the carbon cycle.

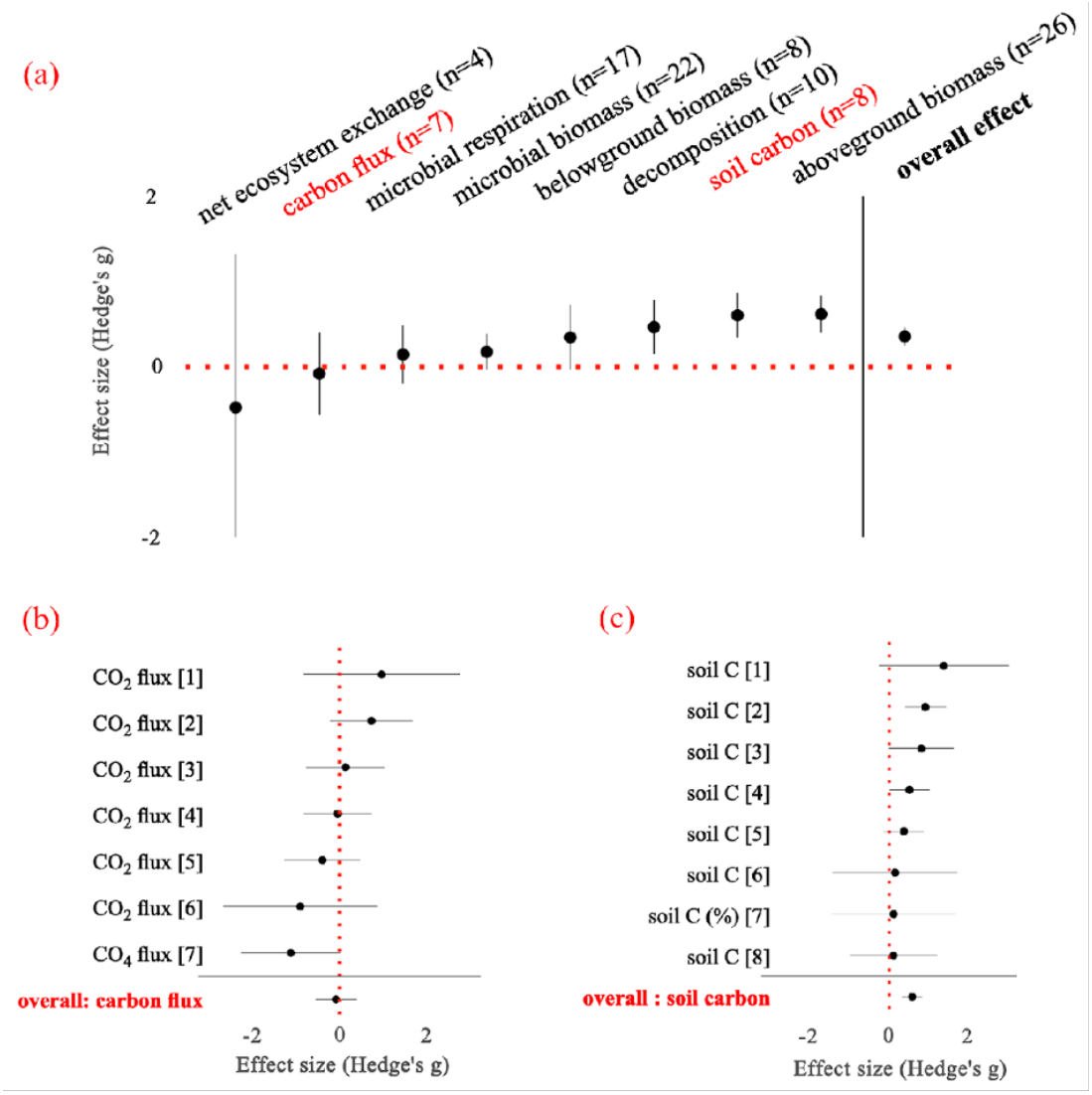

Figure 2.7: a) Average effect size (Hedge's G) and 95% confidence intervals of large herbivore exclusion on eight metrics of ecosystem carbon cycling; effects of large herbivore exclusion are not consistent across metrics. b) and c) illustrate further variability within-metric, with forest plots of collected published data on responses of carbon flux and soil carbon (respectively) demonstrating variation in magnitude and direction of effect. Numbers next to labels in b) and c) reference experimentally unique responses to large herbivore exclusion.

Within each component's analysis, we explored both experimental and biotic explanations for observed variance. First, considering that some effects of large herbivore exclusion on carbon cycling would saturate only over long time periods (e.g. increase

in carbon stored as woody vegetation) or large spatial scales (e.g. interaction of above-ground biomass and fire regime change on carbon storage and flux) (Holdo et al. 2009), we anticipated that experimental plot size and duration would be important moderators of large herbivore exclusion's effects on components of carbon cycling. However, analyses of soil carbon and carbon flux did not provide support for these moderators: neither component's effect size was significantly impacted by plot size or duration (full models: soil carbon (n = 8), P = 0.14, P = 0.28 for duration and size, respectively; carbon flux (n = 7), P = 0.31, P = 0.25).

Given the robust literature on the influence of ecosystem productivity on all functions reviewed here (including carbon cycling) we also expected productivity to be an important moderator. However, individual analyses of soil carbon and carbon flux demonstrated limited and mixed support for ecosystem productivity (here, mean NDVI at each experimental location) as a significant moderator. The effect of large herbivore exclusion on soil carbon was not significantly impacted by productivity (P = 0.13 in full model), though that of carbon flux was (P = 0.02 in full model, P = 0.03 in reduced model) (Fig. 2.8).

The lack of explanatory power of these experimental and biological moderators may be due to lack of true effect; recent meta-analyses also found limited support for productivity in moderating effects of herbivores on plants (Jia et al 2018, Koerner et al. 2018). However, we suspect that small sample size within components (n=8 for soil carbon, n=7 for carbon flux) and unreported variation in other biotic conditions (e.g. herbivore density) limits our abilities to detect their effects on individual components of carbon cycling. Our analysis highlights a need for standardized, comprehensive data collection on all components of an ecosystem function, and detailed reporting of meta-data on exclosure systems, to understand the sources of true effect as well as variation in response to large herbivore exclusion.

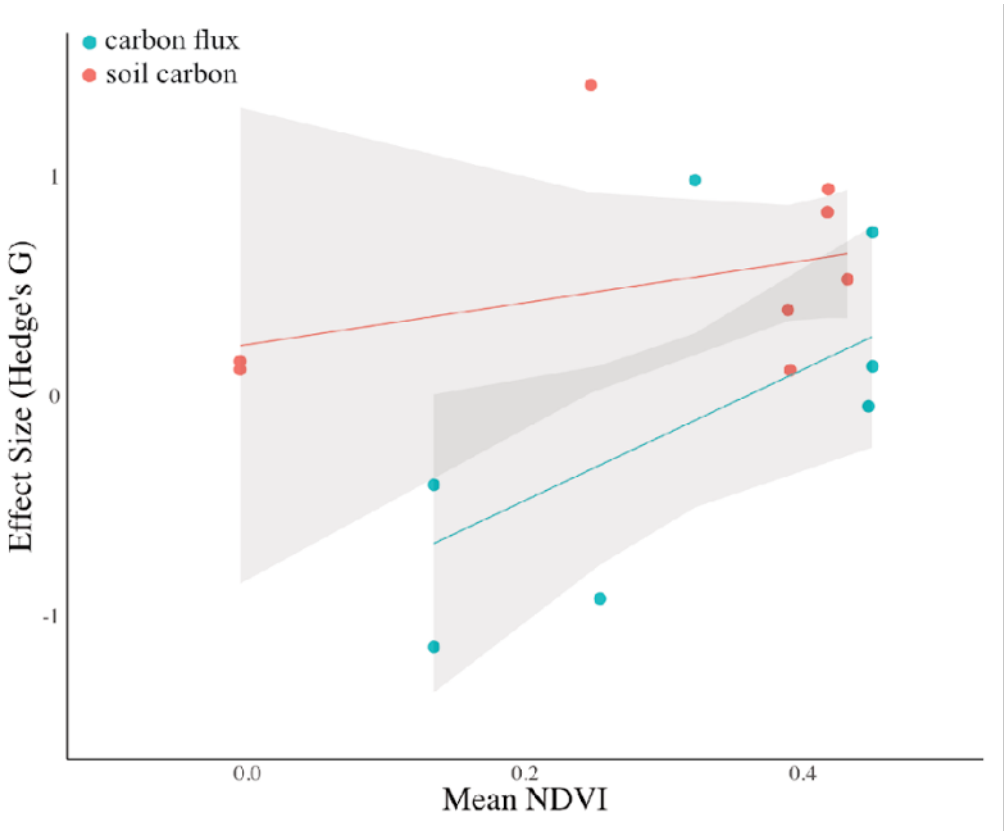

Figure 2.8: Influence of mean NDVI on effect size of large herbivore exclusion on carbon flux ($p = 0.03$, reduced model), and soil carbon ($p = 0.13$, full model). Productivity moderated the effects of large herbivore exclusion on carbon flux (in blue) but not soil carbon (in red).

2.5 Context matters: Possible biological sources of variation

It is clear from the above reviews that there is significant variability in the responses of ecosystem functions to large herbivore exclusion, likely due in part to inconsistency in large herbivores' impacts on ecosystems in general. A recent global meta-analysis of large herbivore exclosure experiments found that effects on plant performance, community composition, and community structure were variable when examined through site characteristics like productivity or climate (Jia et al 2018). The direct effects of

large herbivores on plants (via consumption) result in cascading effects on functions like carbon storage, ecosystem resilience/resistance, and plant regeneration: as the effects of large herbivores on plants are conditional on site-specific characteristics, it is thus reasonable to expect similarly variable effects on functions. Indeed, when analyzed separately, the impacts of large herbivore exclusion on aboveground biomass alone as a proxy for consumption is highly inconsistent (Appendix 1.3, Fig. A.2).

It is therefore important to consider the ecological contexts of an experimental site that likely play influential roles. The presence of predators in an ecosystem may influence the effects of large herbivores and thus of their experimental removal. Large predators in east Africa mediate most large herbivores' habitat selection, likely shifting their cascading effects on functions like plant regeneration (Riginos and Grace 2008). Predation risk alone can prompt stress-induced changes in the body compositions of herbivores, changes which can cascade to impact the composition and quality of their nutrient deposits and significantly impact nutrient cycling (Leroux et al. 2012).

Herbivore density must also be considered when interpreting variability in effects reviewed here. While large wild herbivores are being impacted by global change worldwide, not all populations are declining: modern declines in hunting and predator populations, and shifts in climate and forage availability, have resulted in dramatic deer (and other game species) overabundance (Ripple et al. 2015). This can result in similarly dramatic impacts on ecosystem functions like disease transmission, ecosystem resilience/resistance, plant regeneration, carbon cycling, and nutrient cycling, among others (Côté et al. 2004, Ripple et al. 2015). Herbivore identity also significantly moderates their effects on functions. By dint of their size, megaherbivores like elephants play unique roles in ecosystems and their functioning as compared to other large herbivores, while browsers and grazers also uniquely modify plant communities and the functions that precipitate from them (Fritz et al. 2002). Indeed, as seen in our review of plant regeneration, the presence of

both browsers and grazers in an ecosystem can result in opposite effects, dampening net effects on plant regeneration (Pringle et al. 2014).

Herbivore identity is likely to be particularly influential when considering the effects of domestic herbivores, as wild large herbivore loss is seldom isolated. In nature, it is often driven or rapidly followed by replacement with domestic livestock. As a result, and despite dramatic declines in wild ungulates, total large herbivore biomass on the planet today greatly exceeds historical baselines (Bar-on et al. 2018). In this review, we focused on experiments in which experimental exclosure of wild, native large herbivores occurred; however, approximately 35% of these unique experiments took place where large domestic herbivores exist and were therefore also excluded. Few formal experiments (most notably the Kenya Long-term Exclosure Experiment, KLEE) explicitly explore whether domestic herbivores fill the functional roles of large wild herbivores, by manipulating the presence/absence of both. Though domestic herbivores likely play a functionally different role than wild ones (Charles et al. 2017), major differences in effect appear to be driven more by total herbivore density than by identity (Veblen et al. 2016, Young et al. 2018).

While not included in this review, large herbivores in aquatic ecosystems also significantly impact ecosystem functions (Bakker et al. 2016). In seagrass beds, dugongs, turtles, fishes, and urchins can remove up to 90% of producer biomass (Heck and Valentine 2006) and facilitate productivity by over 50%. Herbivorous fishes and urchins can likewise increase productivity by over 300% on coral reefs, increasing resistance to disturbances like bleaching and resilience to transition to algae-dominated reefs by maintaining lawns of small, productive algae species over macroalgae (Carpenter 1986, Adam et al. 2011). Context like site characteristics, herbivore density, and herbivore identity also mediate the responses of functions in aquatic systems. For example, high herbivore density, like increasing populations of sea turtles in protected areas, can lead to ecosystem collapse (Christianen et al. 2014). However, despite these similarities, the effects of

large aquatic herbivores on ecosystem functions are comparatively unexplored, much less joined to the existing terrestrial literature.

2.6 Conclusions

The effects of large herbivores on vital ecosystem functions are increasingly used to motivate conservation of these taxa (Ripple et al. 2015). While we find strong evidence that large herbivores significantly impact many ecosystem functions, we find limited evidence for clear, predictable patterns of effect for any function (Appendix 1.2), even with a geographically-limited dataset (76% from temperate systems, 50% from grasslands). While this lack of predictability could be driven by inconsistent definitions for ecosystem functions in the literature, in our analysis of the carbon cycle we find similarly little predictability when a function is analyzed by its individual components, and when basic experimental and ecological properties are controlled.

In many ways, this is a surprising finding. Meta-analyses on sessile or smaller herbivore biodiversity, which are generally thought to have lower average effect on ecosystem functioning than large mobile species (Séguin et al. 2014), have shown consistent negative effects of diversity loss on function. What is more, productivity covariates like climate, land-use, and nutrient availability often significantly moderate these effects (Lefcheck et al. 2015, Soliveres et al. 2016, Duffy, Godwin, and Cardinale 2017). However, our results indicate that the functional effects of large wild herbivore removal may be less systematic than those of these smaller taxa, and indeed less predictably moderated by factors like productivity.

One likely cause of the strong variation in functional responses reported is the methodological limitation of exclosure experiments. Experimental exclosures for large taxa typically have lower control on the number and types of large taxa removed and lower

replication than do similar manipulations of smaller taxa. Furthermore, as documented here, existing experiments are insufficient in size (median 400m^2) to capture landscape-level effects like nutrient translocation, which will be better studied in large-scale natural experiments. Also problematically, the average duration of these experiments (median six years) means they frequently assume short-term or linear effects over time, although slow-acting responses (e.g. tree recruitment) and long-term temporal variability is known to substantially influence function (Goheen et al. 2018).

Finally, and crucially, lack of consensus on how to practically define individual functions may amplify in larger field-based experimental systems, where there are multiple metrics with which to measure the different components of a function. A function's components are all meaningful, yet are also potentially confounding when combined or misleading when considered alone. Thus, clear definitions of individual functions and the components they are comprised of is likely an essential next step. Indeed, lack of standardized terminology can be source of complexity in ecology (Fauth et al. 1996), and ecosystem function itself is interchangeably defined as service, process, and function (e.g. Franklin et al. 1981, Lamont 1995, Srivastava and Vellend 2005).

In addition to methodological drivers, it is likely that much of the variation observed here reflects real differences in the effects of large herbivores on ecosystem functions across ecological contexts, and which may not be captured by single covariates like ecosystem productivity. Theory suggests that effects of large herbivores on plants should vary based on a wide range of ecological contexts (e.g. productivity, climate, predator density, food chain length, presence and diversity of smaller consumers). However, these data are often difficult to collect in complex systems or considered unnecessary to a study's aims and are therefore inconsistently reported (Gerstner et al. 2017). It is consequently infeasible to interrogate all these covariates by synthesizing existing data. Relatedly, covariates on large herbivores themselves (identities and densities at a site, diet type, body size, etc.)

are likely also necessary, as it is possible that the common definition of large herbivores (>5kg) is not an ecologically meaningful grouping.

If we seek a more general understanding of the effects of large herbivores on ecosystem functions, globally or across biogeographic zones, two clear needs emerge from these reviews. First, we need more quantitative syntheses on the effects of large herbivore exclusion on individual functions. We anticipate little consensus, considering that functions contain multiple meaningful components (Schmitz et al. 2014) and that data on covariates are often unavailable. However, such syntheses will at minimum explore the extent of variability by function, identify potential drivers of variation, and highlight the suite of components most useful for empirical study of each function.

A second critical need is for increased, systematic empirical exclosure research focusing explicitly on the effects of herbivore exclusion on functions. We recommend the development of a global network of exclosures across ecosystems, for which experimental plots should be at least 100x100m, be replicated at least 3 times per system, and effectively exclude all herbivores >5kg. In addition to collecting functional response data with standardized, synthesis-informed protocols, researchers would collect standardized metadata: herbivore identity and density; site productivity; presence, identity, and density of predators, small consumers, and domestic herbivores; etc. This proposed large-scale network is inspired largely by the Nutrient Network (NutNet), a collaborative experiment run by many investigators, which has leveraged standardized data collected from 5x5m exclosures across a range of environmental conditions (65 grassland sites across six continents) to detect general impacts and context-dependencies of herbivory and nutrient availability (https://nutnet.org/field_sites) (Borer et al. 2014, Borer et al. 2017).

While this effort would be challenging at the plot size and spatial scale we suggest, many suitable experiments exist already. These experiments, like the KLEE, could be incorporated into the network by adopting standardized data collection protocols, in-

formed by synthesis, for each function and relevant metadata. Once established, such a global network could detect general responses of functions to large herbivore exclusion over space and time, including large-scale and non-linear changes, and illuminate the biotic and abiotic covariates that moderate the effects of large herbivore exclusion on individual functions. Coordinated research such as this could provide experimental support for predictions of future ecosystem functioning, and support work in natural systems demonstrating the functional consequences of continued defaunation.

2.7 Acknowledgements and Author Contributions

Thanks to the Ecological Society of America for hosting our session on the cascading effects of large herbivores on ecosystems. Thank you to that session's other participants: David J. Augustine, Tyler R. Kartzinel, Douglas A. Frank, Kari E. Veblen, Robert M. Pringle, and especially Elizabeth T. Borer, who contributed valuable insight into the Nutrient Network. This work was supported in part by National Science Foundation Division of Environmental Biology Long Term Research in Environmental Biology 12-56034, and by the NSF Graduate Research Fellowship Grant No. 1650114. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the NSF. Thank you to Functional Ecology, for inviting us to prepare this review.

All data used in this review are archived on Dryad. This includes data used to generate descriptive statistics of all functional responses recorded within large herbivore experimental exclosures, and data used to conduct a case study analysis on the impacts of large herbivore exclosure on ecosystem carbon cycling. Both datasets can be found at http://doi.org/10.5061/dryad.3tf4mt4

Elizabeth Forbes[1] designed methods for and conducted the quantitative literature

review, case study literature review and data collection, and case study analysis; she led writing of and figure generation for the manuscript. Hillary Young[1] contributed to literature review methods and figure generation. Maggie Klope[1] assisted in quantitative literature review. EF, J. Hall Cushman[2], Deron E. Burkepile[1], Truman P. Young[3], Maggie Klope, and HY each contributed one or more written sections on an ecosystem function or outstanding question. All authors provided substantive input and final approval to manuscript.

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Chapter 3

Fluxbots: A method for building, deploying, collecting and analyzing data from a network of inexpensive, autonomous soil carbon flux chambers

3.1 Abstract

Modeling climate change accurately at the global level requires fine-resolution data on carbon dynamics at local levels. This includes soil carbon flux, the rate at which carbon dioxide cycles between a system's soil and the atmosphere driven by soil morphology, soil microbial respiration, and root respiration. Soil carbon flux rates are essential components of an ecosystem's carbon budget, but vary widely through space and time. Existing modes of collecting high-resolution data are often prohibitively expensive, such that re-

searchers must choose between spatial and temporal resolution. What is more, many ecosystems are underrepresented in global soil carbon flux datasets for logistical reasons, cost, or reflect existing biases in long-term data collection. As such it is essential to develop inexpensive methods to collect high-resolution soil carbon flux rates that are accessible.

We designed, deployed, and collected hourly soil carbon flux data from a network of twelve inexpensive, autonomous soil carbon flux chambers in a savanna ecosystem in Laikipia, Kenya. After processing we had a dataset reflective of 2 months of hourly soil carbon flux rates, across multiple experimental large herbivore community contexts and individual landscape features, and spanning the end of a wet season and the start of a dry season. Our network of 'fluxbots' demonstrated the promise of autonomous, do-it-yourself robotics for large-scale, long-term, high resolution environmental data collection even on relatively limited research budgets. Widescale adoption of these devices would create comparable datasets across ecological contexts, improvements in our understanding of the drivers of local carbon dynamics, and thus improved ability to incorporate local variability into larger-scale characterizations of Earth's carbon cycling.

3.2 Introduction

As the Anthropocene wears on, predicting the impacts of climate change becomes increasingly crucial. In terrestrial systems, carbon dioxide (CO_2) cycles between the soil and the atmosphere, with this flux comprised of biotic soil respiration (hereafter SR, the combined respiration of microfauna, macrofauna, and roots within the soil) and moderated by abiotic factors like air and soil temperature, atmospheric pressure, and soil moisture, type, and morphology (DeCarlo and Caylor, 2020). Soil carbon flux can be used to calculate an ecosystem's carbon budget (Smith et al., 2010), and to validate

or parameterize Earth systems models (Phillips et al. 2017). As soils contain a huge proportion of the world's terrestrial carbon, three times as much as either the atmosphere or vegetation (Schmidt et al., 2011), a high-resolution understanding of patterns in soil carbon flux is essential to understanding drivers of terrestrial emissions. What is more, this resolution must be comprehensive in spatial and temporal scale, and spatial and temporal granularity: soil carbon flux is influenced through time by short- and long-term climatic processes, like individual weather events and seasonality (Munson et al. 2010; Delgado-Baquerizo et al. 2017); by landscape-scale changes to ecosystem structure driven by fauna (DeCarlo and Caylor 2020), fire (Pellegrini et al. 2020), land use (Wachiye et al. 2020), and agriculture (Lohila et al. 2003; Rochette et al. 1991); and by small-scale spatial variability (Holden 2005; Rodeghiero and Cescatti 2008).

Such multi-scale resolution in soil carbon flux data is difficult to achieve, and indeed existing datasets are not distributed evenly across the globe nor always collected with comparable methods (see (Bond-Lamberty et al., 2020)). On a large (landscape or regional) scale, satellite imagery drives modeled estimates of CO_2 exchange based on surface-level parameters (e.g. (Sasai et al., 2011). Eddy covariance infrared flux instruments assess net ecosystem CO_2 exchange from field-based towers (Baldocchi, 2003). Such methods are capable of monitoring flux over large areas; their autonomy ensures continuous measurements and fine temporal resolution. However, these methods measure total ecosystem carbon exchange, and in doing so combine SR and above-ground vegetation respiration and photosynthesis. It is crucial to measure soil carbon flux explicitly to parse how its heterogeneity influences to ecosystem carbon emissions, considering that soil carbon flux can make up between 30 and 80 percent of net ecosystem exchange as measured by devices like those described above (e.g. (Davidson et al., 2006)).

This kind of spatially fine-scale soil carbon flux data (e.g. centimeters to meters) can be taken manually, using small *in-situ* non-steady-state (e.g. monitoring the buildup

of CO_2 inside a sealed volume over time) chambers (Davidson et al., 2002). However, without multiple individual instruments and operators, researchers must prioritize either measuring soil carbon flux frequently at a few sites, or at many sites but less frequently. In addition, high-resolution manual data collection across space or time can be risky or even impossible at times (e.g. in landscapes with potentially dangerous large wildlife).

A final significant hurdle to collecting large-scale, fine-grained soil carbon flux data is data management and analysis. Autonomous chambers, particularly if distributed in a simultaneously-operating network across a large area for an extended period of time, collect huge quantities of raw data. Analyzing these data, while automatic in commercially-available systems, is non-trivial. Such "big data" already require careful parsing to accurately calculate flux from raw CO_2 concentrations; with autonomous chambers it is also necessary to conduct stringent quality control (K. Savage et al., 2008; Rundel et al., 2009). For example, interference by plants growing into or inside the chamber, or wildlife breathing near it prior to closure for measurement, could cause artificially depressed or inflated flux estimates.

Autonomous soil carbon flux chambers can capture all resolutions of interest—they can be installed across a large spatial extent, at fine granularity to capture soil heterogeneity, and programmed to measure at high frequency and continuously through time. Some commercially-available autonomous chamber systems exist. The cost of commercial options, however, is (similar to manually-operated versions) a barrier to their installation across landscape-level extents or in high-risk ecosystems where they may be damaged (e.g. those with large wildlife). For any commercially-available system, autonomous or manual, it is not currently possible to monitor soil carbon flux over landscapes at fine spatial and temporal granularity *without* extensive project funding devoted exclusively to instrument purchase and operation.

Clearly, to capture soil carbon flux heterogeneity across landscapes, an inexpensive

and open-access soil carbon flux chamber design and data protocol is needed. With a cheap autonomous system, many chambers can be made and installed across a landscape and loss (data or financial) from possible chamber damage in the field is negligible. This do-it-yourself or "DIY" design provides capacity for large-scale, fine-grained data collection to research projects with smaller budgets, or that take place in environments that are high-risk for instrumentation. In addition, it makes detailed characterization of soil carbon flux heterogeneity across a landscape more feasible. Here we present a design and analysis plan for an autonomous, non-steady-state (Davidson et al., 2002) carbon flux sensing robot (a "fluxbot"). We demonstrate their use with data collected hourly over 2.5 months with a network of twelve fluxbots in a central Kenya savanna system, a system in which the ecosystem structure is predictably homogeneous on a large scale but the soil characteristics heterogeneous on centimeter to meter scales (DeCarlo and Caylor, 2020).

3.3 Methods

3.3.1 Chamber construction

We constructed each fluxbot with easy-to-find materials, purchased from online retailers, or hardware and electronics stores (Appendix 2, Table A.2). We fabricated the chamber of each from two lengths of 5x5" square PVC pipe, cut to 7" (chamber "body") and 2.5" (chamber "lid"), connected at one side (the chamber's back) with hinges. To ensure an air-tight seal when the chamber was in its closed position (such that CO_2 buildup inside represented SR within the chamber, not wind or leakage), we lined the top edge of the body with a thin strip of neoprene, and glued a 'lip' of rubber weatherstripping material along the outside bottom edge of the front and sides of the lid. We placed a

rectangle of recycled neoprene (salvaged from wetsuits) under the hinges before screwing them in place to connect the body and lid, like a washer, to prevent the screw holes from introducing leakage into the chamber (Appendix 2, Fig. A.3). For more details on this construction, see the extended description in the supplementary information. We tested these three gasket features extensively in the chamber's closed position to ensure a fully sealed closure (Appendix 2, Fig. A.4).

We topped the lid with a custom-cut acrylic plate, using weather- and air-tight glue. We mounted the linear actuator (which has a mounting hole, or clevis rod end, on each opposite end) to the body and lid via long horizontal screws, screwed from the outside to the inside on the left side of each. This mounting allows for free rotational movement on each end of the actuator as it extends (opening the lid to 90 degrees) and contracts (closing the lid and sealing the chamber shut).

3.3.2 Hardware

Once the chambers were built, we installed a custom hardware system in each lid, constructed from a microcontroller (Pycom LoPy4, with Pycom Expansion Board), a calibrated infrared (IR) CO_2 analyzer, a real-time clock (RTC) (set to local time at our field site in Laikipia, Kenya) to ensure synchronized measurement activity across all fluxbots, and a combined air pressure, temperature, and humidity sensor (see supplementary information for more details). We connected these components using a combination of soldering and lever-nut snap-style wire connectors (Fig. 3.1). Data (timestamp, raw and filtered CO_2 in parts per million, temperature in degrees Celcius, humidity in percent, pressure in hPa) was written to a microSD card stored on the microcontroller.

We fixed all electronics in place to a custom acrylic mounting board, cut with screw holes and slots for wiring. We fixed one of these 'electronics systems' to the inside of

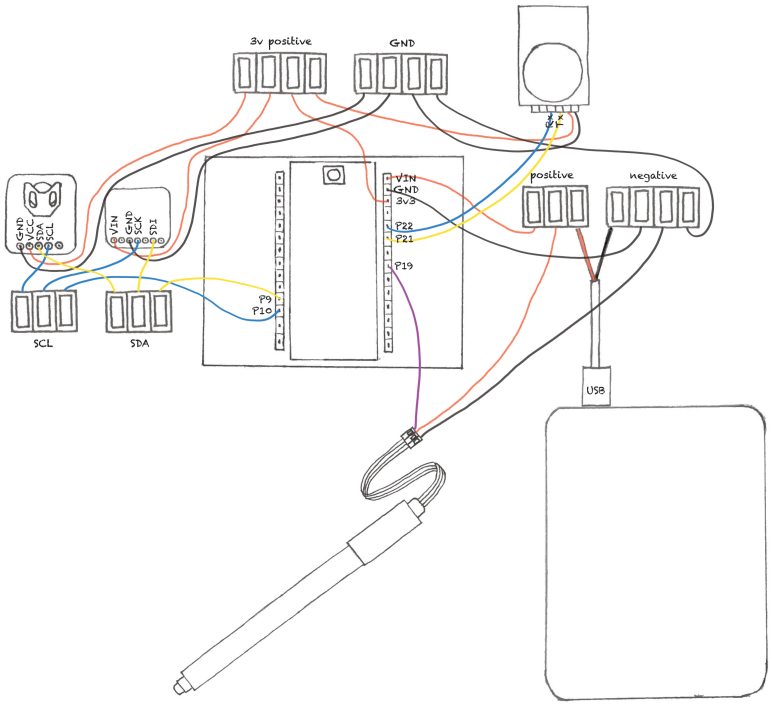

Figure 3.1: Exploded view of the wiring schematic for the fluxbot electronics design, all components displayed on a plane and connections indicated with colored lines.

each fluxbot lid from the top plate, with the CO_2 sensor facing the inside of the chamber volume, using long screws and spacers (Fig. 3.2), with rubber washers to prevent air leakage through the lid. The linear actuator, in place and in its extended position with the lid open at approximately ninety degrees, was then wired into the electronics system via ground, power, and signal cables.

To power the electronics system from an external battery, we threaded a USB cable (with exposed ground and power wires) through a hole in the backside of the chamber body, fitted with a weather-proof air-tight cable gland (Appendix 2 Fig. A.6; Appendix 2, Table A.2). We snapped the exposed ground and power wires into the electronics system's negative and positive lever-nut connections, respectively. We plugged the USB, extending from the back of the chamber body, into a V44 battery to power the system on (fig. 1). We placed the battery into a custom-sewn waterproof 'raincoat', and affixed

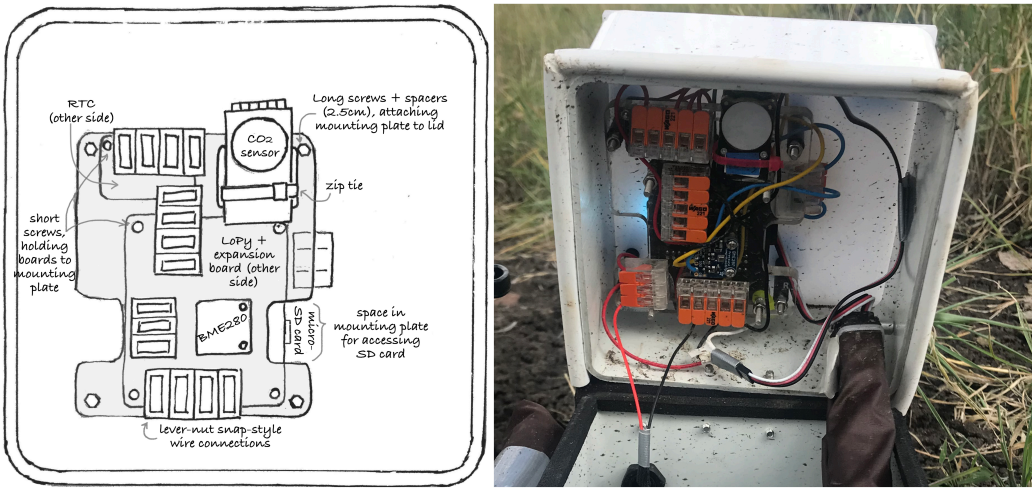

Figure 3.2: Electronics, installed, viewed inside the lid from the underside both in labeled schematic form (left) and photographed in the field (right).

it to the outside of the chamber with heavy-duty Velcro (Appendix 2 Fig. A.6); the linear actuator, which when extended in the fluxbot's open position was also potentially exposed to the elements, was also encased in a sleeve of waterproof fabric that extended and contracted with the actuator's movement (visible in Fig. 3.3, Appendix 2 Fig. A.6). All in all, the cost of materials for one fluxbot as described above is $361.71 (US dollars).

3.3.3 Software

The fluxbots run on a series of MicroPython scripts designed to support synchronous measurements across a network of fluxbots for the duration of their field installation. The fluxbot's electronics system "boots up" upon access to power via the battery, and immediately runs through a series of self-diagnostics to determine if any individual piece of its electronics system are faulty, with an LED light (visible through the top of the fluxbot's acrylic lid) blinking coded colors to indicate potential problems (e.g. a missing microSD card, an unplugged actuator, etc.) (Appendix 2, Table A.1). If an issue is encountered, the LED light will continue blinking the color coded to that error until

the error is resolved, and self-diagnostics can continue. Upon a successful diagnostics procedure, the fluxbot launches its measurement schedule which repeats every hour for as long as power is available. If power is lost temporarily (e.g. extended periods of rainfall or clouds, preventing the solar panels from charging the battery), the fluxbot's measurement schedule picks back up whenever the fluxbot powers back on, thanks to its separately battery-powered RTC which keeps track of time even if the fluxbot is powered off.

During minutes 0-55 of each hour, the fluxbots stand 'open' with the linear actuator fully extended, venting to the atmosphere (Fig. 3.3). At minutes 18, 36, and 54 (while the chamber is open) three rapid (one per second, for three seconds) measurements are taken of ambient CO_2 concentration, temperature, humidity, and pressure. At the start of minute 55 each hour, the actuator contracts, closing the lid of the chamber and sealing the volume inside. From minute 55 to the start of the next hour (five minutes total of closure), the fluxbot takes measurements of CO_2, temperature, humidity, and pressure once per second. All these data (ambient and during the closed period) are written to the microSD card in real time. In addition to this raw data file, a secondary file that documents the fluxbot's history of boot-ups (e.g. after replacement of the microSD card, after power restoration if the battery ran low, after field-based repairs, etc.) is also recorded and stored on the microSD card (for more details, see supplementary information section 3 "software").

3.3.4 Field installation, data collection

We installed 12 fluxbots in a large-scale, long-term large herbivore exclusion experiment (the Kenya Long-term Exclosure Experiment, KLEE), located at Mpala Research Centre and Conservancy, in Laikipia, Kenya. We installed each fluxbot at least 10m apart

to ensure spatial independence, and located them evenly on three *a priori*-identified distinct landscape features (beneath the canopies of the whistling thorn Acacia, *Acacia drepanolobium*; on the surface of active termite mounds; and on open soil patches not influenced by either of the two preceding features) (see Appendix 2, Fig. A.7 for a detailed map of fluxbot locations). The V44 batteries were charged continuously with the 6W solar panel, which we installed adjacent to each fluxbot, horizontally fixed to an additional length of PVC that served as a platform to keep the solar panel exposed to maximum hours of sunlight in this equatorial ecosystem.

Figure 3.3: A fluxbot installed in the field, in its open position, with actuator fully extended. Collar for manual data collection at bottom right.

As described above, upon powering on in the field each fluxbot ran through its series of self-diagnostic tests before initiating its scheduled hourly measurements. The fluxbots remained in the field for approximately 2.5 months, over a period of time encompassing

the end of a dry season and the start of a wet season (August 2019-mid October 2019). We manually collected data from each fluxbot's microSD card every three-four days, and immediately transferred the data to a Panasonic Toughbook laptop and card reader in the field; we immediate copied these data from the Toughbook to a hard drive as well as a cloud storage service upon our return to the lab each data collection day. The microSD cards are accessible when the fluxbot is in its open position; removing the microSD card pauses fluxbot activity for as long as it is removed from the electronics system for, but the data download process takes several minutes at most. Upon replacement of the microSD card each fluxbot resumes its scheduled activity.

In addition to fluxbot data, in August 2019 we opportunistically collected soil carbon flux data manually, from 12 round PVC collars, one 'planted' (3cm deep) adjacent to each fluxbot (visible in fig. 3). We collected these data using a CIRAS gas exchange system with SRC-2 flux chamber attachment, between 9am and 4pm, totalling just over 200 independent manually-collected flux measurements (see supplementary information).

3.3.5 CO_2 Observations

The sensor contained in our fluxbots observes the quantity of CO_2 as a concentration in units of parts per million, $[CO_2]_{\text{Obs}}$ [ppm]. We convert $[CO_2]_{\text{Obs}}$ into a mass density of CO_2 in the chamber according to

$$\rho_{CO_2} = \dot{\rho_a} \frac{[CO_2]_{\text{Obs}}}{1\text{x}10^6} M_{CO_2}, \tag{3.1}$$

where ρ_{CO_2} is the mass density of CO_2 [kg-CO_2/m^3-air], $\dot{\rho_a}$ is the molar density of air [mol-air/m^3-air], and M_{CO_2} is the molar mass of CO_2 [kg/mol] (cf. Table 3.3.7).

The molar density of air, $\dot{\rho_a}$, is derived from air density, ρ_a [kg-air/m^3-air], and the

molar mass of dry air, M_a [kg-air/mol-air] (cf. Table 3.3.7), according to

$$\dot{\rho_a} = \frac{\rho_a}{M_a}. \tag{3.2}$$

Air density within the chamber is determined using observations of air pressure, P_a [Pa], and air temperature, T_a [K] according to

$$\rho_a = \frac{P_a}{R_a T_a}, \tag{3.3}$$

where R_a is the specific gas constant for dry air [J kg^{-1} K^{-1}] (cf. Table 3.3.7). Finally, the time varying mass of CO_2 in the chamber, $C(t)$ [kg] is found using the the chamber volume, V_c [m^3] (Table 3.3.7), as

$$C(t) = \rho_{CO_2}(t) \times V_c, \tag{3.4}$$

where individual values of $\rho_{\mathrm{CO_2}}(t)$ are derived from 20-second averages of $[\mathrm{CO_2}]_{\mathrm{Obs}}$, P_a, and T_a (supplementary information).

3.3.6 Flux Calculations

During an observation event, the system is sealed and the mass of CO_2 in chamber is monitored for five minutes. Throughout the 5-minute observation, we track $C'(t)$ [kg], which is the time-varying mass of CO_2 relative to the initial mass, C_0 [kg], found according to

$$C'(t) = C(t) - C_0, \tag{3.5}$$

where C_0 is the initial mass of CO_2 in the chamber, which is derived from the ambient CO_2 concentration, $[\mathrm{CO_2}]_A$ recorded just prior to the initiation of a measurement. We

estimate the flow rate (mass change per unit time) of CO_2 into the chamber by fitting a first-order regression between every time of observation, t_i, and each ith observation of relative CO_2 mass, C'_i.

$$C'_i = \beta_0 + \beta_1 t_i + \epsilon_i \tag{3.6}$$

Because $C'_{i=0} \equiv 0$, it is given that the expected value of β_0, $\mathrm{E}[\beta_0]$, is equal to 0 as long as we make the standard regression assumption that $\mathrm{E}[\epsilon_i | C'_i] = 0$.

We estimate the average flow of CO_2 mass during each measurement period by taking the derivative of Eq. 3.6 with respect to time, t, which yields

$$\frac{\mathrm{d}C'}{\mathrm{d}t} = \beta_1. \tag{3.7}$$

In many cases, CO_2 concentrations (and, therefore, values of C') in the chamber are changing in a non-linear manner over the course of each 5-minute measurement interval.

Because the CO_2 concentration in the chamber changes over the period of observation, we note that the observed flow rate of CO_2 into the chamber could also change as the gradient in $[CO_2]$ between the soil and the chamber shifts. This time-dependence in the evolution of $C'(t)$ is accounted for by using higher-order terms according our regression between C'_i and t_i.

$$C'_i = \beta_0 + \beta_1 t_i + \beta_2 t_i^2 + \epsilon_i \tag{3.8}$$

$$= \beta_0 + \beta_1 t_i + \beta_2 t_i^2 + \beta_3 t_i^3 + \epsilon_i \tag{3.9}$$

As in the linear case (Eq. 3.6), we can estimate the time-varying flow of CO_2 mass during each measurement period by taking the derivatives of Eqs. 3.8 and 3.9 with

respect to t, which yield

$$\frac{\mathrm{d}C'_i}{\mathrm{d}t} = \beta_1 + 2\beta_2 t_i \tag{3.10}$$

$$= \beta_1 + 2\beta_2 t_i + 3\beta_3 t_i^2 \tag{3.11}$$

Because we are seeking to obtain the best possible estimate of the initial rate of CO_2 accumulation, we focus on our estimators for β_1, which will always describe the initial rate of change in C' within the chamber at the start of a measurement interval (i.e. when $t = 0$). In the case where observed CO_2 accumulation rates are higher at the start of a measurement, the value of β_2 will be less than zero. In contrast, when β_2 is greater than zero, it indicates an observation interval in which for some reason the rate of accumulation of CO_2 in the chamber is increasing during the measurement interval. We therefore use $\beta_2 < 0$ as a quality assurance filter on our measurements. Overall, we find that of the 10085 measurement intervals taken across all 12 flux systems, only 11 percent of observation intervals had β_2 of less than 0.

Equations 3.6, 3.10, and 3.11 provide three different estimates of β_1, the value of $\mathrm{d}C'/\mathrm{d}t$ when $t = 0$. For each observation interval we calculate each of these

Finally, the flux of CO_2 into the chamber for each measurement interval, f_{CO_2} [kg m^{-2} sec^{-1}] is determined as the estimated initial rate of change of CO_2 mass, β_1 [kg/sec], divided by the surface area of the chamber, A_c [m^2]:

$$f_{CO_2} = \frac{1}{A_c}\frac{\mathrm{d}C'}{\mathrm{d}t} = \frac{1}{A_c}\beta_1, \tag{3.12}$$

3.3.7 Key parameters for the fluxbot system

The parameters for the above flux calculations include values like 'chamber volume' and 'chamber surface area' that are specific to the design of these fluxbots and essential to calculation of flux for any chamber-based system. These also include constants related to the properties of CO_2, air, etc. (Table 3.3.7).

Table 3.3.7: parameters needed for calculating flux

Parameter	Symbol	Units	Typical Value
Chamber Volume	V_c	m^3	0.002758
Chamber Surface Area	A_c	m^2	0.01455
Molar Mass CO_2	M_{CO_2}	kg/mol	0.044009
Molar Mass dry air	M_a	kg/mol	0.0289628
Specific Gas Constant, dry air	R_a	J kg^{-1} K^{-1}	287.058

3.3.8 Quality Assurance/Quality Control (QA/QC)

For the final dataset, we removed 250 flux observations that had incorrect timestamps. In addition, we treated the three weeks of data collection that occurred between August 2nd, 2019 (fluxbot installation) and August 23rd, 2019 (on which date the fluxbots were re-calibrated) as a test period, and removed these data from the final dataset. We did this because first, this period occurred immediately after the fluxbot network's installation in the field and therefore likely included time during which the soils were disturbed (e.g. severed belowground roots) and flux could be artificially high for days or weeks (Davidson et al., 2002); second, during this time we required post-installment troubleshooting (e.g. painting the non-sensor electronics components with clear nail polish to protect them from accumulating moisture during several unusually early rainfall events).

We also removed all the data collected in October for a single fluxbot (located in the

wildlife exclosure plot and on open soil), whose hardware shorted at the end of September 2019 likely due to water damage from one of several heavy rain storms that occurred around that time. We continued collecting data at that site by rotating lids from 'donor' fluxbots (e.g. a randomly-selected fluxbot's lid was removed and placed on the broken fluxbot's chamber) for two-three days each. These fluxes are relatively consistent despite being calculated from raw data collected by different lids. However, the associated ambient CO_2 values vary according to which donor lid collected them, indicating some between-fluxbot variation in absolute CO_2 detection (Fig. 3.4). While fluxes are calculated with net change in CO integrated over a set period of time, and not absolute CO_2, we removed these data (229 total observations) from the final dataset in case they occlude an overall characterization of the fluxbot network's performance. However, visualizing these 229 fluxes separately allows us to demonstrate that flux assessments across fluxbots is likely to be relatively consistent, despite the possibility of variation in absolute measurements of CO_2.

To identify and remove spurious flux calculations remaining in the dataset (e.g. that were calculated with raw data that was faulty or compromised in some way), we implemented a stringent QA/QC procedure. All flux measurement intervals were examined for a total of seven possible errors, listed in increasing order of severity:

- *net change in atmospheric pressure greater than 10hPa*;
- *net change in temperature greater than 2.5C*;
- *extraordinary maximum CO_2 values (e.g. that could be expected from errors ranging from electronic failure to heterotrophic respiration inside the chamber from a trapped invertebrate)*;
- *whether the measurement interval encompassed a minimum of 4.5 minutes*;

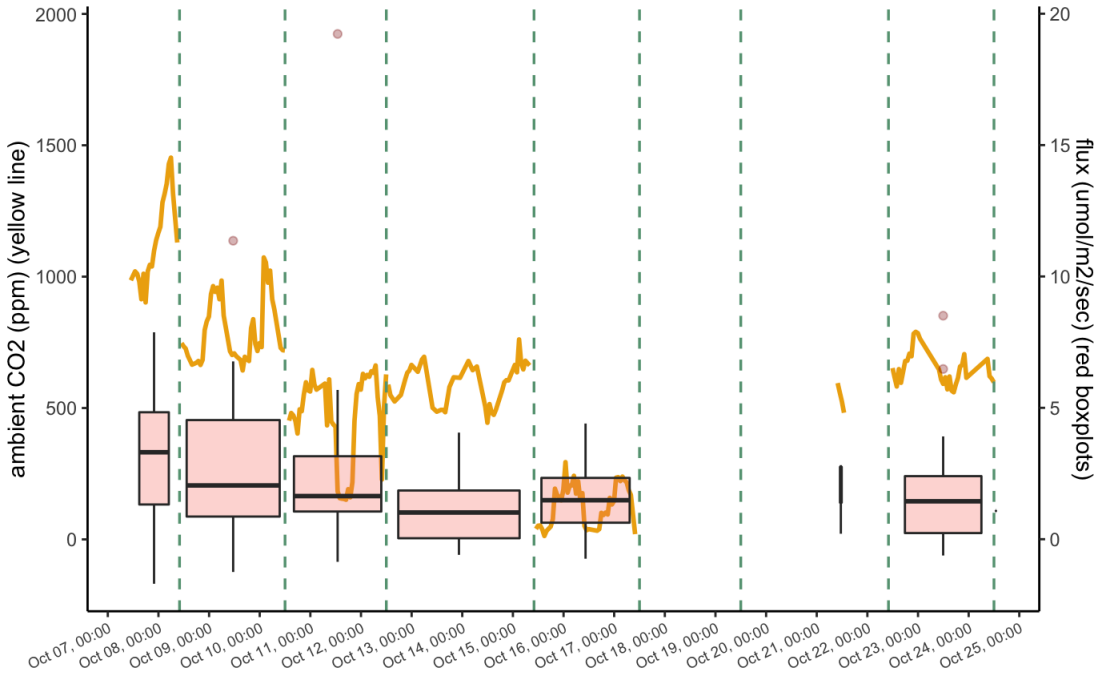

Figure 3.4: Fluxes (salmon boxplots, right axis)) and ambient CO_2 (yellow line, left axis) collected at the fluxbot location "no wildlife, open soil site 1" which had a new 'donor' lid (and therefore a different internal sensor) every 2-3 days after its own lid was irreparably damaged by heavy rainfall. Green dotted lines indicate approximate date/times for when a new donor lid was placed on the site's chamber body, from field notes.

- *if the net change in CO_2 over a measurement interval was less than 10ppm, indicating possible leakage or imperfect seal*;
- *if the net difference in CO_2 from start to finish of a measurement interval was negative, indicating a greater possible likelihood of leakage or CO_2 uptake from an errant photosynthesizing plant inside the chamber*;
- *if the last recorded value of CO_2 was less than the mean, and the first recorded value was greater than the mean.*

These seven errors were each assigned a value of [1 * an increasing order of magnitude]; a single flux calculation could therefore 'earn' up to seven figures of QA/QC 'flags' (e.g.

1, 10, 100, 1000, 10000, 100000, and 1000000 for a total possible maximum of 1111111). Any flux calculation that accrued a value of more than 11 was discarded (e.g. fluxes that had either high pressure *or* temperature buildup, as well as those that had *both* high pressure *and* temperature buildup, were kept in the final dataset). We removed a total of 719 total observations for failing this QA/QC check process (Fig. 3.5).

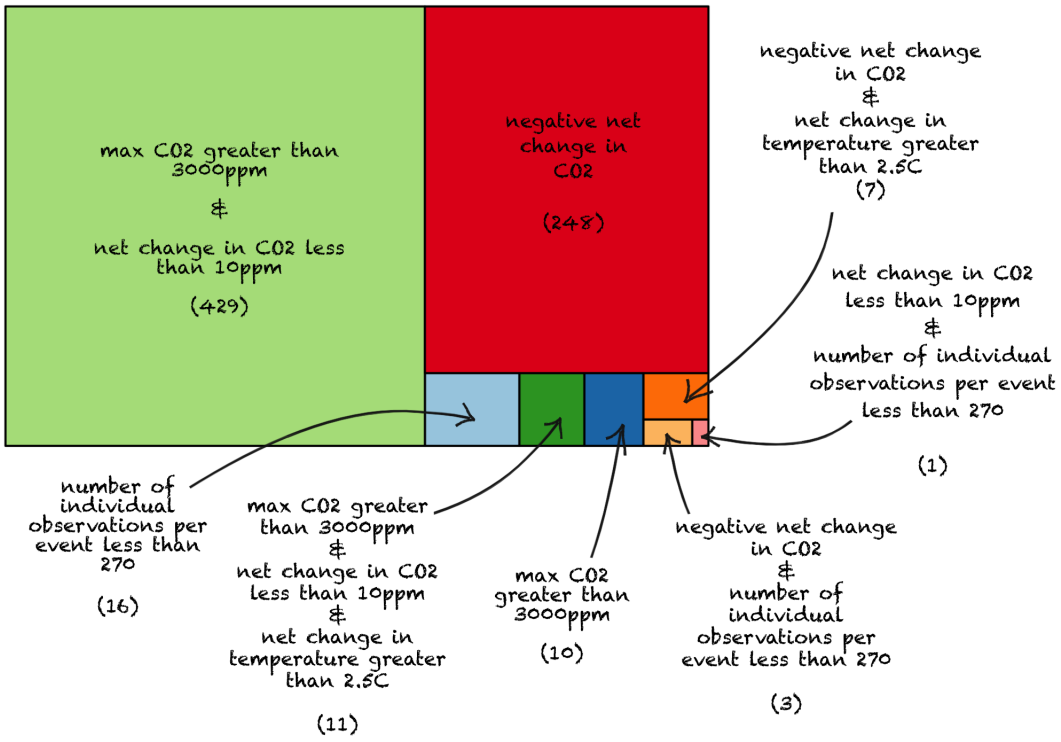

Figure 3.5: Breakdown of fluxes removed from the final dataset after QAQC procedure.

3.3.9 Flux calculation error

We found the projected line of minimum and maximum slope for each flux interval using the standard error of its fitting function in kg to find minimum and maximum $C_{intercept}$ and C_{final}, with 'C' as concentration of CO_2.

We can envision possible flux error as a parallelogram around the linear projection with slope β_1, $C_{intercept} = 0$, and C_{final} using the "box method": with $C_{intercept-max} = (0$

+ error_{kg}) and $\text{C}_{intercept-min}$ = (0 - error_{kg}), and $\text{C}_{final-max}$ = ($\text{C}_{final-max}$ + error_{kg}) and $\text{C}_{final-min}$ = ($\text{C}_{final-min}$ - error_{kg}). Therefore possible minimum and maximum slopes can be simplified to:

$$\beta_{min} = \frac{C_{final} - 2(error_{kg})}{t_d} \tag{3.13}$$

and

$$\beta_{max} = \frac{C_{final} + 2(error_{kg})}{t_d} \tag{3.14}$$

with 'C' defined as CO_2 concentration in kg and 't' as total duration of the flux observation interval. Because we chose the fitting function (between quadratic and linear) with the higher initial slope, β_1, to calculate flux as a linear function, we also project the error in flux as linear.

3.4 Results

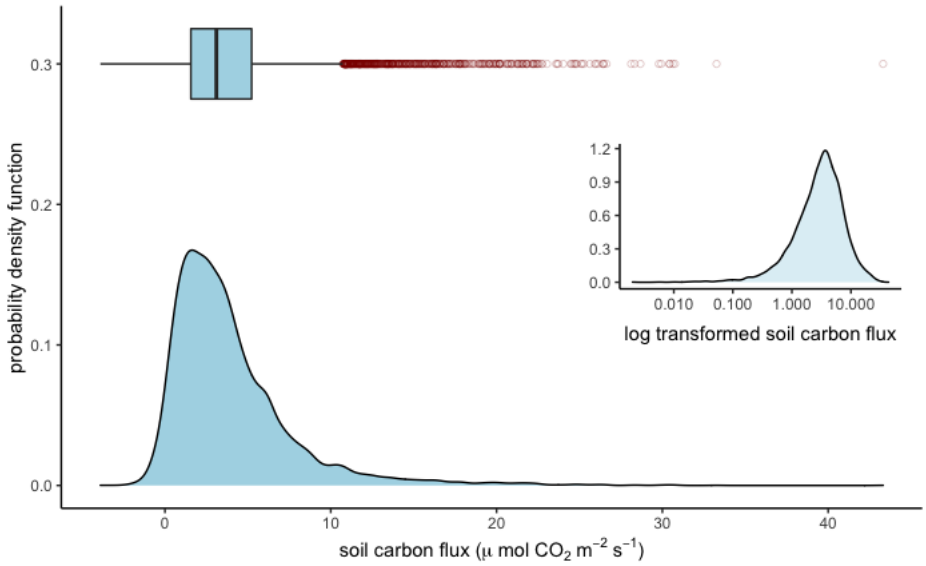

Figure 3.6: Density distribution of fluxes from entire deployment period.

Our network of 12 fluxbots in the KLEE collected a total of 10085 cleaned and quality-controlled individual observations of soil CO_2 flux. Mean flux across all 12 fluxbots and over time was 3.97 µmol/m^2/sec, and ranged from -3.893 to 43.313 µmol/m^2/sec (Fig. 3.6). However, only 330 of the 10085 observations were below zero and of those, the vast majority (291) occurred between 0 and -1, indicating a net efflux of carbon from our 12 sites on average.

3.4.1 Linear vs. Quadratic fit

As described in the methods, for each flux observation interval we calculated both a linear and quadratic regression. For closed-chamber systems, not all accumulation curves are best described by a linear regression (e.g. Fig. 3.7); in cases where linear regression is inappropriately applied to non-linear accumulation curves, fluxes can subsequently be underestimated (Kutzbach et al., 2007). Therefore, between the initial slopes ($\beta 1$) from each regression for a given measurement period, we chose the larger of the two to calculate flux for that period.

Because the fluxbots are closed-chamber systems, it is unlikely that our method of choosing regressions (either linear or quadratic) overestimate the rate of flux, due to the diffusion gradient of CO_2 inside the chamber compared to outside. Indeed, abiotic interference in the rate of CO_2 accumulation inside the chamber would likely only result in a slower rate of increase (e.g. a bad seal resulting in CO_2 leakage out of the chamber or CO_2 accumulation plateauing due to increased pressure.) Biotic interference would also likely facilitate a slower rate of increase (e.g. plant growth inside the chamber reducing chamber CO_2 through photosynthesis, plant material growing between the chamber body and lid and causing leakage, etc.). While negative fluxes (e.g. carbon uptake to the soil from the atmosphere) could be overestimated using this method, we placed the fluxbots

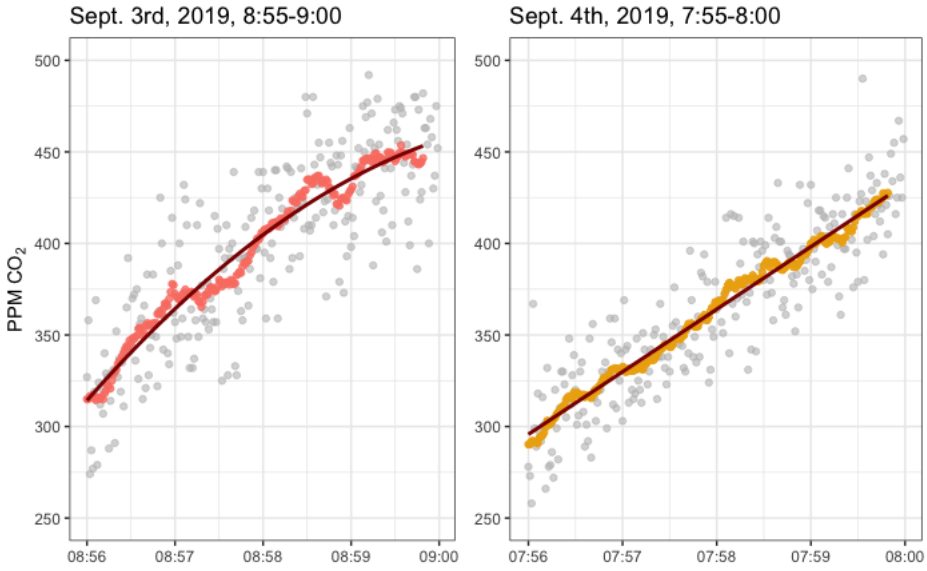

Figure 3.7: Two different measurement intervals taken by the same fluxbot (located in the 'total wildlife exclusion' plot, at 'open soil' site 2), one day apart at similar times in the morning. The left panel best fits a second-order polynomial regression, while the right panel best fits a linear regression. Gray points are raw data; salmon (left) or yellow (right) points are averaged with a 20s rolling mean and the regression fitted to that averaged data.

on plant-free soil patches; as a result, most measured fluxes are likely to be positive (e.g. no photosynthetic uptake of CO_2).

Of all the fluxes, two-thirds were calculated using the quadratic regression (Fig. 3.8a); these fluxes were generally higher than those calculated using the linear regression (Fig. 3.8b).

3.4.2 Patterns of flux over time

Raw CO_2 data demonstrated distinct diel patterns, as exhibited by fluctuations in raw concentration of CO_2 (ppm) over time (Fig. 3.9a), with atmospheric CO_2 higher in nighttime hours than day.

Fluxes calculated from these raw data *also* demonstrated distinct diel patterns, with the highest fluxes occurring at night and lowest tending to occur midday or mid-afternoon

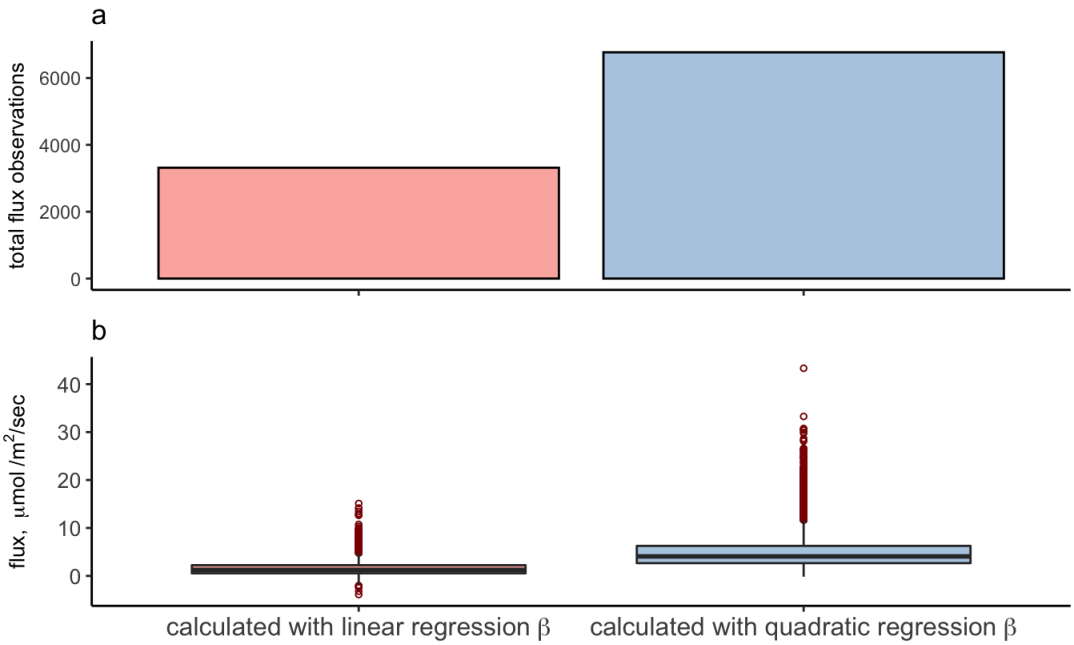

Figure 3.8: a) The total number of flux observations made, and proportion calculated with a beta value derived from a linear regression versus a quadratic regression. b) The spread of flux values calculated with a beta value derived from a linear regression versus a quadratic regression.

(Fig. 3.9b); a simple t-test comparing daytime fluxes (8am to 4pm, or 2 hours after sunrise and 2 hours before sunset) and nighttime fluxes (8pm to 4am) revealed consistently and significantly higher fluxes in nighttime hours (mean 4.28 μmol/m2/sec) than in daytime hours (mean 3.72 μmol/m2/sec) ($p < 0.001$).

For each flux interval we plotted the ratio of flux error to flux (hereafter relative flux error), against the regression fit (R^2) value of the regression from which its β_1 was selected. We found that relative flux error is extremely small at high R^2 values (as expected; e.g. $\sim 0.0045\%$, or e^{-10}). As R^2 approaches zero, however, the relative error per flux observation remains extremely small (e.g. $\sim 0.25\%$, or $e^{-6)}$); at its highest, with R^2 of zero, the relative flux error is $\sim 3.02\%$ (e.g. $e^{-3.5)}$) (Fig. 3.10).

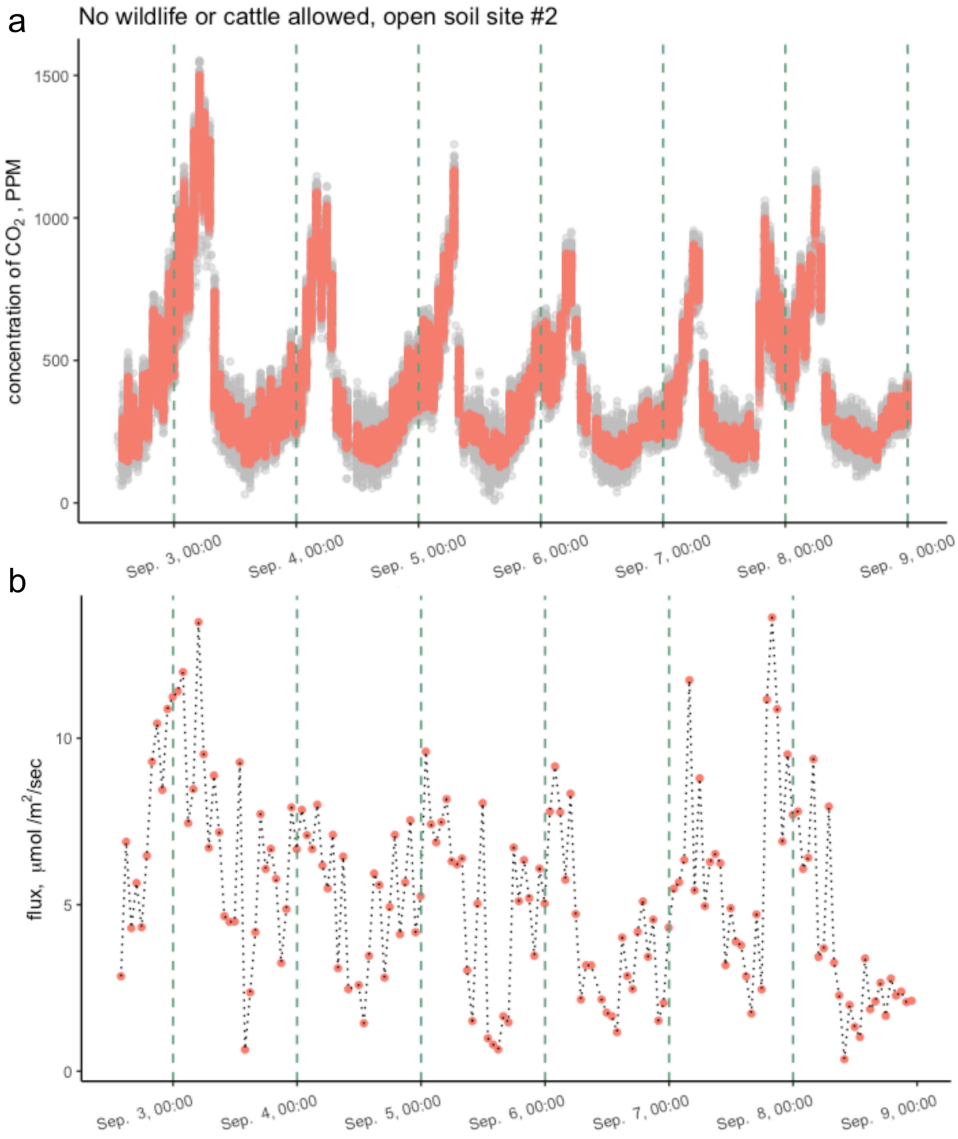

Figure 3.9: a) Raw CO_2 concentration data, collected from a single fluxbot over six days in September 2019. Gray points are untransformed raw co_2; salmon points are transformed with the 20sec averaging window. b) Fluxes, calculated from the 20sec averaged CO_2 data. For both a) and b), green dotted lines indicate each day's midnight hour.

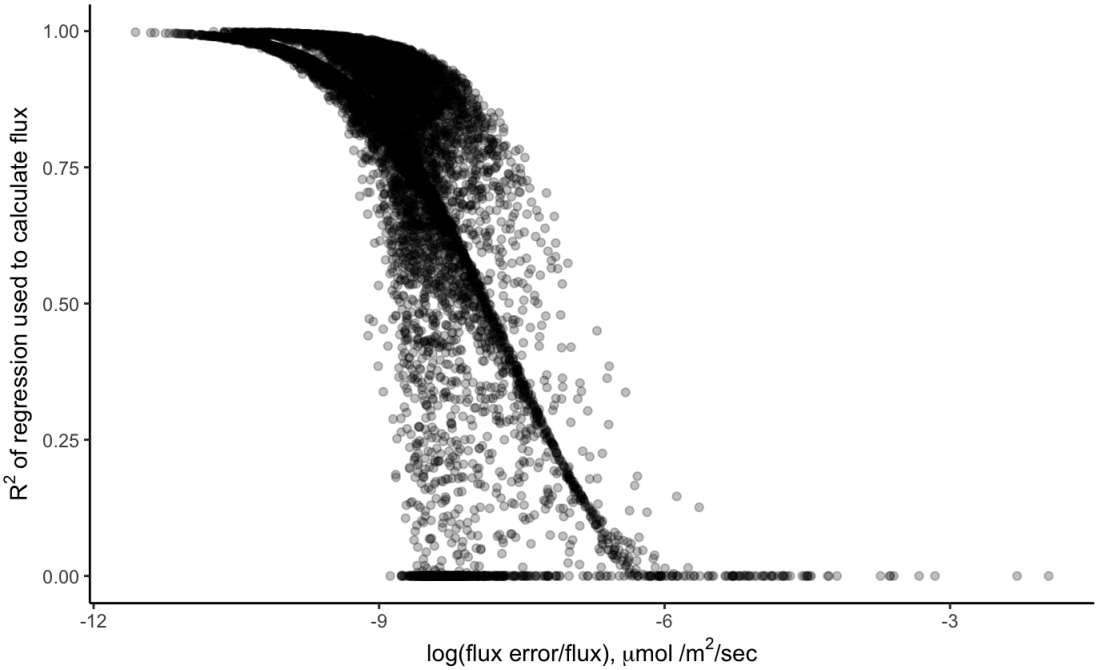

Figure 3.10: The natural log-transformed relative flux error (e.g. flux error/flux) relationship to R^2, or goodness-of-fit, of the regression used for each flux calculation.

3.5 Discussion

3.5.1 Comparison of methods

In the KLEE, it takes an average of 10 minutes to collect a flux observation using a manually-operated soil carbon flux chamber, including travel between sites (observation from previous (*in situ*) soil carbon flux data collection in this system, Forbes et al. unpublished data). Using this assumption, it would take over 1,680 working hours to manually collect the same number of flux observations as collected by our fluxbot network. While a high-cost, manually-operated commercial sensor may have less noise per flux observation, the inability to take highly-resolved flux data means variation across space and time will inevitably be high. It is possible to install a network of commercial autonomous chambers to resolve that variability by collecting data across space

and time; however, a network of fluxbots can be much larger at a fraction of the cost of an commercial autonomous sensor network, thus allowing for more interesting and ecologically-relevant deployment strategies (e.g. across an experiment like the KLEE; across ecologically-distinct landscape features, like tree canopies; etc.). The ability to collect such a computationally 'big' dataset using inexpensive, autonomous fluxbots allows for a more comprehensive understanding of fluxes *on average* over time, across a larger and more ecologically complex spatial extent.

Commercially-available soil carbon flux chambers, whether manually-operated or autonomous, have extremely high-quality infrared CO_2 sensors, design features to scrub excess CO_2 or moisture from inside the chamber, and integrated data analysis systems that allow for flux to be calculated automatically in real time. These features, and the internal quality-control processes of these sensors' software, reduce noise and variability in soil carbon flux measurements. While we calibrated each of our fluxbots' infrared gas analyzers to known concentrations of CO_2 prior to deployment, the sensors were variable in their detection of absolute CO_2, demonstrated by the fluxbot-specific detection of absolute ambient CO_2 by different 'donor' fluxbot lids on a single site over time (fig. 5). However, because flux is calculated using the net difference in CO_2 over a given observation interval, variability in the fluxbots' gas analyzers assessments of absolute atmospheric CO_2 concentration would not influence final flux calculations (and did not do so in the case of the site with the donor lids) (Fig. 3.4).

It was not possible to validate fluxbot performance with a commercial manually-operated system (by measuring at the same exact locations as the fluxbots within an hour of a fluxbot measurement (Savage et al. 2003), due to differences in size and shape of each instrument. What is more, these soils are extremely physically and biologically variable in space and time, thus resulting in high (on the centimeter scale) spatial and temporal variability in flux (Davidson et all, 2002; DeCarlo and Caylor, 2020) (see Appendix 2 for

discussion of data comparison).

However, if a research goal includes between-fluxbot comparisons of absolute ambient CO_2 *in addition to* flux, a solution is to increase the quantity of ambient measurements. The Allan variance assessment, conducted post-collection of all the data, demonstrated that a 20-second rolling average optimally reduced noise for the raw data (SI fig. 6). This indicates that we significantly under-sampled ambient CO_2 (e.g. three periods of ambient CO_2 monitoring, for three seconds each, per hour) relative to flux. Because of the (therefore inherently) noisy ambient data we collected, we could not confidently correct for differences in ambient CO_2 detection across the fluxbot network. To assess cycles of ambient CO_2 in *addition* to soil carbon flux, we therefore suggest that users of our method adjust the software's ambient data collection schedule to one single period of CO_2 measurement for 60 seconds immediately prior to chamber closure, a collection schedule that would provide enough data for noise-reduction in ambient data. While we expect soil carbon fluxes to differ from site to site, because the fluxbots 'share' general ambient atmospheric CO_2 a dataset of hourly ambient CO_2 would allow researchers to cross-validate and correct variable ambient CO_2 detection across the fluxbot network. This strategy would fully leverage the network advantages of the fluxbot system, by collecting non-noisy ambient data that is easily comparable across all fluxbots deployed in the network.

It is important to note that our cost breakdown (supplementary information, table 2) does not include labor costs, as the design/build/deployment of the fluxbot sensor network was conducted in-house by the research team. In addition, crucial labor hours were spent in the lab and in the field post-deployment solving problems that would not need to be de-bugged (*in situ*) in the future. As such, and because of the low materials cost, a refinement of the methods presented here would reduce the labor time (and thus costs) involved in manufacturing and troubleshooting a fluxbot network.

3.5.2 Relative flux error

Projects using other DIY autonomous sensor designs have all relied on slightly different analytical methods to calculate flux and reduce error. For example, in Carbone et al. (2008), the authors fitted a quadratic regression to their CO_2 accumulation data starting at the time where the internal CO_2 concentration was equal to the ambient, and used this regression's β_1 to calculate flux. In a subsequent study using the same chamber design, the authors fitted multiple, overlapping, three-minute-long linear regressions to each 20 minute accumulation interval, choosing the regression with the highest R^2 value (over 0.90) with which to calculate flux representative of that period (Carbone et al., 2011).

As described here, for the linear and quadratic regressions describing a single accumulation interval, we selected the higher β_1 to estimate flux regardless of its R^2. In this way we operated under the assumption that with a closed-chamber system, the initial rate of increase is most representative of real flux, due to the risk of pressure buildup inside the chamber reducing the diffusion gradient of CO_2 to the soil and causing underestimates of flux (Pumpanen et al., 2004; Kutzback et al., 2007). Because of this assumption, and because low R^2 values are statistically certain when slopes are at or close to zero, we also did not remove any fluxes based on a threshold for its associated regression's R^2 value.

This is not to suggest that R^2 does not matter as an indicator of a regression's ability to explain a trend; however, for the purposes of estimating flux, we are more interested in the trend (i.e. slope) itself than the regression's predicted values. It is not possible to know how variably a fluxbot detected CO_2 concentration each second while its chamber was closed; a low R^2 may simply indicate noisy data, which could occur for reasons ranging from environmental variability; to cracking in the soil (DeCarlo and Caylor, 2020); to chamber interference; etc. We therefore assume that noisy accumulation data

has a trend that still represents a true and valid flux. Or, a low R^2 could simply mean a regression's slope was close to or at zero, since statistically we would expect R^2 to be low (or even zero) at times when CO_2 accumulation was best described by a horizontal regression. Considering that many of our selected β_1 values were close to zero (fig. 8b) and subsequently as were many corresponding fluxes (fig. 6), we argue that relative flux error provides a greater assessment of flux calculation quality than R^2. Because the relative flux error for the fluxes with small (even zero) R^2 was quite low, we chose to keep all flux observations without pruning based on their associated regressions' R^2 values.

3.5.3 Inter-fluxbot variability in ambient CO_2 detection

There is clearly variability across the fluxbots in their ability to detect *absolute* values of atmospheric CO_2; however, we are confident that the fluxbots estimate soil carbon fluxes over space and time reliably. The overall distribution of fluxes collected with our 12 fluxbots (fig. 6) is consistent with those seen in other studies of this system and other tropical sites (DeCarlo and Caylor, 2020; February et al., 2020; Courtois et al., 2019; Konaté et al. 2003; Poth et al., 1995). In addition, in our study of rotating 'donor' lids at a single site in October 2019, the different lids collected consistent fluxes despite collecting visibly lid-specific values of ambient CO_2 (Fig. 3.4).

While we did not test each fluxbot lid on each fluxbot body, we feel that our confidence in the fluxbot data is well-supported. The fluxbots examine flux as a function of *change* in CO_2 over a set time, not absolute CO_2; the distribution of fluxes we observed is consistent with studies here and in other tropical systems; and, we have evidence of consistent detection of flux despite variable detection of absolute ambient CO_2 at a single site over time. Thus, we feel confident that variability in ambient CO_2 detection across the network does not negatively impact the individual fluxbots' ability to accurately

measure soil carbon flux.

Benefits of the fluxbot network We argue above that noise within single observations and variability in absolute CO_2 detection between fluxbots did not negatively impact our final dataset. Even without this argument, however, reducing noise and variability for *single* observations of flux becomes comparatively less important than improving the spatial and temporal resolution of observations when characterizing soil carbon flux heterogeneity for a landscape. Soil, and soil carbon flux, is notoriously heterogeneous, particularly in dry grasslands (Fotí et al., 2016) and rangelands (Wang et al., 2021). As argued here, characterizing soil carbon flux at high spatial *and* temporal resolutions requires increased soil carbon flux collection abilities, which is only feasible with a large numbers of autonomous sensors. A sufficiently large quantity of commercially-available autonomous sensors can be prohibitively expensive. Given the low cost of a single fluxbot it is possible to significantly increase the resolution of flux observations in space and time, and therefore a network of fluxbots' ability to converge on a landscape's 'true' flux. We also have high confidence in their ability to detect patterns in and deviations from this mean — for a given location, or time of interest, or both. Therefore we suggest that large numbers of DIY fluxbots, installed systematically across a large spatial extent and *a priori* known landscape features, can provide a more comprehensive and accurate assessment of an ecosystem's soil carbon flux than existing options, despite any possible increase in variability for a single flux observation.

The number of, and spatial and temporal resolution in, soil CO_2 flux observations that we collected is to our knowledge greater than that of any dataset previously collected in this system. Due to the site's inherent challenges (large wildlife; heavy rainfall; higher risk for in-person measurements during dusk, dawn, and nighttime hours; etc.), previous studies of soil CO_2 flux in this site (the KLEE) have been taken at high spatial resolution only in daylight hours (Forbes et al. unpublished data) or at low spatial resolution over

24 hours (DeCarlo and Caylor, 2020). While we also experienced some 'gaps' in our data collection, due to environmental factors like moisture buildup on the electronics at dew-points (dusk and dawn) or cloudy days causing occasional power losses, we ultimately collected data more or less hourly across our 12 sites, compiling over 10,000 individual observations of flux. This dataset reveals a distinct diel pattern in mean soil carbon flux in this seasonally dry savanna ecosystem, with the highest fluxes occurring at night and the lowest during the day. We compounded this increase in temporal resolution with increased spatial resolution *and* granularity, by installing fluxbots across several hundred meters of savanna, within two large-scale experimental wildlife community contexts, and on ecologically distinct landscape features. By installing the fluxbot network across, and collecting data from, such an ecologically meaningful extent we demonstrated the utility of inexpensive fluxbot networks for collecting much needed highly-resolved, ecologically meaningful soil carbon flux data.

3.5.4 Conclusions:

Manual flux chambers are incredible tools that allow for extensive flexibility while collecting carbon flux data in the field; however, the requirement that a researcher be present while collecting these data precludes collection either during large windows of time in which this is infeasible, or across a large spatial extent. Data collection over a large area or at fine-grained resolution is therefore difficult unless a research team is equipped with multiple instruments, which can be prohibitively costly. While autonomous devices exist on the commercial market currently, they are also extremely expensive. By building and installing this network of autonomous, inexpensive, fluxbots we have demonstrated the possibilities for collecting "big" soil carbon flux data that is large-scale, high resolution, and at ecologically-relevant granularity.

By making this fluxbot design and raw data analysis plan open access, we hope to further demonstrate that large-scale, high-resolution flux data collection capacity is accessible even on a limited project budget. Indeed, such improvements in soil carbon flux data in ecosystems worldwide would help illuminate not only its dynamics but also its connections to and influence on those ecosystems' carbon budgets on an Earth Systems scale.

3.6 Acknowledgements and Author Contributions

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thank Alfred Ekaaz for his invaluable assistance in the field in testing and deploying the beta version of the fluxbots, especially for his essential knowledge of and experience with electronics in the field.

Elizabeth Forbes[1] and Kelly Caylor[2] conceived of the original project aim and designs. Mark Hirsch[3] designed the software and hardware for the beta version of the fluxbots (tested in 2018; data not presented here). KC designed and wrote the data processing code that produces flux calculations from raw data streams. EF collaborated on data processing code, led the building and deploying of fluxbots in the KLEE (beta version and the current version presented here), and led data analyses and writing of this manuscript. Grace Lewin[4] deployed, maintained, and collected data from the beta version of the fluxbots. Vincent Benenati[5] designed the software and electronics wiring for the current version of the fluxbots (presented here) and provided real-time software updates as needed during their deployment in the KLEE in 2019. Spencer Frey[6] designed the current fluxbot version's hardware, and deployed, maintained, conducted troubleshooting, and collected data from the system in the KLEE. Naiskie Mantas[7] and George Koech[7] deployed, maintained, conducted troubleshooting on, and collected data from both the beta version of the fluxbots and the current system presented here, including *in situ* electrical and engineering work, and the rotating-lid data collection for comparison of multiple sensors in one location.

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Chapter 4

Wild and domestic grazing controls on landscape assembly, and influence on landscape-scale soil carbon availability and mineralization in a Kenyan savanna.

4.1 Abstract

Large bodied, wild vertebrate herbivores face unique pressures in the Anthropocene and thus often selectively experience local-scale declines in abundance or even local extinction. However, the decline or loss of these herbivores in ecosystems worldwide can drive significant alterations to a system's structural arrangement, and subsequently how carbon is stored and cycled throughout that system. Despite these indirect influences on carbon cycling, large herbivores are frequently left out of estimations of an ecosys-

tem's carbon budget. As biodiversity loss and climate change both advance, it is crucial to identify and quantify the connections between large herbivore (and their loss) and carbon dynamics.

We investigated soil carbon storage and cycling in a central Kenyan savanna ecosystem, within the context of a large-scale, long-term large herbivore exclosure experiment that excludes different size classes and identities (e.g. wild vs. domestic) of large vertebrate herbivores. We studied microbial respiration across three climatic contexts (e.g. three years with varying drought conditions), and dissolved organic carbon (DOC), nitrogen (DON), and microbial biomass in one of those years. We found that respiration, soil DOC, DON, and microbial biomass differed significantly across key landscape features; specifically, all are generally the highest beneath the canopy of the dominant nitrogen-fixing tree species, Acacia drepanolobium, indicating that these soils are where carbon pools are largest and carbon cycling occurs the fastest.

However, while the relative area of canopy cover decreases as large herbivore density increases (regardless of identity), the overall pool size of soil DOC, DON, and microbial biomass does not change. In addition, while large herbivore community composition did not have consistent effects on microbial respiration rates across years, rates were significantly impacted by years' ambient climate conditions, indicating that temporal variability is a significant overarching driver of soil carbon dynamics. Together these results indicate that while large herbivore community composition and experimental loss does not affect net pool size of soil DOC or cycling rates, the spatial patterning of where carbon is stored and cycles on the landscape changes depending on which herbivores are present.

4.2 Introduction

Large-bodied herbivores have a substantial influence on ecosystem structure, here defined as the arrangement of physical (living and nonliving) components that make up an ecosystem. These influences are driven primarily via their consuming primary production (plants), physically manipulating their environments, transporting nutrients long distances, and creating regular patterns in the landscape (Schmitz et al. 2018). For example, in Kenya, elephants browsing on woody plant species can prevent savannas from becoming *Acacia* woodlands (Owen-Smith et al. 2019). White rhinos can maintain grazing lawns, creating patchy landscapes of short grasses that do not burn as easily as taller patches, thus influencing local-level fire regimes (Waldram, Bond, and Stock 2008). By feeding on land at night and retreating to rivers during the day, hippopotamuses transfer vast quantities of nutrients via their excretions into rivers, richly subsidizing downstream riparian and aquatic communities with carbon and nitrogen (Subalusky et al. 2015). The use of temporary corrals (bomas) by pastoralists to keep domestic livestock in at night results in the development of nutrient-rich grassy glades, that can endure as distinct and highly productive landscape features for centuries after the bomas are moved (Porensky and Veblen 2015).

The structure of an ecosystem has been long accepted as a major determinant of its functions: its pools and cycles of nutrients, energy, and matter (Lamont 1995; also see Bradshaw 1984, fig. 2). Because large herbivores are both a part of an ecosystem's biological composition and can moderate its physical assembly, they strongly influence key ecosystem functions. These include the carbon cycle (Forbes et al. 2019), and as such it is likely that large herbivores influence climate via their control on ecosystem assembly, and subsequent effects local-scale carbon dynamics (Schmitz et al. 2014; Schmitz et al. 2018). Indeed, there is evidence of the influence of large-bodied herbivores on ecosystem

structure, subsequently influencing carbon cycling and climate across geologic time. The Pleistocene megafaunal extinctions may have caused global cooling due to a drop in methane emissions (Malhi et al. 2016; Smith et al. 2016) or warming due to increases in Arctic tree cover and decreased albedo effect (Doughty, Wolf, and Field 2010); in either case, megafaunal collapse's effects on ecosystem assembly is likely to have driven global scale climate changes. Relatively recently, the eradication of the rinderpest pathogen in the mid-20th century revitalized a decimated wildebeest population in the Serengeti, whose subsequent grazing may have reduced fire frequency via removal of flammable undergrowth, increasing carbon stored in trees and soil (Holdo et al. 2009). In the near future, the extinction of critically endangered forest elephants from Central Africa's rainforests could result in a loss of 7% of the forests' aboveground biomass carbon due to a likely proliferation of the fast-growing, softwood tree species that is their preferred browse (Berzaghi et al. 2019).

Despite such evidence that large herbivores' impacts on ecosystem structure percolate to impact the carbon cycle, ecologists have only recently called for the influence of large-bodied animals to be explicitly incorporated into characterizations of it (Schmitz et al. 2014; Bar-on et al. 2018). This is a challenging task, as large herbivores effects' on ecosystem structure and functions are often ecosystem- and herbivore-dependent (Forbes et al. 2019). Moreover, empirically demonstrating such an influence often requires large-scale, long-term empirical experiments (Lamont 1995) because many of the potential pathways (e.g. changes in physical structure of ecosystems) occur only over long time periods and large spatial scales. Thus, it is still difficult to reliably integrate their effects on an Earth systems scale.

It is increasingly pressing to identify and incorporate large bodied herbivores' impacts on ecosystem structure into studies of ecosystem carbon because in the Anthropocene, large-bodied wildlife species are going locally, globally, or functionally extinct — faster

than at any other time in history (Ceballos et al. 2015; Young et al. 2016). At the same time, and often as a driver of wild large herbivore loss, the abundance of large domestic herbivores is increasing dramatically in ecosystems worldwide, with little understanding of whether they provide the same functional effects as their wild counterparts. Such reassemblies to large herbivore communities could result in difficult-to-predict outcomes on local-level carbon cycling and ultimately, climate dynamics. While scientists now recognize that biodiversity loss (including spatial replacement with domestic species) and climate change are inextricably linked, the implications biodiversity loss are currently difficult to predict much less legislate around (Pörtner et al. 2021). Systematic, experimental characterizations of the impacts of large herbivores on the carbon dynamics of the ecosystems in which they remain are urgently needed to incorporate the impacts of their loss (and possible replacement by domestic analogs) into local-scale carbon cycle models.

To better understand the ecosystem-level influences of large herbivores and their loss on carbon cycling, we need long-term, large-scale manipulations of wildlife communities (and their domestic counterparts) that allow ecologists to methodically assess the impacts of experimental losses of species from a landscape (Forbes et al. 2019). One such experiment is the Kenya Long-term Exclosure Experiment (hereafter 'KLEE'), located in Laikipia, Kenya at the Mpala Research Centre and Conservancy. Constructed in 1995, KLEE uses different barriers to selectively exclude three size classes of wild vertebrate herbivores from inside large experimental treatment plots. Notably, KLEE incorporates three analogous treatments that include periodic cattle grazing in addition to size-selective exclusion of wild herbivores, allowing additional examination of the effects of herbivore identity on ecosystem response. KLEE is located in savanna in which pastoralism occurs but the wildlife community remains largely intact, making its exclusion plots a realistic representation of large herbivore species' functional extinction and their

relationship to a domestic analog.

KLEE has been operation for more than 20 years, long enough that significant changes to savanna structure in response to varying large herbivore community composition have been observed (Sitters et al. 2020; Charles et al. 2021). As a result, we can examine if and how changes to ecosystem structure, induced by large herbivore community composition, influence soil carbon dynamics. We used four of KLEE's six treatments to assess whether different assemblages of wild and domestic large herbivores indirectly influence carbon storage and cycling via their effects on savanna assembly. We ask: does carbon storage and mineralization differ across distinct landscape features within treatments? Is carbon storage and mineralization affected by herbivore community composition, given changes in landscape assembly? Do changes vary by herbivore identity (wild or domestic)? And, what is the overall effect of large herbivore community composition on carbon storage and cycling? We measured soil dissolved organic carbon (DOC) and nitrogen (DON) storage, microbial biomass, and microbial activity across treatments. DOC and DON are the most active and available pools of carbon and nitrogen; their proportions are thus intimately tied with ecosystem productivity and decomposition. Microbial biomass and activity (i.e. mineralization of soil organic matter, making it available as nutrients for plant growth) is metabolically dependent on the size of these two pools. Quantifying these pools helps identify their contribution to the ecosystem's carbon storage capacity and cycling rates.

Changes to the spatial assembly of key, ecologically-relevant landscape features in KLEE are driven by the size-selective loss (exclusion) of large wild herbivores, and their replacement with domestic livestock in KLEE; given that, we focus on the assembly of two vital landscape features, *Acacia drepanolobium* trees and termite mounds, in each treatment. We hypothesize that pools of DOC, (DON), and microbial biomass in soils beneath tree canopies and on termite mounds will be larger than background

matrix soil, as well as on termite mounds. We hypothesize that a decrease in large herbivore biomass (regardless of wild/domestic identity), promoting greater proportional tree canopy cover (Sitters et al. 2020), will result in larger net pools of DOC, DON, and microbial biomass at the plot scale, and that these increases will be accompanied by faster rates of microbial carbon mineralization. We hypothesize that increased termite mound frequency in plots with cattle (Charles et al. 2021) will result in larger plot-level pools and faster mineralization in plots with cattle as compared to their non-cattle analogs (Charles et al. 2021).

4.3 Methods

4.3.1 Experimental site:

KLEE is located at Mpala Research Centre, Ranch and Conservancy (0° 17' N, 36° 52' E) in Laikipia County, west-central Kenya. KLEE is located on clay-rich, volcanic, 'black cotton' vertisol soils. The semi-arid, seasonally dry ecosystem experiences weakly trimodal rainfall, with a distinct dry season December to March, and a mean annual rainfall of 600mm/year. These soils 'swell' in wet conditions due to their ability to take up significant volumes of water, then 'shrink' in dry conditions as the soils dry out (Muchena and Guchene 1988). These seasonal cycles of swelling and shrinking (along with swell/shrink dynamics from more frequent, regular variations in environmental moisture) result in soils with a characteristically cracked soil morphology (DeCarlo and Caylor 2020).

The vegetation at KLEE is a wooded savanna, with an understory dominated by five grass species (Young et al. 1998, Porensky et al 2013). Notably, woody biomass in KLEE is dominated by a single tree species, *Acacia drepanolobium*, which makes up 97%

of tree canopy cover (Porensky et al. 2013). *A. drepanolobium* trees are a foundation species in this ecosystem, both as a major structural constituent of the site and also as a legume that has a mutualistic relationship with nitrogen-fixing bacteria in their root nodules (Fox-Dobbs 2010). Their nitrogen-fixation capacity creates hotspots of nitrogen availability in the otherwise nitrogen-poor tropical savanna (Gichangi et al. 2016). The wildlife community is phylogenetically and functionally diverse, hosting year-round communities of large-bodied, vertebrate herbivores including plains zebra (Equus quagga), Grant's gazelle (*Gazella grantii*), eland (*Taurotragus oryx*), hartebeest (*Alcelaphus buselaphus*), African elephant (*Loxodonta africana*), giraffe (*Giraffa camelopardalis*), African buffalo (*Syncerus caffer*), oryx (*Oryx beisa*), and Grevy's zebra (*Equus grevyi*). As an operating livestock ranch, Mpala also stocks domestic cattle (*Bos taurus*) at low to moderate densities (Veblen et al. 2016).

The invertebrate community's biodiversity is also considerable, and notably includes an ecosystem engineer: the fungus-cultivating *Odontotermes* sp., whose subterranean mounds (which are only slightly convex at the soil surface) are distributed evenly across the landscape (Pringle et al. 2010). These mounds are hotspots for relatively high nutrient concentrations, and have lower clay content and greater water infiltration than surrounding non-mound soils. Subsequently, termite mounds support a different community of vegetation as compared to surrounding soils (Brody et al. 2010).

KLEE itself consists of six herbivore community composition treatments using barriers to exclude two size classes of large wild herbivore, and that either allow or preclude domestic cattle grazing. Each replicate of the experiment is constructed in a 2x3 grid of square, 200x200m (4ha) treatments. The wild herbivore exclosures include non-fenced treatments where all wild herbivores are present ('MW', or all mega- and meso-herbivores); semi-permeable fenced treatments where all large wild herbivores smaller than 'mega' (e.g. giraffes and elephants) are present ('W', or just meso-herbivores); and

entirely fenced treatments where no large wild herbivores (e.g. >15kg) are present ('O', or no mega- or meso-herbivores) (Fig. 4.1).

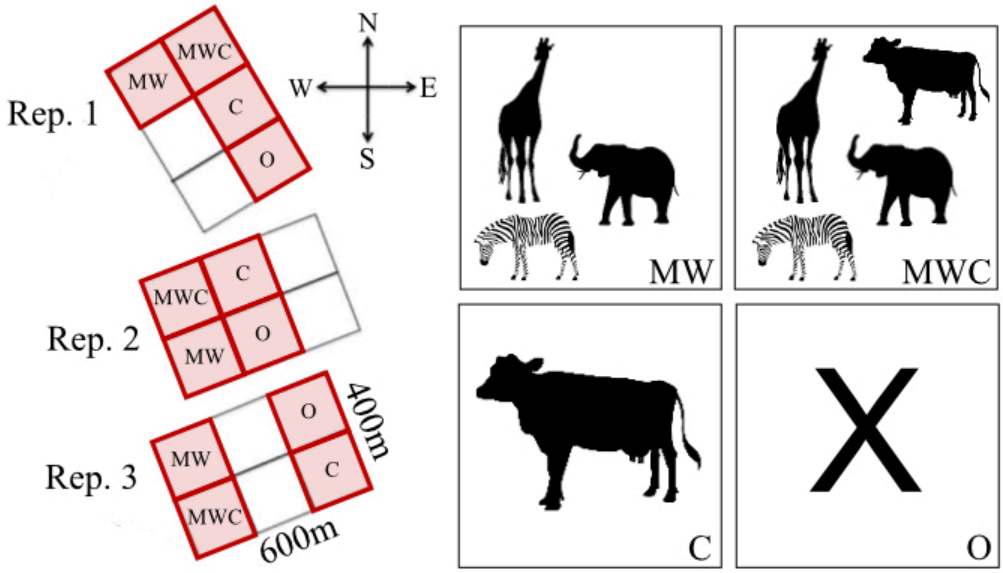

Figure 4.1: Schematic diagram of the KLEE, with the four treatments used in this study highlighted in red (left). These four treatments are (right, counterclockwise from upper-right): MWC, all large herbivores allowed; MW, just wild large herbivores allowed; C, cattle-only; O, no large herbivores allowed, wild nor domestic.

The other three KLEE treatments are identical to those just described, with the additional presence of domestic cattle grazing. A controlled grazing regime occurs three to four times per year: over two to three consecutive days, 100-120 head of Mpala cattle are herded into the 'MWC' (all wild mega- and meso-herbivores, plus cattle), 'WC' (wild meso-herbivores only, plus cattle), and 'C' (no mega- or meso-wild herbivores; cattle only) plots and grazed for several hours (Fig. 4.1). Cattle are grazed in each cattle-treatment plot for an equivalent amount of time during each cattle grazing event. This regime results in a stocking density and grazing intensity that is similar to that at Mpala Ranch (0.10-0.14 cattle/ha) (Young, Palmer, and Gadd 2005; Odadi et al. 2007) and other nearby properties where grazing occurs. Its episodic interval is consistent with traditional East African herding practices, where herders graze cattle in an area for

several days before herding them elsewhere for new forage, allowing forage to recover. The abundance of key landscape features in study at KLEE, namely *A. drepanolobium* trees abundance and canopy cover as well as the abundance and size of termite mounds, is influenced by the community composition of large herbivores including their identity as wild or domestic (Sitters et al. 2020; Charles et al. 2021).

This grid of six treatments is replicated in three blocks across the landscape. Of the six KLEE treatments, we focused this study on the four most extreme in terms of large herbivore density: no wild or domestic large herbivores (O), only cattle (C), only wild large herbivores (MW), and both wild large herbivores and cattle (MWC) (Fig. 4.1). We chose these four treatments specifically to interrogate the impacts of the 'loss' of the largest wild herbivores in this ecosystem, and their spatial 'replacement' with domestic cattle.

4.3.2 Soil sample collection

We collected soil samples over three dry seasons (in 2015, 2016, and 2018), between Jun-Aug each time. This study was not initially set up to test for interannual effects, but rather, we hoped that by sampling over multiple years' dry seasons we would detect a stronger signal of variation across landscape feature and treatment. However, the three years each exhibited distinct ambient climatic patterns including an historic, multi-year drought. For microbial carbon mineralization rate (described in the next section) we thus included year of soil collect in our analyses. We used a stratified within-treatment sampling method to collect soils from three distinct landscape features within each treatment: beneath the canopy of living *A. drepanolobium* trees, from the surface of active termite mounds, and from open soil patches not influenced by either a mound or a tree canopy, e.g. the background savanna matrix. We took all samples from the top 0-5cm

layer of soil, and all were collected from the "inner hectare" of each 4ha-sized plot to exclude edge effects.

In 2015 sampling intensity was substantially more limited than in 2016 and 2018: we collected three samples per soil type within each of the four treatments, selecting random locations at least 75m apart (108 total samples). We increased sampling intensity in 2016 and 2018 to address high variation we observed in 2015's samples: to collect samples in 2016 and 2018, we placed a 100m transect down the center of each plot's inner hectare. For open soil samples, starting at 0m, we collected one sample every 10m along the tape, from 0m to 90m. We ensured each open soil sampling point was not within 10m of a termite mound or tree canopy edge. For under canopy soils, we used the same transect method; at each 10m stopping point, we walked perpendicularly away from the tape to the nearest live *A. drepanolobium* tree to sample beneath its canopy, alternating walking left or right to maximize distance between under canopy samples. For termite mound soils, we used a preexisting map of active termite mounds, created by Grace Charles et al. (2021), and randomly selected up to ten mounds within each plot from which to sample (if there were fewer than 10 mounds in a plot, we sampled every mound). Across all three sampling years, samples were 10m apart to ensure spatial independence (e.g. Folorunso et al. 1988).

We air-dried each sample upon returning to the lab (e.g. same day) and as soon as possible after (e.g. within 1-3 days) we sieved each to 2mm to eliminate non-soil particulate matter. We stored each sample in an air-tight Whirlpak bag then stored in secondary, sealed and airtight plastic bags inside of airtight, opaque drum-style plastic buckets with lids, inside a closed cabinet isolating samples from possible impacts of light. We stored inert (isolated from moisture, light, air) samples until their use (0.5-5 months).

4.3.3 Soil microbial carbon mineralization

In order to assess soil microbial activity (e.g. the mineralization rate of bioavailable carbon by soil microbes), we incubated each soil sample for 144 hours (six full days). First, in 2015, we determined water holding capacity (WHC); for 36 samples (three samples from each of the three features, within each of the four herbivore treatments), we calculated its gravimetric water content. There was not an appreciable difference in WHC across herbivore treatment, but there was across landscape feature, we used average WHC of landscape feature to establish the volume of water needed per sample to reach 50% for rehydration. We used these values for all incubations, as WHC is not expected to change significantly without significant changes to texture.

We conducted each incubation by measuring a constant mass of each soil sample into 1L Mason jars; for the 2015 incubation, we used 20g dry soil per sample, and for 2016 and 2018 incubations we used 10g dry soil per sample due to limitations on total weight for importation under the USDA permit. We re-wet each individual soil sample with enough deionized (milli-Q filtration system) water to bring it to 50% of its WHC, after which we immediately sealed the jar with a standard Mason jar lid which had been fitted with a rubber septum. We then took daily 1ml gas samples from the headspace of each jar via this septum using a syringe fitted with a non-coring needle.

We measured the carbon dioxide (CO_2) content of these 1ml samples using a flow-through Licor-brand infrared gas analyzer (IRGA) (Li-6252). With this device, a constant flow of carbon- and moisture-scrubbed air is pushed through the IRGA (with a motor and fan) such that its baseline detection of CO_2 is zero. When a sample is injected into a rubber septum in the through-line after the carbon-scrubber but before it reaches the IRGA, a peak is produced in the IRGA's readout of CO_2 concentration proportionate in area and height to the concentration of CO_2 in the sample. In this way we monitored

daily microbial respiration via emissions (and total accumulation) of CO2 from each sample over the course each six-day incubation. We converted each peak height (e.g. concentration of CO_2 per unit volume of headspace), to CO_2 expressed in µg carbon using ideal gas laws and jar volume. We assembled each sample's time series data, and divided each timepoint by the dry soil mass associated with it to calculate total µg C produced per gram of soil each day. We divided total accumulated CO_2 at the end of the incubation for each sample by six (total length of incubation in days) to calculate the rate of soil microbial carbon mineralization in µg C per gram of soil, per day.

4.3.4 Extractable (e.g. bioavailable) organic carbon, nitrogen, and microbial biomass

The three incubations described above measured soil microbial activity, but to measure the soils' extractable carbon and nitrogen concentrations, we conducted a second six-day incubation of the 2016 dry soils only. At the conclusion of that incubation we extracted the incubated soils' remaining dissolved organic carbon and nitrogen (DOC and DON), including DOC and DON associated with microbial biomass. We extracted DOC and DON from the incubated soils using standard chloroform-fumigation extraction methods. We took a small subsample of each incubated soil to determine soil moisture content, measured its wet weight, oven-dried it to a constant mass, and measured its dry weight (with soil moisture content being [wet weight - dry weight]/dry weight). We split the remaining sample in half, weighed each half, then subjected one half to fumigation for 24hrs with chloroform (which lyses microbial cells) before homogenizing in a slurry with 20ml of a salt solution (0.05M K_2SO_4; previously determined to be the most effective extractant type and concentration for this soil (E. Forbes and K. Marchus, unpublished data)). We homogenized the other, un-fumigated half in the same salt solution. We used

a vacuum manifold system to filter each thoroughly-mixed slurry through a glass fiber filter, extracting the liquid containing the split samples' soluble carbon and nitrogen.

We analyzed each liquid extract for DOC and DON on a Shimadzu-brand Total Organic Carbon analyzer (TOC). By splitting each sample in half and fumigating one of the two, we determined concentrations of soil DOC and DON, and microbially-sourced DOC and DON (a proxy for microbial biomass) by subtracting the values of the non-fumigated subsample from those of the fumigated subsample (which extracted DOC and DON from both the soil and from lysed microbial cells). We used the sample's soil moisture content to calculate each extracted subsample's mass in grams of dry soil, then standardized the concentrations of soil DOC, DON, microbial C, and microbial N to mg/kg soil.

4.3.5 Conversion of concentrations of DOC, DON, microbial biomass to pools

To convert site-based concentrations of DOC, DON, and microbial biomass to site-based pools we used existing data from KLEE on soil bulk density per landscape feature (Brody et al. 2010). We multiplied each response variable by the average bulk density of soils for its corresponding landscape feature, and by the constant 5cm sampling depth, to determine pool size of each response variable (of each landscape feature, within each treatment) in kg/m^2.

4.3.6 Conversion of site-based pools to plot-level pools

To convert site-based pools of DOC, DON, and microbial biomass to total plot-level pools (kg/ha), we used existing data from KLEE on proportional area of each landscape feature in each treatment (Fig. 4.2) (Charles et al. 2021). For each response variable

we multiplied each sample's site-based pool size (kg/m^2) by the total area (m^2) of each landscape feature, within each treatment, to calculate the total mass (kg). We averaged mass size by landscape feature, within each treatment. For each individual plot (n = 12), we summed the masses of each of its three component landscape features, and divided by plot size (4ha) to determine total plot-level pool size (kg/ha).

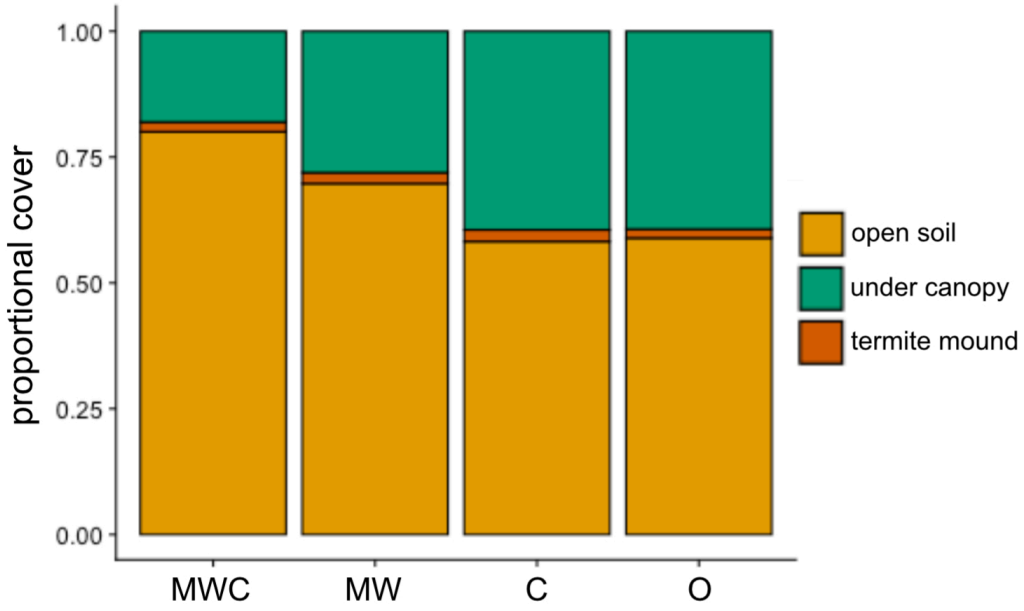

Figure 4.2: Proportional cover of each landscape feature within each treatment examined in this study. Data from Sitters et al 2020 (under canopy) and Charles et al. 2021 (termite mound).

4.4 Data Analyses

For all analyses below, we considered MWC the treatment-level control and OS the feature-level control. For each linear model, including in model testing, we assessed residual diagnostics using the DHARMa package for hierarchical models (Hartig 2021), and conducted post-hoc Tukey pairwise comparisons across all significant terms using the emmeans package (Length 2021).

4.4.1 Soil microbial carbon mineralization

We used a linear mixed effects model to assess differences in microbial respiration rate, by herbivore treatment and landscape feature, using the lmer function in the R package lme4 (Bates et al. 2015) on log-transformed rates with treatment (four levels), feature (three levels), year of sample collection (three levels), and the pairwise interactions between all three as fixed effects, with KLEE replicate (three levels; North, Central, or South) as a random effect. We decided to include year of sample collection as a fixed effect, because of the strikingly different climatic conditions in each of the years during which sampling occurred, despite each sampling period occurring in what is typically the summer dry season. In 2016 (the second year of sampling), a multi-year drought began which devastated the region's water supply. In 2018 (the third year of sampling), the drought had ended and the year was unusually wet, with the spring rainy season lasting longer into summer than is typical. Because drought can impact soil organic carbon inputs as well as soil microbial growth and carbon mineralization (Deng et al. 2021), we felt it was likely an important driver of microbial respiration rate in this system.

We subsequently also assessed yearly soil microbial activity to assess impacts of herbivore treatment and landscape feature: for each year's incubation, we log-transformed the mineralization rate and used linear mixed-effects models, assessing during model selection whether to use treatment, feature, and the interaction between the two versus just treatment and feature as fixed effects, with KLEE replicate as a random effect. We conducted post-hoc Tukey pairwise comparisons across all significant terms.

4.4.2 Soil DOC, DON, and microbial biomass pools at sampling sites

We compared pool sizes of soil DOC, DON, and microbial biomass C and N across landscape feature and treatment to determine differences in average concentration at each sampling site (e.g. not area-weighted). We log-transformed each of these four response variables and used linear-mixed effects models, with feature + treatment as fixed effects and KLEE replicate as a random effect to assess relative effects of each fixed effect on pools. We assessed overall effect of treatment and feature on each response variable with ANOVAs, and conducted post-hoc Tukey pairwise comparisons across any significant terms.

4.4.3 Total plot-level soil DOC, DON, and microbial biomass pools

We compared total pools of DOC, DON, and microbial biomass in kg/ha across herbivore treatments to determine differences in pool size at the plot scale. We used linear-mixed effects models with treatment as the fixed effect, and KLEE replicate as a random effect to conduct this comparison across treatments. We assessed overall effect of treatment on each response variable with ANOVAs, and conducted post-hoc Tukey pairwise comparisons across any significant terms.

4.4.4 Power analyses

Because treatment-level analyses in KLEE inherently have a sample size of n=3 (with three replicates of the experiment across the landscape), we ran power analyses for each of the four models analyzing plot-level pools of DOC, DON, MBC, and MBN, respectively.

We used the mixedpower package in R (Draschkow 2021) and increased the number of hypothetical KLEE replicates by three for eight steps total (up to 24 total simulated KLEE replicates), for 1000 simulations each. We chose a t-statistic of 2 for determining detection of treatment effect (because t = 1.96 reflects an alpha level of 5%). We assumed that computed sample sizes showing statistical power of $\geq$ 80% (e.g. the probability of correctly rejecting the null hypothesis, that treatment has no effect on total pool) would represent sufficient empirical sample sizes to detect an ecologically real difference in pool size across treatments (Kumle, Võ, and Draschkow 2021).

4.5 Results

4.5.1 Microbial carbon mineralization rates

Microbial respiration rate (e.g. rate of soil carbon mineralization) varied significantly by year, both as a fixed effect ($p < 0.001$) and as it interacted with both treatment ($p = 0.003$) and landscape feature ($p < 0.001$). Soils collected in 2016 (the first year of the drought) had the lowest rate of microbial respiration: 15% lower than in the preceding year, 2015, and 27% lower than two years later in 2018 (the extremely wet year post-drought). Respiration rates from soils collected in 2018 were also 15% faster than respiration rates from those collected in 2015 (all pairwise comparisons $p < 0.001$). Because treatment ($p < 0.001$) and landscape feature ($p < 0.001$), were also significant drivers of differences in soil respiration rates, we examined each year individually with treatment and landscape feature as fixed effects (Fig. 4.3).

In 2015, herbivore treatment (and not landscape feature) drove differences in rates of microbial carbon mineralization (table 2). Soils collected from MW plots respired the slowest, at 26% lower rates than soils collected from C plots ($p = 0.031$; no other pairwise

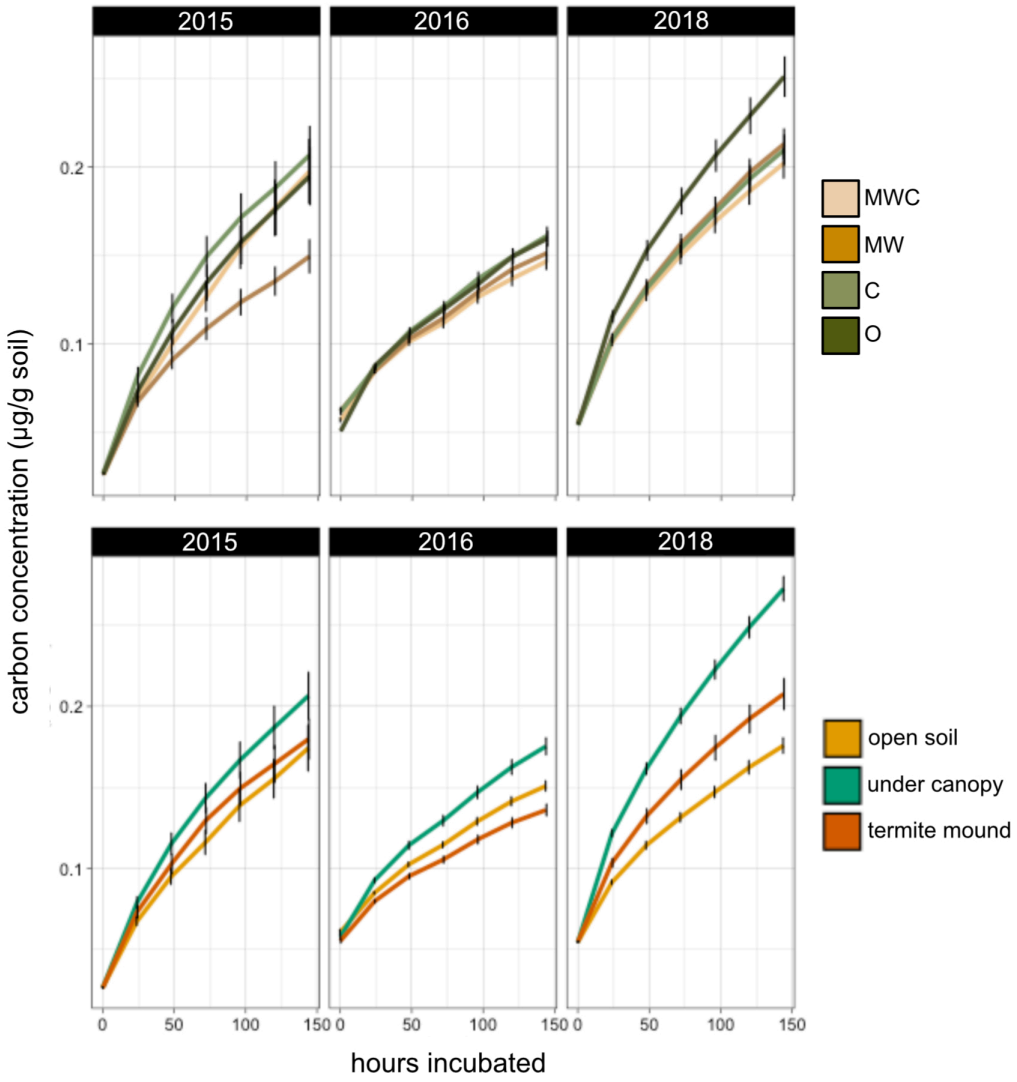

Figure 4.3: Accumulation curves, e.g. the emission rate and total accumulation of carbon dioxide from soil microbial mineralization by treatment (top row) and landscape feature (bottom row) for each of the three years' soil incubations. Error bars represent one standard error of the mean. Samples incubated in 2016 were twice the mass as samples incubated in 2017 and 2018, hence the lower starting point at "hours incubated = 0".

comparisons across herbivore treatments were statistically significant). While differences were not significant, soils from under tree canopies respired faster than soils from open soil patches or termite mounds. In 2016, both herbivore treatment and landscape feature drove differences in rates of microbial carbon mineralization (table 1); however, post-

hoc comparisons revealed no significant pairwise differences in mineralization rate by treatment. Across all treatments, each of the three landscape features had significantly different rates of microbial carbon mineralization. Soils from under tree sites had 12% higher rates than those from open soil patches ($p < 0.001$), and 22% higher than those from termite mounds ($p < 0.001$). Soils from open soil patches had the second highest rate of respiration, at 11% higher than those from termite mounds ($p = 0.003$) (Fig. 4.3).

In 2018, herbivore treatment, landscape feature, and the interaction between the two drove differences in rates of microbial carbon mineralization (table 2). Pairwise comparisons revealed that soils from O plots had 15% higher rates of respiration than those from C plots ($p = 0.003$), 14% higher than those from MW plots ($p = 0.006$), and 18% higher than those from MWC plots ($p < 0.001$). However across all herbivore treatments, soils from under trees once again had the highest rate of microbial respiration, 27% higher than from termite mounds ($p < 0.001$) and 35% higher than from soils open patches ($p < 0.001$). Interestingly, in 2018 the trend between soils from open soil patches and from termite mounds reversed that we saw in 2016: soils from termite mound sites had 11% higher rates of carbon mineralization than those from open soil sites ($p = 0.015$) (Fig. 4.3).

4.5.2 Feature pools

After conversion of sample concentrations to pools with bulk density and sample depth (which eliminates the effect of sample compaction from comparisons), the strongest driver of differences in soil DOC, DON, MBC, and MBN pools at sampled locations was landscape feature. After 21 years of KLEE's herbivore treatments, samples taken from termite mounds had an approximately 30% larger DOC pool than samples taken from open soil patches (Fig. 4.4a), though the differences in soil DOC between samples taken

from termite mounds and under tree canopies, and from open soil patches and under tree canopies were not significant.

As for available soil nitrogen, samples taken from beneath tree canopies had the largest pool, with a 20% greater DON pool than those taken from termite mounds and 46% greater than those taken from open soil patches. Soils from termite mounds had a 32% larger DON pool than those from open soil patches (Fig. 4.4b). There was a slight herbivore treatment effect on feature-specific soil DON pools, which pairwise comparisons revealed were 25% greater in total exclusion plots (O) than in all herbivores allowed (MWC) plots ($p = 0.005$), but the main driver of effect on sampled pools for both soil DOC and DON was landscape feature. In both cases, being neither beneath a canopy nor on a mound drove a decrease in bioavailable carbon and nitrogen in the soil.

Neither soil MBC nor MBN pools at the sampled locations were influenced by herbivore treatment. However, both were influenced by sample feature, like soil DOC and DON. Soil MBC was 26% greater in samples taken from beneath tree canopy than from open soil patches and 52% greater than in samples taken from termite mounds. It was also 35% greater in samples taken from open soil patches than from termite mounds (Fig. 4.4c). In a similar pattern, soil MBN was 27% greater beneath tree canopies than open soil patches and 41% greater than termite mounds, and 20% greater at open soil patches than termite mounds (Fig. 4.4d).

4.5.3 Feature pools: plot-scale DOC, DON, MBC, MBN

Once we converted pools at sampling locations to proportional plot-level pools (table 1), herbivore treatment effects on the size of each feature's DOC, DON, MBC, and MBN pools were revealed. The total 'open soil' DOC pool was 26% smaller in cattle-only plots (C) than all herbivores allowed plots (MWC) ($p = 0.02$). The total 'under canopy' DOC

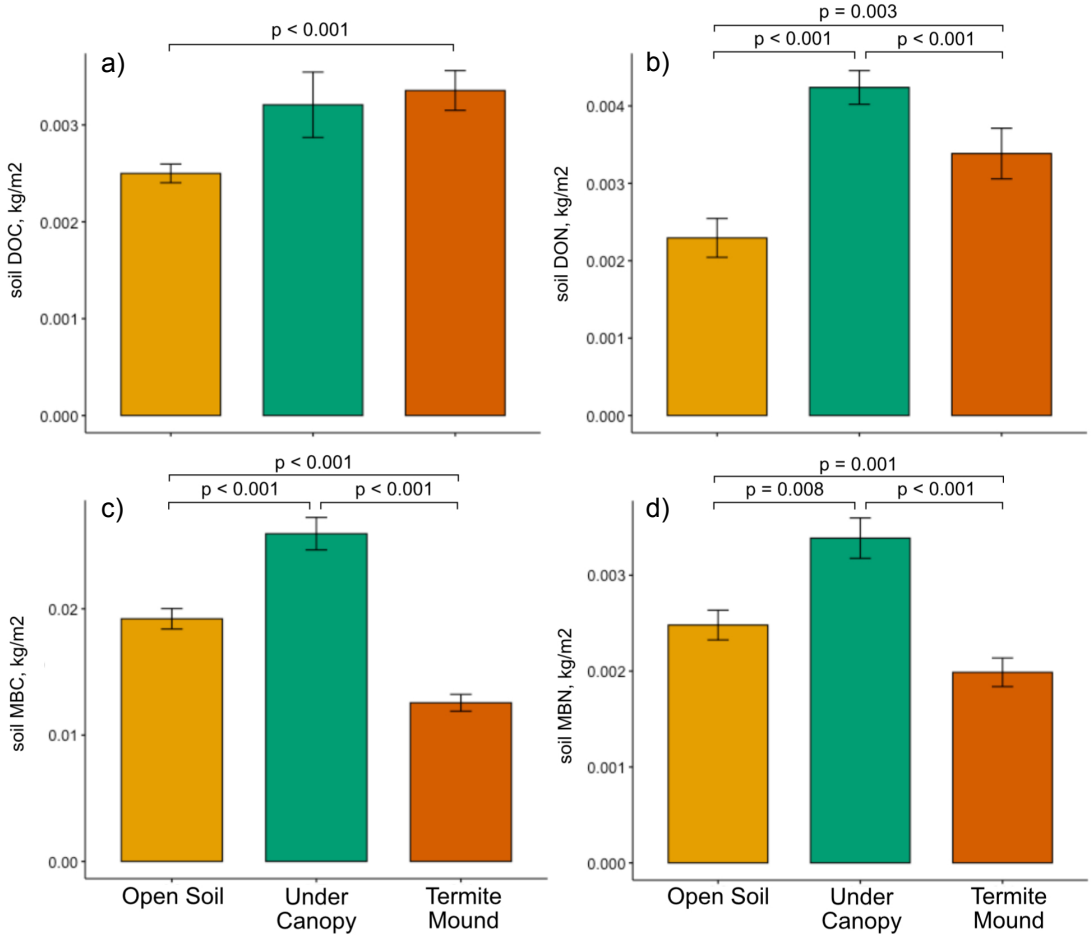

Figure 4.4: Mean pool sizes of a) soil DOC, b) soil DON, c) soil microbial carbon (MBC), and d) soil microbial nitrogen (MBN) at each landscape feature in the KLEE. Error bars indicate one standard error. Significant pairwise comparisons indicated with brackets and associated p-value.

pool increased with decreasing herbivore density (from MWC to O), but the difference pool was only significant between MWC and O, with the total exclusion of herbivores driving a 71% increase in total soil DOC under *A. drepanolobium* canopies (p = 0.009). There was no significant difference in pool size across herbivore treatments for the termite mound soil DOC pool (Fig. 4.5a).

The soil DON pool at open soil patches and on termite mounds did not differ significantly across herbivore treatments. However, the soil DON pool under tree canopies

was significantly affected by herbivore treatment, with total plot-level soil DON generally increasing with decreasing herbivore density. Pairwise comparisons revealed that the total under-canopy pool was 60% smaller in MWC plots compared to O plots, and 61% smaller compared to C plots. Excluding cattle in MW plots increased the size of the soil DON pool, but its pool was still 33% smaller than that in C plots. Similar to the soil DOC pool, there was no significant difference in termite mound soil DON pool size across herbivore treatments (Fig. 4.5b).

Soil microbial biomass carbon (MBC) pools at open soil patches decreased in size with decreasing herbivore density. Only the difference between the pools in MWC and O were significant, with the MWC pool 29% larger than that in O. Interestingly, the presence of domestic cattle alone seemed to increase the under-canopy MBC pool, which was by far the largest in the cattle-only (C) plots: 63% larger than in MWC plots (where MBC pools were smallest), 48% larger than in MW plots, and 24% than in O plots. Once again, the size of the termite-mound MBC pool did not differ significantly across herbivore treatments (Fig. 4.6a).

Soil microbial biomass nitrogen (MBN) pools predictably followed similar patterns as MBC pools across herbivore treatments: generally decreasing in size as herbivore density decreased, but with no significant differences in pool size across treatments. And similarly to MBC pools, the presence of domestic cattle as the sole herbivore seemed to increase the under-canopy MBN pool, which were again the largest in cattle-only plots: 63% larger than in MWC plots (where again, MBN pools were smallest), 51% larger than in MW plots, and 27% larger than in O plots. Lastly, and again, the size of the termite-mound MBN pool did not differ significantly across herbivore treatments (Fig. 4.6b).

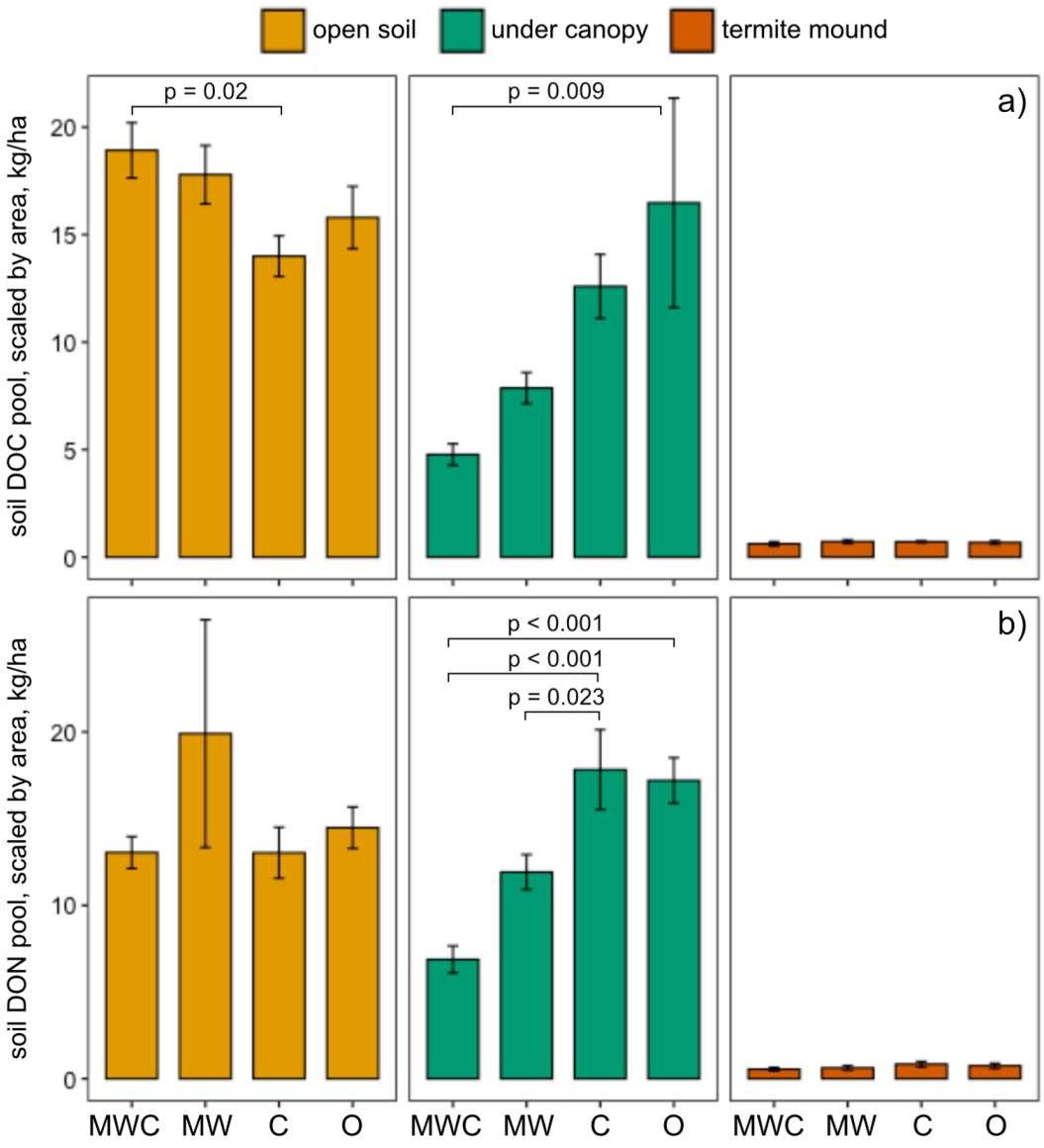

Figure 4.5: Mean total pool sizes of each landscape feature, within each herbivore treatment for a) soil DOC and b) soil DON. Error bars indicate one standard error. Significant pairwise comparisons of pool size within each feature, across treatments, indicated with brackets and the associated p-value.

4.5.4 Plot-level pools: total DOC, DON, MBC, and MBN

Once the above feature-level pools were summed to calculate plot-level total pools of DOC, DON, MBC, and MBN, none differed significantly across herbivore treatments

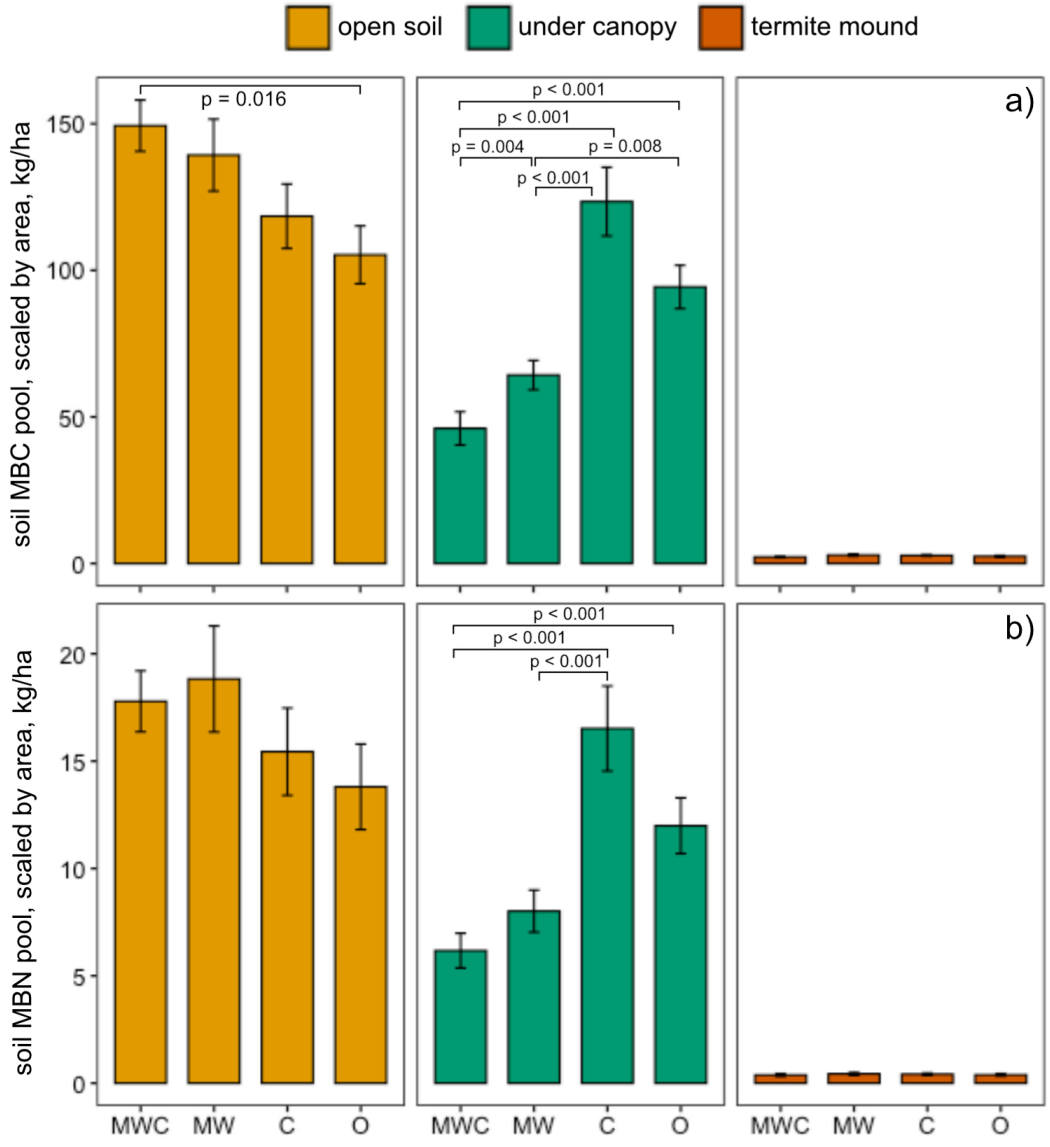

Figure 4.6: Mean total pool sizes of each landscape feature, within each herbivore treatment for a) soil microbial biomass carbon and b) soil microbial biomass nitrogen. Error bars indicate one standard error. Significant pairwise comparisons of pool size within each feature, across treatments, indicated with brackets and the associated p-value.

(table 4). However, there were several (non-significant) trends. Total DOC pool size generally increased as herbivore density decreased (Fig. 4.7a). Total DON pool size was generally smallest in MWC plots as compared to MW, C, and 0 (Fig. 4.7b). Again,

while the difference was not significant, the significantly larger under-canopy pools of MBC and MBN in cattle-only plots resulted in slightly larger total MBC and MBN pools in cattle-only plots compared to those in all herbivore plots, cattle-only plots, and total exclusion plots (Fig. 4.8a, b).

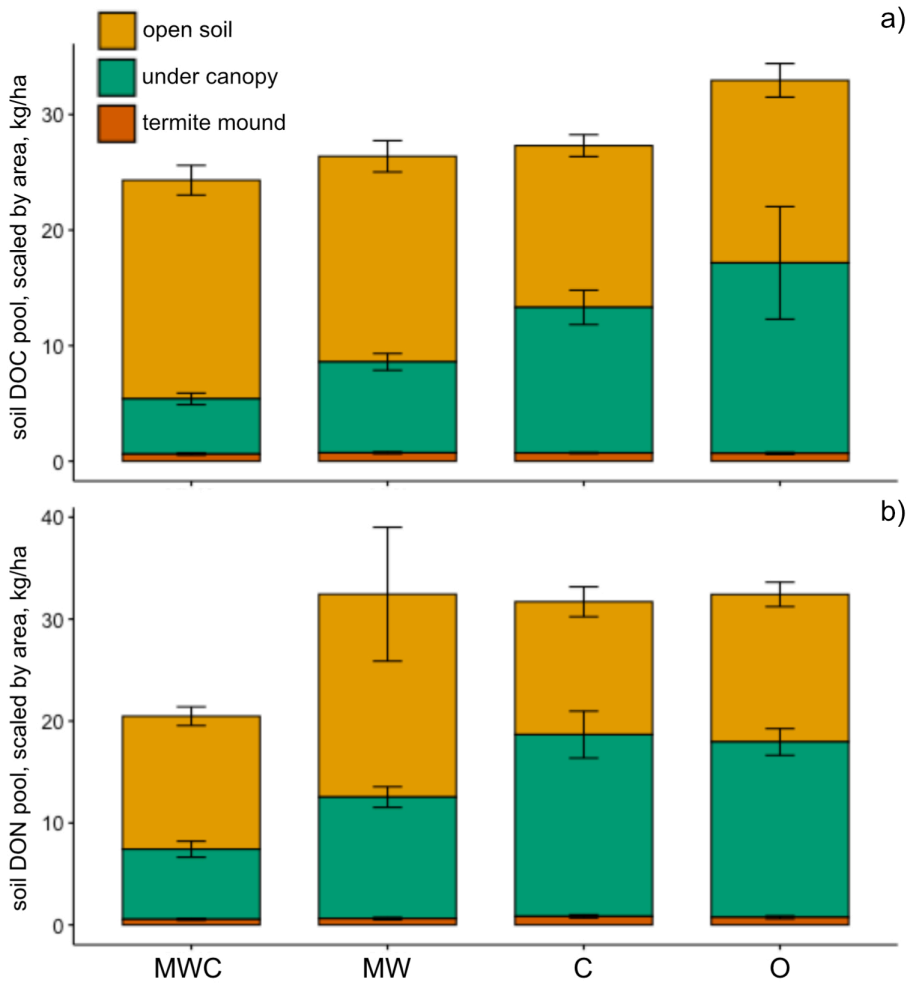

Figure 4.7: Total mean pool sizes of a) soil DOC and b) DON across herbivore treatments, calculated using the proportional area of each landscape feature in each treatment. Error bars indicate one standard error, for each component landscape feature within each treatment.

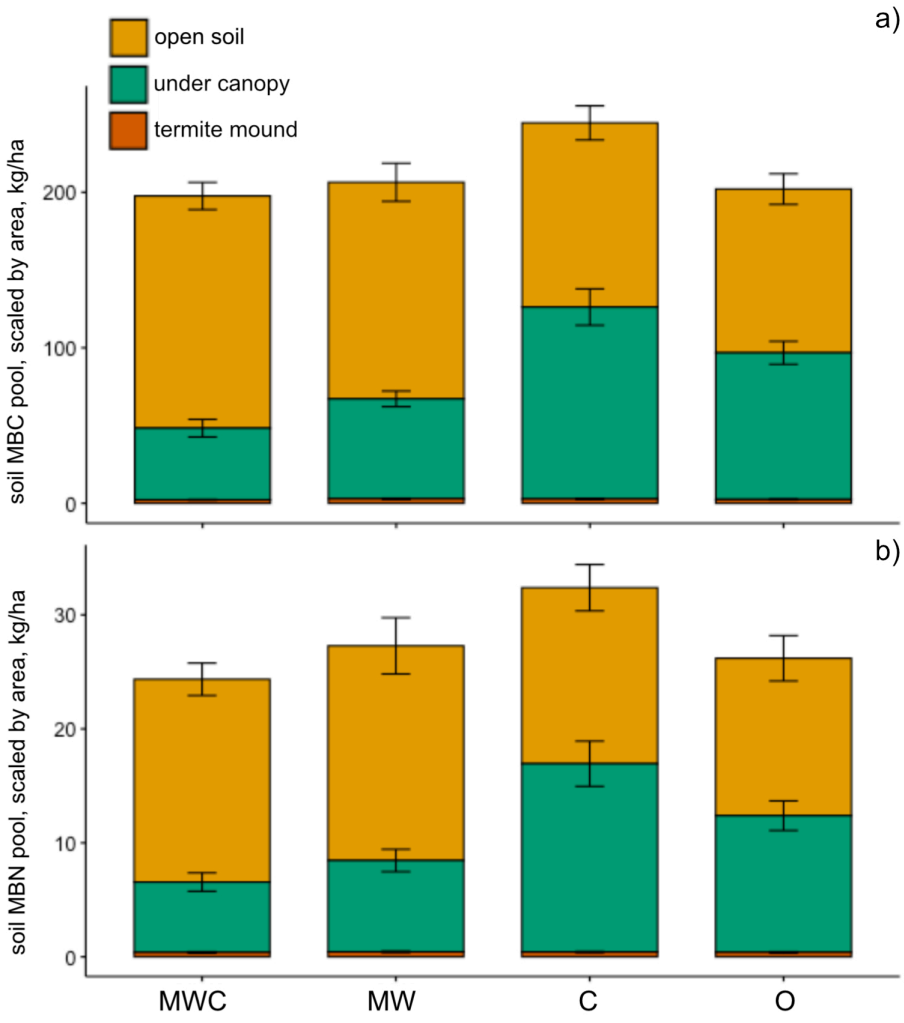

Figure 4.8: Total mean pool sizes of a) soil microbial biomass carbon and b) nitrogen across herbivore treatments, calculated using the proportional area of each landscape feature in each treatment. Error bars indicate one standard error, for each component landscape feature within each treatment.

4.5.5 Power analysis

While none of the linear mixed effects models analyzing total, plot-level pools of DOC, DON, MBC, and MBN revealed significant effects of herbivore treatment, we were able to further explore the non-significant trends we observed in the data using power analyses. Power analysis of the linear model for total plot-level DOC revealed that, with a sample size of nine or more (but at least more than six) KLEE replicates, we likely

would have detected a significantly larger soil DOC pool in the O plots as compared to the MWC plots (Fig. 4.9a). Similarly, with a sample size of at least six KLEE replicates or more, we likely would have detected significantly larger soil DON pools in MW, C, and O as compared to the MWC plots (Fig. 4.9b). The trends we observed for total MBC and MBN pools were also supported by power analyses. In both cases, with a sample size of 12 or more (but at least more than nine) KLEE replicates, we likely would have detected larger pools in C plots as compared to MWC plots (Fig. 4.9c, d).

4.6 Discussion

4.6.1 Microbial carbon mineralization rates

Soil microbial carbon mineralization, as measured by rate of respiration, varied significantly across the three years of soil sampling that comprised our incubations. As described in the methods section, we did not originally intend to study interannual effects on soil microbial respiration; however, given the historic drought in the middle of our three sampling years, we chose to include year that samples were collected as a fixed effect in our analyses in anticipation of likely climatic impacts on soil DOC and DON availability, as well as microbial biomass.

We saw significant effects of year on soil microbial respiration, with soils collected in 2015 and 2018 having higher rates of respiration than those collected in 2016, the start of the multi-year drought. This is to be expected, as drought depresses soil microbial decomposition rates, and thus their rates of carbon mineralization and subsequent respiration (Munjonji et al. 2020). Similarly, respiration rates were higher in 2018 than in 2016 as well, likely due to the fact that 2018 was a particularly wet year, immediately post-drought; such a wet year after a long drought likely resulted in rainfall-induced in-

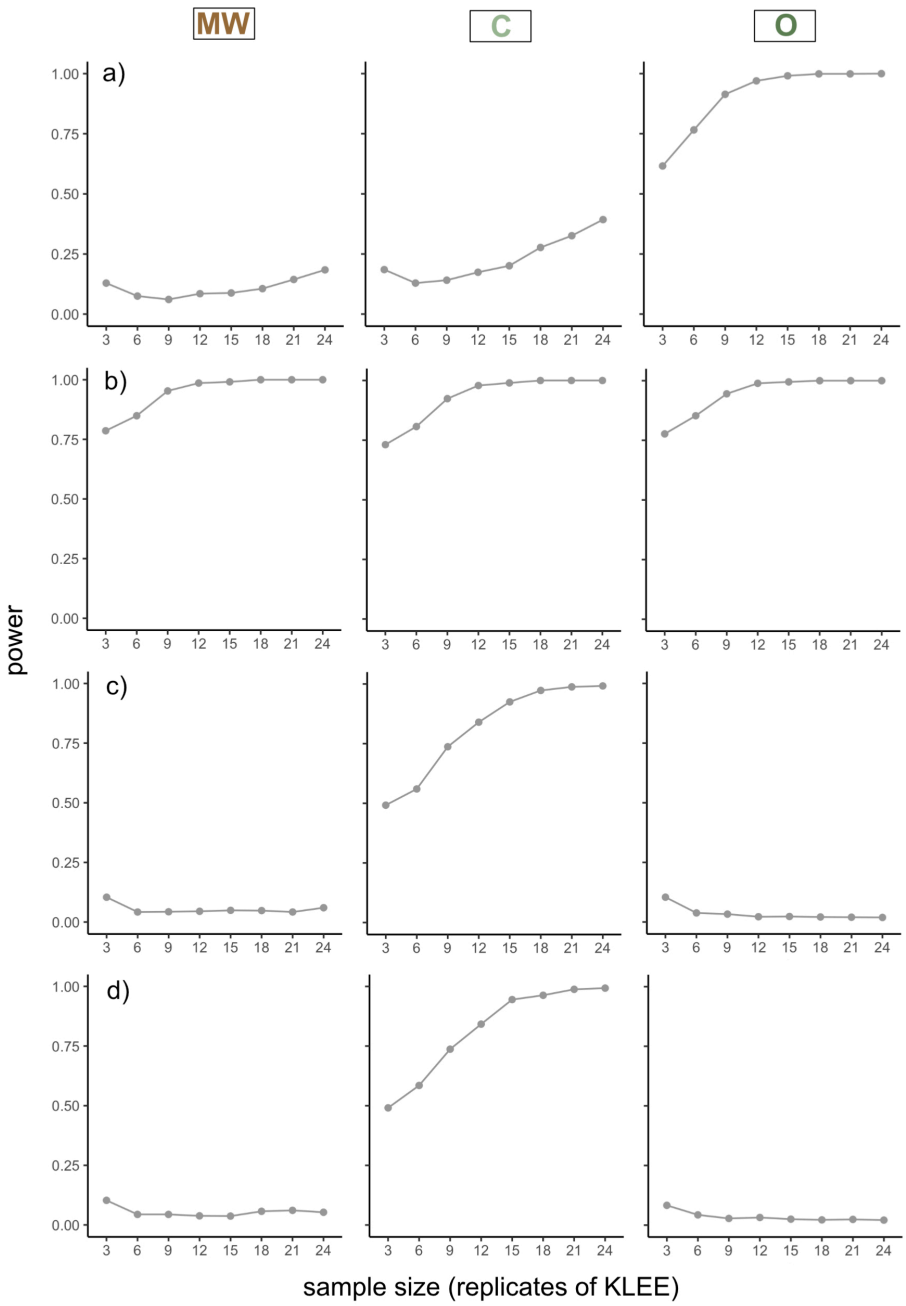

Figure 4.9: Power analysis of each linear mixed effects model comparing total pool sizes of each response variable across herbivore treatments (MWC as control): a) soil DOC, b) soil DON, c) soil microbial biomass carbon, and d) soil microbial biomass nitrogen. We chose 80% power as indicative of a likely rejection of the null (that there is not difference in pool size across treatments) for each analysis.

creases in microbial biomass, and pulses of microbial activity and respiration after the (more frequent and sustained) rainfall events (or the Birch effect) from this savanna's drought-adapted microbial community (Leizeaga et al. 2020).

By splitting our analyses by year, we were able to examine year-specific microbial respiration rates and disentangle climatic effects from those of grazing and landscape feature identity. In 2015, for example, there was no significant effect of landscape feature on respiration. Herbivore treatment was the driver of differences in mineralization, with soils from MW exhibiting lower rates of soil microbial respiration than soils in other treatments (though only significantly less so than soils in C plots) (Fig. 4.3). We hypothesize that a relative lack of inputs in MW may have spurred this result. Soils in O plots have aboveground primary productivity inputs as it is not exported by large herbivores, remaining as a resource within the plots; soils in C and MWC plots have inputs of large quantities of nutrient-rich dung over 3-4 days several times a year. Indeed, our sampling period in 2015 took place immediately after cattle grazing treatments in C and MWC. It is possible that sampling during this time period captured the relatively short-term positive effects of dung deposition (Augustine and McNaughton 2006), leaving MW as the only treatment without substantial inputs to the soil.

Conversely, soils collected in 2016 (the first year of the drought) exhibited no effect of herbivore treatment on respiration rates (Fig. 4.3); it is likely that all four treatments were equally parched by the extremely dry ambient conditions (Munjonji et al. 2020). There were, however, significant effects of landscape feature on respiration rates, with the highest rates of microbial respiration coming from soils that were sampled from under tree canopies. This aligns with what we know about soil characteristics beneath *A. drepanolobium* in this experiment and other leguminous trees in Sub-Saharan African savannas. Not only is there higher organic matter input through litterfall, and shade which positively impacts microbial activity, there is also enrichment of nitrogen through

fixation (Belsky et al. 1989). The second fastest rate of soil respiration was from open soil patches, and the lowest from soils collected from active termite mounds. This result corresponds with the soils analyses from this same year, wherein soil microbial biomass was lowest on termite mounds and highest under tree canopies (Fig. 4.4c, d).

In 2018, both treatment and feature significantly affected soil respiration rate (Fig. 4.3). The rate of soil respiration was highest in total exclusion plots (O). While we do not have yearly soil DOC, DON, and microbial biomass data to assess this, we hypothesize that a post-drought proliferation in primary productivity led to an influx of organic litter into the soil in O; the abundance of soil moisture, in addition to these resources, would have prompted increases in microbial biomass and activity (Munjonji et al. 2020). However, once again, landscape feature was the primary driver of soil respiration rate in 2018. There were significantly greater rates of respiration in soils from under trees as compared to open soil or termite mounds, again likely because soils beneath canopies are enriched in carbon and nitrogen. This result again correlates with our soil extraction results from two years previously, that soils under canopies have elevated microbial communities compared to non-canopy soils (Fig. 4.4c, d).

For open soil and termite mound soils, the pattern from 2016's incubation flipped in 2018. In 2018, soils from termite mounds had the second-fastest rate of microbial respiration, and soils from open soil patches the lowest (Fig. 4.3). This switch could be drought related. There is evidence that termite mounds increase ecosystem resilience in droughts with mounds serving as hotspots for plant survival, leading to greater nutrient concentrations and soil moisture as compared to non-mound soils (Ashton et al. 2019). Indeed, other studies in this system have suggested that termite mounds mitigate drought as refugia for plants that would otherwise wither, allowing for mounds to be bases for revegetation once droughts end (Bonachela et al. 2015). It is therefore possible that mounds maintain their comparatively small microbial community in droughts due to

their status as plant and nutrient refuges, leading to a relatively large surge in microbial biomass (and activity) in response to rainfall.

4.6.2 Site-specific pools

In this Kenyan savanna ecosystem, the biggest driver of bioavailable soil carbon, nitrogen, and microbial biomass is the identity of the landscape feature where soils are located, not the prevailing large herbivore community composition (either wild or domestic). For example, pools of soil DOC and DON at termite mounds are greater than at background matrix soil (e.g. our "open soil") (Fig. 4.4a, b), which is consistent with previous research at this site demonstrating that mounds are enriched in carbon and nitrogen (Brody et al. 2010).

This pattern is logical, when considering the role that termites play in this and other ecosystems. Termites import plant and woody detritus to their mounds (Kihara, Martius, and Bationo 2015), where it is broken down by the termites themselves and their obligate fungal mutualists (Vesala et al. 2017). In addition, termites digest organic matter into stable aggregates, which tend to be protected from soil microbial decomposition (Jouquet et al. 2016; Kihara et al. 2015; Six et al. 2002). For labile soil carbon, these two interacting factors likely lead to a buildup at termite mounds, resulting in a larger net pool: first, the elevated input of organic matter to the soil on termite mounds, and second, the slow rate of soil carbon mineralization there. Soil DON is higher at mounds compared to open soil as well, likely because of both the nitrogen-fixation abilities of termites' gut microbiomes (Sapoutzis et al. 2016) and the concentration of nitrogen-rich nodules on the fungal combs these termites grow within their mounds (Nobre, Lefèvre, and Aanen 2010). These factors together result in termite mounds serving as hotspots for nutrient enrichment in the savanna, including bioavailable carbon and nitrogen.

The relatively slow rate of microbial mineralization at termite mounds in 2016 (the year these samples were taken) is reflected in the significantly smaller pools of MBC and MBN at termite mounds, as compared to both open soil patches and under tree canopies (Fig. 4.4c, d). While *Odontotermes* spp. cultivate fungal combs in their mounds, and mounds in this ecosystem have distinct and more diverse bacterial communities than surrounding soils (Baker et al. 2020), our study demonstrates that this increase in biodiversity does not translate to an increase in microbial biomass, and instead that biomass is lower on termite mounds than either open soils or beneath tree canopies. This observation is not unprecedented; in Australian clay soils, microbial biomass is negatively correlated with termite mound abundance (Holt 1996). Indeed, in these soils (including vertisols) microbial biomass is positively correlated with soil clay content. Given that termite mounds in KLEE have lower clay content that surrounding soils (Brody et al. 2010), it stands to reason that mounds would have lower microbial biomass. Another possible explanation is competition with termites. While microbes and termites break down litter into different size classes, a relatively high abundance of either limits fresh litter availability for the other (Holt 1996). Lastly, studies of some fungus-farming termites have demonstrated they express antifungal and antibacterial peptides in their saliva, which termites use as a building material and apply on their eggs. Such evidence suggests that fungus-cultivating termites have developed protective defenses against deleterious microbes (Lamberty et al. 2001), which may contribute to lower microbial biomass on mounds.

Site-specific soil DON pools, while higher at termite mounds than in the open soil patches, is significantly higher beneath *A. drepanolobium* canopies than at either open soil patches or termite mounds. This is also a logical pattern in this ecosystem, considering that the trees are able to fix atmospheric nitrogen gas into organic, bioavailable forms thanks to their mutualism with nitrogen-fixing bacteria in their root nodules (de Faria

et al. 1989; Fox-Dobbs et al. 2010). As such the soils beneath the canopies of these trees are enriched in nitrogen, which is otherwise limiting in this tropical savanna. The less dramatic effect of soil DOC enrichment beneath canopies, as compared to the highly elevated pool DON, could be explained by the trees' own litterfall; nutrient-rich leaves contribute to a lower C:N ratio beneath the canopy (Treydte et al. 2007) but still result in an input to the soil of both carbon and nitrogen (Ludwig et al. 2004; Kunhamu, Kumar, and Viswanath 2009).

The pools of MBC and MBN were similarly elevated underneath tree canopies, significantly more so than at open soil patches and termite mounds. There being higher microbial biomass beneath *A. drepanolobium* canopies is likely explained by the high availability of labile carbon and nitrogen, which are necessary for microbial metabolic processes like growth and reproduction (Belsky et al. 1989). In other semi-arid African savannas and in similar grasslands, microbial biomass is positively influenced by existing pools of nutrients as well as dynamic inputs, both of which are present beneath tree canopies (Mlambo, Mwenje, and Nyathi 2007).

4.6.3 Plot-level pools: by feature

Once scaled to their relative area within each plot of the KLEE, the differences in the total size of feature-level pools across the herbivore treatments became clear. For example, there was a greater relative area of open soil patches in the MWC plots compared to the other three herbivore treatments (Fig. 4.2), and thus these plots had a larger pool of soil DOC from open soil, with the difference between MWC and C plots significant (fig. 5a). Similarly, soil DOC pool size beneath tree canopies grow larger with decreasing herbivore density, closely tracking the increase in relative area of canopy coverage from MWC to C and O (Fig. 4.5a). While soil DON plot-level pool sizes at open soil patches

did not seem to be influenced by herbivore treatment, they increased significantly at under-tree patches as relative canopy area increased from MWC to C and O, which again corresponds with *A. drepanolobium*'s role as a nitrogen fixer (Fig. 4.5b). The size of neither soil DOC nor DON pools at termite mounds were significantly influenced by herbivore treatment; considering that the total area covered by active termite mounds was relatively consistent across all KLEE treatments, this is to be expected.

The relative size of the soil MBC pool at open soil patches decreased from MWC to O, reflective of the decreasing proportional area occupied by open soil as herbivory declines (Fig. 4.6a). While they are not significant differences, a similar trend can be seen in MBN pool size as herbivore density decreases (Fig. 4.6b). Similarly, as herbivore density decreases (and proportional canopy cover increases from MWC, to C and O), there is a larger pool of under-canopy MBC and MBN, indicating successively more microbial biomass as tree canopy cover rises.

While the difference between C and O plots in under-canopy microbial biomass is not significantly different, compared to the other three plots the under-canopy pool in C plots seems to be relatively large (Fig. 4.6a, b). This is an interesting possible outcome and implies that the presence of 4x yearly cattle grazing somehow influences plot-level under-canopy microbial biomass differently than just wild herbivore grazing, or no grazing at all. Indeed, at a site in southern Kenya, Belsky et al (1993) observed that microbial biomass was positively correlated with grazing pressure, but that it only differed between canopies and open soil patches in the presence of light grazing from wild herbivores. While grazing in C plots is from solely large domestic herbivores, and not wild ones, it is possible that the relatively light, rotational grazing in those plot enhanced under-canopy microbial biomass similar to Belsky et al. 1993.

4.6.4 Total plot-level pools

Once feature-level pools were summed, we saw no net difference in DOC, DON, and microbial biomass across treatments in KLEE. This is despite major reassemblies of the ecologically distinct landscape features, and subsequent significant differences in each features' pool size within each treatment. While we expected this reassembly of features to result in different sizes of plot-level pools, we instead see relatively even pool sizes across treatment. While unexpected, 21 years of herbivore community manipulation producing no plot-scale changes to labile soil carbon, nitrogen, and microbial biomass is not necessarily unique. In the Great Plains of North America, 74-year exclosures found that grazing's interaction with ambient climate conditions is the biggest driver of effect. In wet years, grazing increases soil organic carbon content, while in drought years the opposite occurs (Derner, Augustine, and Frank 2019).

It is also possible that the prevailing grass community in KLEE is responsible for the observed evenness in plot-scale soil DOC, DON, and microbial biomass pools. Globally, grazing in tropical grasslands dominated by C4 plants is positively associated with soil organic carbon content (McSherry and Ritchie 2013; Abdalla et al. 2018), as compared to the opposite in C3 grasslands. C4 grasses compensate for grazing by shuttling resources belowground, resulting in more fine root biomass growth. Grazing also stimulates root exudation of carbon and nitrogen compounds to the rhizosphere where they support microbial communities. Enhanced allocation of grasses' resources to their root systems also results in faster root turnover, creating greater inputs of organic root detritus to the surrounding soil and microbial decomposers (Wilson et al. 2018). Thus, moderate to heavy grazing in C4-dominated grasslands can lead to enrichment of soil organic carbon, nitrogen, and larger and more active soil microbial communities (McSherry and Ritchie 2013). In the Serengeti (another East African C4-dominated grassland) grazing has also

been shown to enhance soil carbon, nitrogen, and microbial biomass and activity; this pattern is perhaps also due to large herbivore dung deposition on the soil surface, where it is quickly incorporated as bioavailable nutrients belowground by macroinvertebrates like dung beetles (Ruess and McNaughton 1987).

While we did not find herbivore treatment effects on sampling site-specific pools of DOC, DON, or microbial biomass (figs. 7, 8), it is possible that grazing stimulated grasses' organic inputs to the soils in plots where large herbivores were present in insignificantly detectable quantities. This remains a hypothesis, but could help explain the lack of net difference in total pool sizes across herbivore treatments: the positive effect of grazing in herbivore plots dulling the positive effect of greater proportional tree canopy coverage in exclusion plots. A recent study from KLEE indicates that grazing by wild herbivores, or wild herbivores and cattle, resulted in higher total carbon and nitrogen pools as compared to exclusion or cattle-only plots, irrespective of whether samples were taken from open soil or beneath canopies (Sitters et al. 2020). While our study examines only the most labile fraction of organic carbon, it is worth further exploring whether grazing-induced increases in soil nutrient inputs by C4 grasses compensates for the relatively low relative coverage of tree canopies in plots where grazing is allowed, and whether grazer identity (wild or domestic) effects bioavailable nutrient pools differently.

4.6.5 Power analysis

While we acknowledge and discuss the non-significant effect of herbivory-induced landscape reassembly on total pools of DOC, DON, and microbial biomass above, we also sought to explore the patterns in our data that were potentially obscured by low sample size (n = 3 total KLEE replicates).

We had hypothesized that soil DOC would be lowest in MWC and highest in O

plots due to the reassembly of landscape features, decreasing relative open soil coverage and increasing relative tree canopy coverage as herbivore density decreased. While the effect of treatment was not significant, we did see a small trend indicating an increase in total soil DOC pool size from MWC to O. A power analysis revealed that increasing the sample size of the KLEE from three to nine would give us <80% power to detect a significant effect of treatment on soil DOC pool size, specifically O compared to MWC (Fig. 4.9a). Such a result indicates that the observed bump in soil DOC pool size in O is possibly a real trend, and that more statistical power could have revealed it. This result is generally what we would have expected; canopy coverage is associated with greater quantities of nutrients, carbon, and biological soil activity in tropical savannas, given elevated litter inputs, root exudates, nitrogen fixation, and moisture levels from hydraulic lift and shade (Thomas et al. 2018). Increased proportional canopy cover as a result of large herbivore exclusion would thus result in greater plot-level pool sizes of the soil constituents measured here.

We had also hypothesized that soil DON would be lowest in MWC and highest in O plots, again due to the reassembly of landscape features, specifically an increase in tree canopy cover. While the effect of treatment on soil DON pool size was not significant, the power analysis indicated that increasing the KLEE sample size from three to six or more would likely give us the power to detect significant effects of treatment on soil DON pool size, specifically greater pools in MW, C, and O as compared to MWC (Fig. 4.9b). Given that there is greater relative tree canopy coverage in every other treatment plot, and that soils beneath leguminous tree canopies are enriched in nitrogen (Brody et al. 2010; Piñeiro et al. 2010; Thomas et al. 2018), it follows that MWC would have the smallest overall DON pool. While pastures in other regions can be prone to significant loss of DON through leaching, there is currently little empirical research on DON leaching rates from Sub-Saharan African pastures and grazed savannas. In addition, grazing and

inputs of urine from grazers can accelerate nitrogen cycling, thus disallowing buildup of a DON pool (Carbonell et al. 2021). In any case, this is an interesting pattern considering the trend in Sitters et al. (2020), who observed larger total nitrogen pools in MWC and MW plots.

We hypothesized that, similar to soil DOC and DON, we would observe larger soil microbial biomass pools as herbivore density decreased. Instead, we observed that C plots had slightly, non-significant elevated microbial biomass pools compared to the other three treatments. Power analysis demonstrated that increasing the sample size of the KLEE from three to 12 would likely give us the power to detect a significant effect of treatment on soil microbial biomass pool size, specifically in the C plots compared to MWC (Fig. 4.9c, d). Such an effect has been observed elsewhere. Studies in clay-rich savannas in South Africa have shown that rotational grazing of domestic livestock can stimulate microbial biomass and activity due to a combination of large-scale nutrient deposition (dung, urea) and the 'resting time' that rotational grazing affords wherein soil and vegetation recover and deposited nutrients are highly available. (Amelung et al. 2017).Thus it is possible that the slight increase in soil microbial biomass pools in C plots observed here is a real trend, the result of light rotational grazing from domestic cattle.

4.7 Conclusions

In this study, we demonstrate that changes in large herbivore community composition that drive reassembly of key landscape features percolate to control the size of bioavailable soil carbon and nitrogen pools within each landscape feature, across herbivore treatments. We saw significant differences in soil DOC, DON, and microbial biomass pool sizes at each landscape feature. When scaled to reflect the proportional area each feature occupies in each treatment, feature-level pool sizes differed significantly across herbivore treatments.

While the total pools of DOC, DON, and microbial biomass did not differ significantly across treatments once feature-level pools were summed, we saw patterns that may have been detectable with a larger number of KLEE replicates: an increase in DOC when all large herbivores are excluded, a decrease in DON when all large herbivores are present, and an increase in microbial biomass in plots with only rotational cattle grazing. Given the different functional roles that each feature plays, these changes to landscape assembly could percolate to larger-level changes in nutrient cycling and microbial activity.

We also clearly demonstrated that the biggest driver of soil microbial activity is landscape feature; specifically, microbial respiration is always higher beneath *A. drepanolobium* tree canopies than at open soil patches or on termite mounds. Given our analyses across years of soil collection, we determined that larger scale, treatment-level effects on soil microbial activity are likely mediated by drought conditions: drought years minimized differences in herbivore treatment effects, and plots without any large herbivores experienced high microbial activity post-drought. We also demonstrated that drought may reverse patterns in microbial activity between open soil patches and termite mounds, perhaps due to termite mounds' status as refugia for plants and nutrients in drought years.

While our initial hypotheses were not fully supported by these data, what is clear is that large herbivores interact with savanna function via their significant effects on savanna structure. It is also possible that different kinds of large herbivore (wild or domestic) have different plot-scale effects on nutrient cycling and microbial activity, due to the ways in which their herbivory occurs (continuous or rotational). All told, our study reveals nuance in how large herbivores impact savanna structure, and function. Untangling these nuances will be essential to understanding how wild large herbivore loss, and their possible spatial replacement with domestic herbivores, impacts savanna carbon storage and cycling in the future.

4.8 Acknowledgements and Author Contributions

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Truman Young[1] constructed KLEE in 1995 and served as its lead PI until 2018. Eliz-

abeth Forbes[2] conceived of the original project aim and designs, with substantial input from TY, Hillary Young[2], and Josh Schimel[2]. TY and HY consulted on sample collection scheme. EF collected, prepared, and shipped the samples. EF conducted the three soil incubations and data collections with assistance from JS. EF and Dana Moore[3] conducted the soil extractions with assistance from JS. EF conducted all statistical analyses and interpretations, with input from HY, JS, and TY. EF wrote the manuscript with substantial input from DM, JS, HY, and TY.

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Chapter 5

Thesis discussion

In this chapter I will briefly synthesize and integrate the three preceding research chapters, and summarize the challenges, questions, and results from each. I will identify the common threads across the chapters and knit their findings together into a cohesive story about large herbivore loss, and its implications for landscape-scale carbon cycling.

This dissertation specifically explores how loss of large herbivores influences carbon cycling (and thus climate) (Pörtner et al. 2021). Both large wildlife community shifts and climate change are advancing rapidly; identifying and quantifying the ways in which large wildlife loss impacts climate on local scales will strengthen our ability to understand how the two processes interact on a global scale.

5.1 Chapter 1 – Review of existing exclosure experiments to inform practical studies of large herbivore 'loss' on ecosystem functions

In this first chapter (Forbes et al. 2019), we asked specifically how large, wild herbivores influence ecosystem function on a global scale, though a comprehensive literature review. While it is widely understood that these consumers influence ecosystem function, it is challenging to observe empirically, and especially experimentally. Ecosystem function as a term (much less individual functions of interest) has not been easily or consistently defined. In addition, functions, once defined, are frequently measured using a range of methods and metrics, making comparisons across studies difficult. In addition, experiments examining large wild herbivores and their loss on ecosystem function are not distributed evenly across ecosystems or latitudes. Even where data exists, teasing apart context-specific outcomes of herbivores (and the experimental loss of herbivores) on a given function is difficult, given the many direct and indirect ways that large herbivores affect ecosystem structure and function.

We explored the existing literature on three fronts. First, we conducted a quantitative literature review to identify the five ecosystem functions that have been studied most frequently using large-scale (>25 m2), large (>5 kg) wild herbivore exclosure experiments as a proxy for large wild herbivore decline or loss. Second, we reviewed the literature qualitatively for these five functions, assessing the overall effects of wild herbivore exclusion on each. We found that the effects of large herbivore exclusion on any function are dependent not only on the ecosystem and latitude in which the experimental loss is occurring, but also on seasonality (e.g. reindeer exclusion decreasing tundra nutrient cycling rates in summer months, but increasing it in winter months); herbivore density

(e.g. the proliferation, rather than decline, of some large herbivores in some locations, like moose in North American boreal forests); and herbivore identity (e.g. domestic herbivores, which were examined briefly in this chapter but which spatially replace or coexist with wild large herbivores in ecosystems worldwide).

Third, our quantitative case study revealed similarly context-dependent outcomes of wild herbivore exclusion on carbon storage and cycling, with effects of large wild herbivore exclusion dependent on characteristics like ecosystem productivity and herbivore size (elephants knock down trees, whereas smaller large herbivores browse on tree seedlings). Adding to the complexity, metrics of carbon cycling can describe either pools of stored carbon themselves or processes that cycle carbon between pools. Pools can be split further into ecologically meaningful fractions like aboveground biomass carbon versus belowground soil carbon, or recalcitrant, mineral-adsorbed soil carbon versus biologically active soil carbon that is consumed and respired to the atmosphere quickly. There are also multiple ways to measure rates of carbon cycling, like the flux of carbon between the soil to the atmosphere via biological respiration and physical soil cracking, or the drawdown of atmospheric CO^2 by the ecosystems' photosynthesizing organisms.

We found that large wild herbivore exclusion results in variable outcomes on carbon dynamics, with effect sizes ranging from negative to positive depending on which metric is being measured. It is crucial to place the metrics one is measuring within the larger context of the carbon cycle, and interpret the results within that context. We concluded that to gain a synthetic understanding of the effects of large herbivores (and their experimental loss) on ecosystem functions, it is essential to clearly identify the metrics with which a function will be measured. We suggest that a network approach in data collection is needed at existing and new exclosures across the globe to optimize dataset comparability (as described in Chapter 1, inspired by the infrastructure of NutNet). A network of data collected with identical methods will allow ecologists to disentangle context-dependencies

of large herbivores' effects on functions, and make it easier to predict outcomes of large herbivore decline or loss within given ecological parameters.

5.2 Chapter 2 – Identifying when tools, and thus information, are missing

In Chapter 2, we focused on networks as a tool to monitor carbon cycling in experimental contexts. Our recommendation in Chapter 1, that ecologists better sync their research methods in large-scale long term exclosure experiments to create comparable datasets on functionality across ecosystems, requires that tools and methods to do so are accessible. In this chapter, we specifically explored current methods available for monitoring soil carbon flux, or the cycling of CO^2 between the soil and the atmosphere from biotic soil respiration and abiotic factors like temperature, pressure, and soil texture (DeCarlo and Caylor 2020). Soil carbon flux is an essential input into an ecosystem's carbon budget, and reflective of its biologically active soil carbon pool. Soil carbon flux varies spatially, on the scale of centimeters to meters, and reflecting the ecological heterogeneity of an ecosystem; it also varies through time over days, seasons, and years.

Therefore it is essential to measure soil carbon flux on fine spatial and temporal scales, which requires autonomous sensors that can collect data continuously over long periods without an operator. However, existing autonomous tools sacrifice either spatial or temporal resolution, or are too expensive to install on a fine scale across a large scale experiment like those reviewed in Chapter 1. This kind of barrier (e.g. lack of funds) leads to global biases in the distribution studies' on large herbivore effects on ecosystem functions, like those observed in Chapter 1. We therefore developed an inexpensive, do-it-yourself (DIY) soil carbon flux sensor to make the collection of high-resolution soil

carbon flux datasets more broadly accessible.

We collaborated across disciplines (ecology, geography, engineering, and computer science) to design and construct a network of autonomous carbon flux sensing chambers. We installed the network in a large-scale, long-term large herbivore exclosure, at ecologically distinct landscape features within each herbivore treatment. The exclosure experiment is located in a savanna in central Kenya, in which continuous (i.e. 24hr) soil carbon data collection done by hand, and access during the rainy season, is logistically infeasible. We successfully collected 2.5 months of hourly data, comprising the end of a dry season and the start of a rainy season, creating the largest and most fine-scale (across both space and time) soil carbon flux dataset to date in this ecosystem and potentially the tropics. We demonstrated that DIY technologies can and should be explored for collecting large-scale, long-term, computationally big datasets that describe functions like carbon cycling in understudied locations, and that are comparable across systems.

5.3 Chapter 3 – Methods in ecosystems science to examine how large herbivore loss influences community structure and carbon dynamics

In Chapter 3, we further explored carbon cycling in the large herbivore exclosure experiment introduced in Chapter 2: how do large herbivores, both wild and domestic, influence carbon cycling at the landscape scale in a Kenyan savanna? We explored carbon cycling within the same large-scale, 21-year large herbivore exclosure experiment: the Kenya Long-term Exclosure Experiment, or KLEE. A rich community of large herbivores (including functionally unique megaherbivores like elephants) persist there, and a millennia-long history of pastoralism in the region suggest domestic cattle herds have

long coexisted with wildlife (Boles et al. 2019). We explored carbon storage and cycling in four of KLEE's experimental treatment plots: those that are unfenced and allow both wild large herbivores and domestic cattle, those that only allow wild large herbivores, those that only allow cattle, and those fully-fenced that do not allow any large herbivores (wild or domestic).

Within each plot, we were interested in how large herbivores indirectly influence savanna carbon cycling via reassembly of two ecologically important landscape features: Acacia drepanolobium trees and termite mounds, in addition to the background matrix of the savanna. We determined that each feature within each herbivore treatment had a different size net pool of bioavailable carbon, nitrogen, and microbial biomass. We also found that landscape feature was the primary driver of the rate of soil microbial activity, with soils beneath tree canopies always respiring at faster rates than soils from the other two features. Herbivore treatment was also a driver of microbial activity, though the effect direction and size was heavily influenced by ambient drought conditions. These findings underscore our driving motivation in Chapter 2, illustrating that it is necessary to measure metrics of carbon cycling at the spatial resolution of ecologically important features, and a temporal resolution that captures change in ambient climate conditions.

Because we saw significant differences in how carbon cycles and is stored in each landscape feature, we asked how changes to the relative coverage of each feature on the plot scale would ultimately result in different total pool sizes in each herbivore treatment. However, these differences did not ultimately sum to significant differences in pool sizes at the treatment scale. While changes to large-bodied herbivore community composition, density, and identity may not be creating the distinct changes in pool sizes we hypothesized, they do clearly drive consistent patterns of landscape reassembly. These results supplement existing research in KLEE that found significant differences in pool sizes of total carbon across the herbivore treatments. This study indicates that despite the total

carbon storage in KLEE changing in response to long-term herbivore community manipulation, the pool of carbon that is most readily available for uptake and cycling appears to remain consistent regardless of the degree of large herbivore loss.

5.4 Limitations of this research

This dissertation includes a conceptual approach on the global scale, but narrows its empirical scope significantly to specific landscape features within a single large herbivore exclosure experiment. There are thus limitations to the conclusions that can be drawn. For example, the research site occurs on a relatively fertile soil type (vertisol) with a particular vegetation (monodominant acacia wooded grassland) that may not be generalize-able locally, regionally, or globally.

In addition, we were unable to statistically confirm the treatment-level trends we observed in total DOC, DON, and microbial biomass pools across the four herbivore treatments studied here. This result could be real, as discussed both in Chapter 3 and briefly above; it also could be an artefact of KLEE's experimental design, which constrains statistical analyses to a sample size of three. Such a constraint could be obscuring other context dependencies that muddied a clear result, a possibility we explored in power analyses.

Considering the drought's impacts on soil microbial activity in 2016, it is also clear that year effects can be profound (Werner et al. 2020) and that data collected over longer, more continuous time periods (e.g. beyond the dry season) would better describe within- and across-year drivers of change in carbon cycling. The conclusion we can draw from these data, and that underscores those we drew in Chapter 1, is that context dependencies (time, season, weather, climate, ecosystem structure, herbivore identity, etc.) matter in empirically assessing how large herbivores and their loss influence ecosystem functions

like carbon cycling. Identifying these context dependencies remains a challenge.

5.5 Contributions of this research

Chapters 1 and 3 of this dissertation underscore that context dependencies are not simply complications of determining an ecosystem's functional response to large herbivore loss, but important features of those functions in their own right. Given the context dependencies revealed in chapter one on a global scale, and on a local scale in chapter three, the innovation of a novel instrument that can monitor carbon flux at fine resolutions in Chapter 2 is an important contribution to this research. Our fluxbots, and similar inexpensive DIY tools, collect datasets large enough for ecologists to see regular patterns that identify environmental factors that modulate functions. For example, our fluxbots successfully captured cyclical patterns of carbon flux over multiple 24hr periods across a large and heterogeneous spatial extent; we identified that ambient temperature and pressure drive cyclical patterns in flux rate. Once identified within the data, these contexts can be used to characterize 'normal' soil carbon flux rates, and subsequently, deviations from normal. Interdisciplinary collaborations like those we pursued in Chapter 2 to design our fluxbots are therefore an opportunity to increase our data collection and interpretation capacities at large-scale, long-term large herbivore exclosure experiments.

Chapter 3 also demonstrates that the effects of large herbivore loss (and, possible replacement with domestic cattle) on carbon cycling are likely indirect, and predicated on those herbivores' direct effects on landscape assembly and structure. We show that the biggest driver of variability in soil DOC, DON, microbial biomass, and microbial activity on a plot-level is the identity of the landscape feature from which a sample was taken. Given the effects that large herbivores have on ecosystem structure via consumption, trampling, and nutrient deposition worldwide, it is likely that our observation (reassembly

of landscape carbon cycling heterogeneity based on reassembly of characteristic landscape features) is not isolated to this Kenyan savanna ecosystem.

5.6 Conclusion

As ecologists in the Anthropocene, our observations are inherently contained in the dynamism of a changing world, amid processes like anthropogenic biodiversity loss and climate change. We must continue to explore the interactions between these two distinctly modern phenomena to better predict how local-level large herbivore loss, and their replacement with domestic analogs, will impact the carbon cycle. We need to clearly define and measure ecosystem functions like carbon cycling consistently across experimental contexts, particularly in large herbivore exclosure experiments to assess their losses' possible impacts. We need to collect high-resolution data, across space and time, to identify the context dependencies (landscape features; climatic events; diurnal patterns; etc.) that can obscure or interact with the effects of wildlife loss on carbon storage and emissions. More detailed local-scale understanding of these large herbivore community reassemblies, and how carbon storage and emissions change as a result, can feed into more nuanced (and accurate) predictions of large herbivore loss's impacts on carbon budgets and climate at the global scale.

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Appendix A

Appendices

A.1 Chapter 1

A.1.1 Chapter 1 Appendix 1: Ecosystem function

While ecosystem function is a very commonly used term, there is little agreement on its definition. The broadest definitions are capable of encompassing nearly every ecosystem feature (Srivastava and Vellend 2005): commonly, however, ecosystem functions are defined as either (or both) the pools of material in an ecosystem (nutrients, biomass, genetic material, etc.) and the fluxes of those pools (e.g. including cycles of material like carbon and regimes like fire; a subset often called 'ecosystem processes'), and are inclusive of functions that are beneficial to humans (ecosystem services) (Franklin et al. 1981, Lamont 1995, de Groot et al. 2002, Hooper et al. 2005). Given the generality and lack of consensus on a definition, the task of synthesizing herbivore loss's impacts across ecosystem functions requires careful consideration of whether a given response qualifies as a function.

For our analyses we choose to use a broad definition of ecosystem function, and

prepared a list of potential functions from syntheses published on other taxa in the biodiversity-ecosystem function literature. Specifically, we explored recent published reviews and meta-analyses on this topic (Cardinale et al. 2009, Lefcheck et al. 2014, Hooper et al. 2012, Solivares et al. 2016, Delgado-Baquerizo et al. 2016) and judged which of the functions explored were potentially applicable to large, mammalian herbivores. We also cross-referenced our list with several published by conservation organizations that seek to preserve ecosystem functions (IUCN Millennium Ecosystem Assessment, Maynard et al. 2010). From this effort, we compiled a list of potential functions, adding in several others were likely impacted by large herbivores, based on published studies in both the ecosystems services and ecological cascade literature (Ripple et al. 2014, Malhi et al. 2015, La Notte et al. 2017). In total, we identified 17 ecosystem functions (listed in Appendix 1.2) likely to be impacted by large herbivores.

A.1.2 Chapter 1 Appendix 2: Review methods

To explore the impacts of experimental large herbivore loss via fencing/exclosure on ecosystem functions, we first identified a list of ecosystem functions likely impacted by large herbivores (Fig. A.1). Next, we performed WoS searches for each function on this list. For clarity, we specifically targeted only those studies that used exclusion (fencing) with unfenced controls to experimentally explore large mammalian herbivore loss on ecosystem function. We searched with the following string: "(verte* or mammal* or wildlife) and (graz* or herbiv* or brows*) and (exclud* or exclus* or exclos* or fenc* or extinc* or loss)". For each function's individual search, we also added specialized search terms to the end of this string (list of search terms by metric in Fig. A.1). All searches were conducted between Jan 1, 2018 and May 1, 2018. We then filtered results by the following criteria. We excluded studies on ancient or extinct megafauna, studies

Function	Search terms	Total results	Viable results
Carbon cycling	and (carbon* or CO2 or (aboveground biomass) or (belowground biomass) or methane)	216	22
Nutrient cycling	and (nutrient or eutr* or nitr* or phosph*) and (cycl* or content or stor*)	106	24
Soil quality	and ((soil quality) or compaction)	46	7
Nutrient diffusion	and (nutrient*) and (diffus* or translocat* or translat* or subsid*)	9	0
Seed dispersal	and (seed) and (dispers*)	122	5
Primary productivity	and (primary producti*)	73	11
Plant regeneration	and (germinat* or propagat* or (seed predat*))	176	29
Pollination	and (pollinat*)	37	3
Resistance/resilience	and (resistan* or *resilien* or equilibrium or regime)	250	33
Fire regime maintenance	and (fire regime)	44	3
Raw material provision	and (timber or mineral or (fossil fuel))	71	0
Disease transmission	and (disease) and (transmi* or vector* or spread)	42	6
Pest control	and (pest) and (control or reduc*)	35	1
Food provision	and (bushmeat or meat)	25	0
Water quality	and water and (quality or purif* or filt*)	40	2
Air quality	and air and (quality or purif* or filt*)	1	0
Pharmaceutical/medicinal provision	and (pharma* or medicin*)	16	0

Figure A.1: The 17 ecosystem functions investigated in this review, and the specialized search terms used to identify publications from the large herbivore exclosure literature on each in Web of Science. The total number of returned publications for each function were read and viable publications (those that fulfilled the requirements of our search for each function; appendix 2) were retained for inclusion in the review. Of these 17, after assessment of total results 12 returned viable publications.

that included only invasive herbivores, domestic herbivores, small herbivores (<5kg), non-mammalian herbivores (e.g. geese, swans), aquatic herbivores, and studies with exclosures smaller than 5x5m. A summary of the filtered results for each function are provided (Fig. A.1). For each study the relevant meta data (location, biome, exclosure

size and replicate number, exclosure age, and function measured as response variable) were collected from each study. Studies that collected data on multiple functions were included in each function's data. The data (including publication references) is included in the Data Sources section.

Biomes were determined for each experimental site by recording the reported ecosystem type from each publication, then grouping these into broader biome categories (e.g. savanna, prairie, rangeland = 'grassland'; taiga, birch forest, boreal forest = 'forest'; etc.). Only two (of 177) unique experimental sites did not report a biome description. Ten unique sites were described in multiple ways, or did not objectively indicate which broad biome the system should be categorized; in these cases, we examined the entire methods section to find context clues with which to subjectively decide which biome would be used as the general descriptor (e.g. a mountain watershed site was categorized as a grassland once methods section describing the watershed as a rangeland and with diet selectivity of resident large herbivores being grasses, forbs, and sedges was examined).

A.1.3 Chapter 1 Appendix 3: Case-study on carbon dynamics

To uncover possible mechanisms of variability encountered in our reviews of individual functions, we conducted a meta-analysis of the impacts of experimental large herbivore loss on a single function: the carbon cycle. We read each of the papers identified through our WoS search closely to determine whether they reported data on carbon storage or cycling. To ensure comprehensive data collection, for this function only, we additionally backwards-cited any reviews or meta-analyses that appeared in the WoS search for additional publications. We also reviewed the literature from several known experimental herbivore exclosures that were not identified in the initial search and included data on carbon dynamics when they existed.

For each study included in this dataset, we recorded all metadata described in appendix 2, as well as: metric measured, mean values with and without large herbivores, the standard error of the mean, and average productivity gleaned from Earth Explorer satellite data. If not provided in-text mean values of carbon response variables (and associated standard errors or deviations) were collected from figures using Web Plot Digitizer. If standard deviation from the mean was provided, that was preferentially collected over standard error. If neither SE nor SD were provided, p-values were collected. If p-values were reported as general estimates (e.g. $p < 0.05$), conservative values were recorded (e.g. $p = 0.05$). Studies that reported data on more than one metric of carbon dynamics were included multiple times, once for each metric.

Studies that did not report SE, SD, or p-value were discarded; studies that reported a $p > 0.05$ were also discarded, for lack of ability to make a conservative value estimate; this resulted in the loss of only four total responses (from three publications). If studies reported multiple time points, we consistently collected data from the last-reported time point. If studies reported data from a factorial experiment in which non-native, domestic, or small herbivores were also excluded, we collected data only from those plots excluding large, native herbivores. Several studies reported grouped data (e.g. for aboveground biomass, separated by plant functional group). For these data, means were summed; standard deviations were calculated using reported standard errors, with (n = number of experimental exclosures), then recalculated according to equation 1.

To best ascertain the direction of effect in carbon cycling in response to large herbivore exclusion, we separated all data by metric. We used the R package 'metafor' to calculate Hedge's g (standardized mean difference) and sampling variances for each metric's data. For those studies that provided only p-values or p-value estimates, we calculated Hedge's G and sampling variance according to the code included in the Data Sources section. To aid intuitive visualization of the effects of large herbivore exclusion, we multiplied

Hedge's G by (-1) to demonstrate change in effect size (e.g. a negative standardized mean difference indicates an increase in the metric measured in response to large herbivore exclusion; multiplied by (-1) allows for graphing a positive effect of exclusion on said metric).

For the two components of the carbon dataset (soil carbon and carbon flux) with a large enough sample size, we ran a mixed-effects rma model (in R, package: metafor), first with an omnibus test of parameters (exclosure size, exclosure duration, and mean NDVI as a proxy for productivity), then with reduced models for significant moderators of model variance (soil carbon: none, carbon flux: mean NDVI). To graph these relationships, we again multiplied the effect sizes for each component by (-1) to intuitively demonstrate the change in response to large herbivore exclusion (please see above explanation).

To understand the sources of the variation in functional responses, we examined the sources of variation of large herbivore exclusion on consumption (a direct effect of large herbivores on ecosystems) across exclosures. To do this we isolated aboveground biomass (one metric of the carbon cycle analyzed in this case study), and analyzed it separately (Fig. A.2). We found the variation in the data to be significantly moderated by plot size ($p=0.03$) and mean NDVI ($p<0.01$) of experimental exclosures, accounting for 99.9% of the heterogeneity in effects. Thus, the effects of large herbivore exclusion on aboveground biomass are significantly moderated by plot size and site productivity. It is reasonable to hypothesize that downstream effects on other functions that interact with consumption will also likely be highly variable, thus potentially explaining providing a mechanistic reason for variation observed in other functional responses.

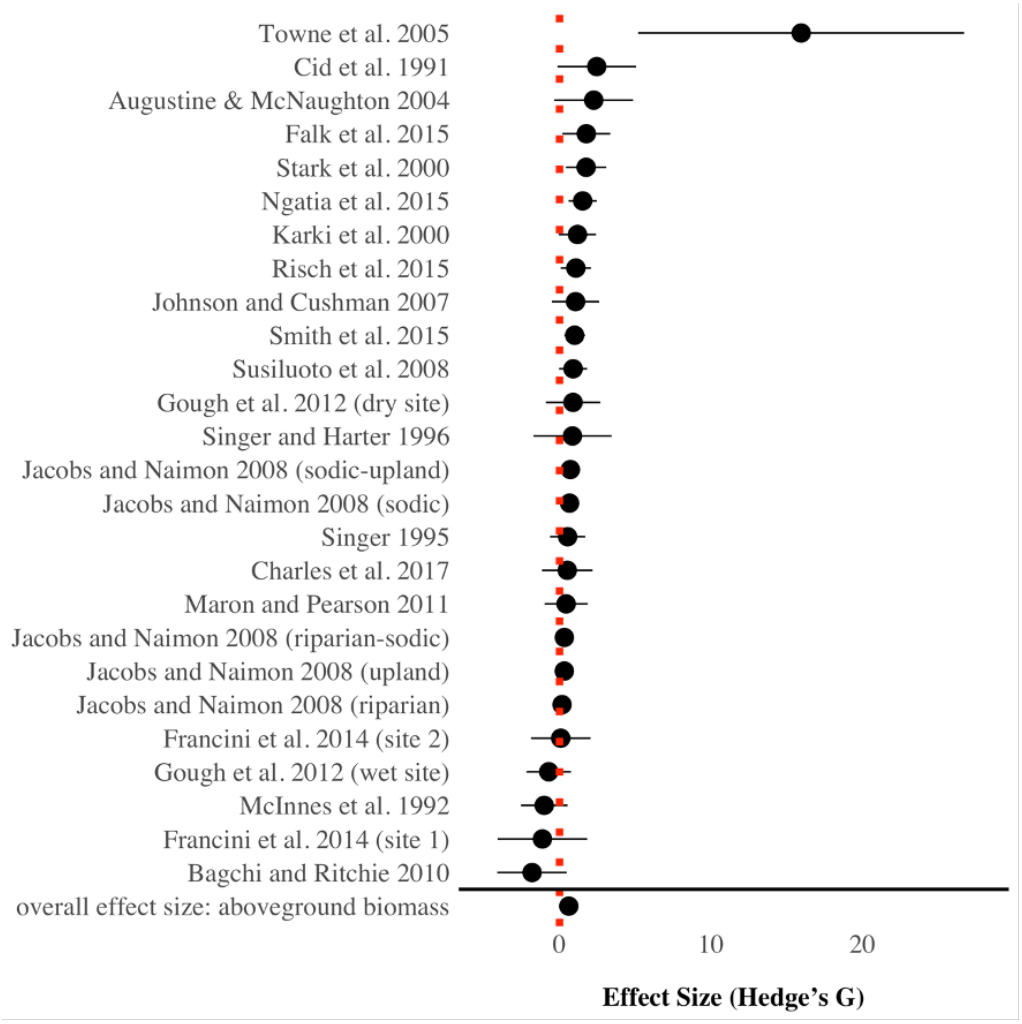

Figure A.2: Effects of large herbivore exclusion on aboveground biomass in 26 functionally independent experimental exclosure sites included in the case-study. There is strong variation in effect, signaling that large herbivore exclusion has variable direct effects on ecosystems (e.g. consumption); high variation in this direct effect may suggest an expectation of variability on subsequent and indirect outcomes on functions.

A.2 Chapter 2

A.2.1 Chamber construction

We fabricated each chamber from two lengths of 5x5" square PVC pipe, cut to 7" in length (chamber "body") and 2.5" (chamber lid). The body and lid were connected with hinges, and the lid was topped with a piece of 1/4"-thick acrylic laser-cut to 5"x5" dimensions, glued in place with air-tight, weather-proof permanent sealant.

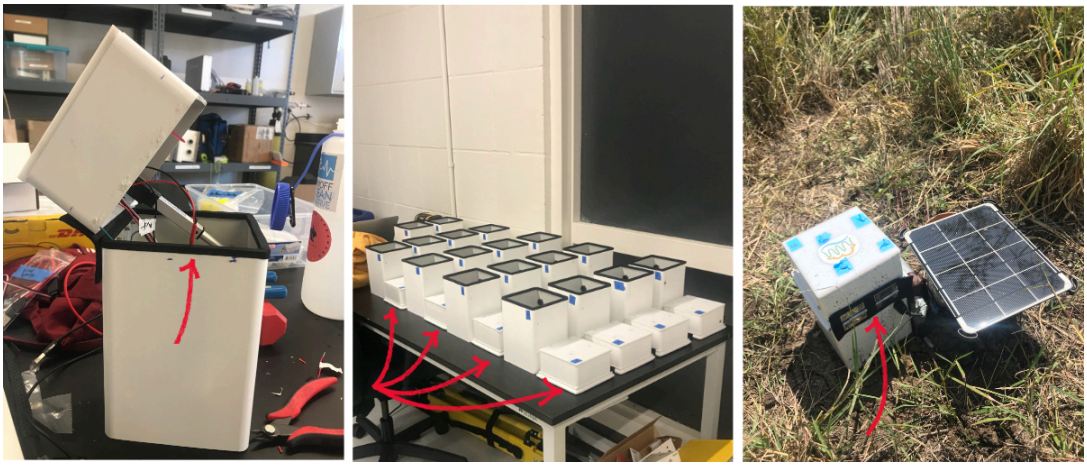

Figure A.3: The three components of the air-tight seal between chamber body and lid when closed, indicated with red arrows on images from left to right: the thin neoprene foam strip where the body and lid meet when closed; the white rubber 'lip' on the outside edge of the chamber lid on the three non-hinged sides, as seen on the unassembled fluxbots; and, as seen on an installed fluxbot in the field, the layer of wetsuit neoprene as a washer between the hinged connectors and the PVC of the chamber lid and body.

We installed three components to the join between the body and lid to ensure an air-tight seal when the chamber was in its closed position. First, we lined the top edge of the body with thin (1/4 x 1/4") strips of neoprene joined at the corners. We glued a 'lip' of rubber weatherstripping material along the outside bottom edge of the front and sides of the lid, extending several millimeters over the edge, joined at the corners with 90 degree cuts and weatherproof silicone sealant.

A.2.2 Leakage

We considered two things about chamber leakage when determining if and how to calculate this coefficient. First, the question "how fast does air turn over in the chamber?". If the air turns over quickly, then tests like blowing on the outside edge of the closed chamber would be visible in the data. Second, "how high is the concentration of CO_2 in the chamber?". If there was extremely high CO_2 in the chamber, we would be able to see it diffuse out. To test these two questions, and reduce leakage when the chambers are closed to functionally zero, we conducted the following "breath test" experiment while adding a series of three successively more intensive seals between the chamber lid and body.

We first lined the top edge of the chamber body with a thin strip of neoprene (Fig. A.3, left); we then glued a 'lip' of rubber weatherstripping material along the outside bottom edge of the front and sides of the lid (Fig. A.3, middle). Last, we placed a layer of recycled wetsuit neoprene between the hinge and the PVC before screwing the hinges in place to connect the chamber body and lid, to prevent the screws from introducing the possibility of air leakage (Fig. A.3, right). We tested the performance of these three features individually and together by conducting a 'breath test' with each successively added seal feature: when the chamber was in its closed position and a steady-state concentration of CO_2 inside it was reached, we breathed around all four edges of the junction between the lid and the body. As exhibited here (Fig. A.4), we achieved an increasingly leak-free seal with each successively-added seal.

A.2.3 Hardware (electronics system)

Once the chambers were built, we installed our custom electrical hardware system into each lid. We constructed the electronics system from a Pycom LoPy4 microcon-

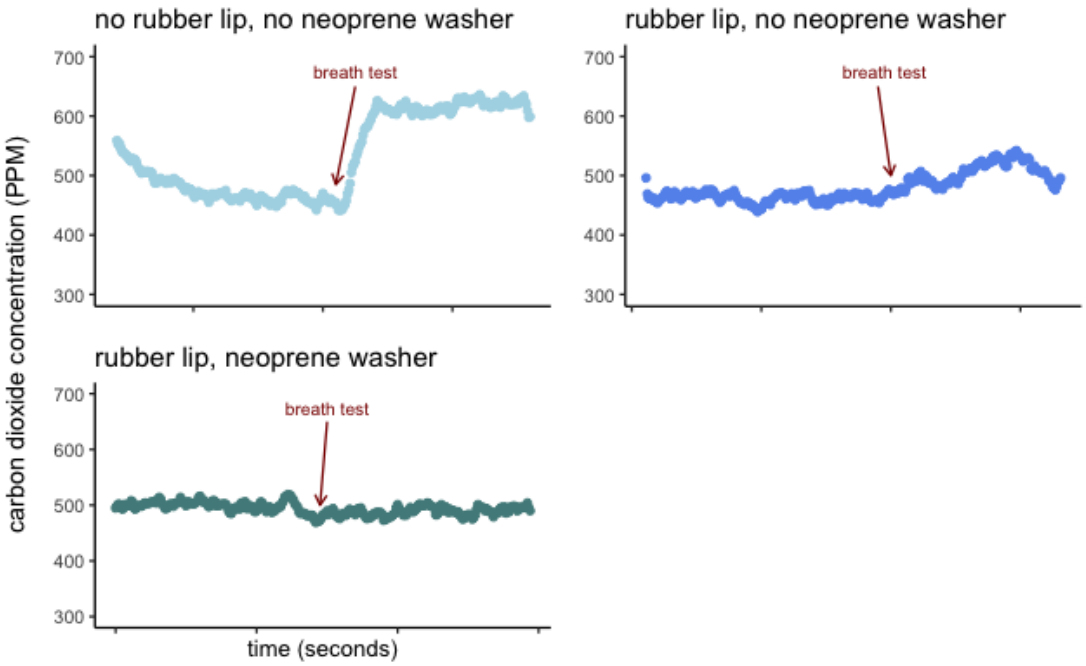

Figure A.4: Experimental comparison of progressively added components of the fluxbot's seal when closed, from left to right, top to bottom: just the foam edge between the lid and body; the addition of a rubber lip around the three non-hinged edges; the final addition of a strip of neoprene as a washer on the back side between the hinges and PVC. Arrows indicate the time at which the seal was tested by breathing around the edges of the fluxbot's closure.

troller, with a Pycom Expansion Board. In addition to an infrared (IR) CO_2 sensor, we also included a real-time clock (RTC) to ensure synchronized measurements across all chambers, and a sensor to measure air pressure, temperature, and humidity inside the chamber. The microcontroller (via the expansion board) controlled all other electronics; wiring was accomplished by a combination of soldering and lever-nut snap-style wire connectors. Data was directly written to a microSD card, stored on the expansion board. All electronics were fixed in place to a custom mounting board, which we pre-cut with screw holes and slots for feeding wires through (Fig. A.5). We fixed this 'electronics system' to the inside of the lid from the top plate with the CO2 sensor facing out, using long screws and spacers, with rubber washers to prevent leaks.

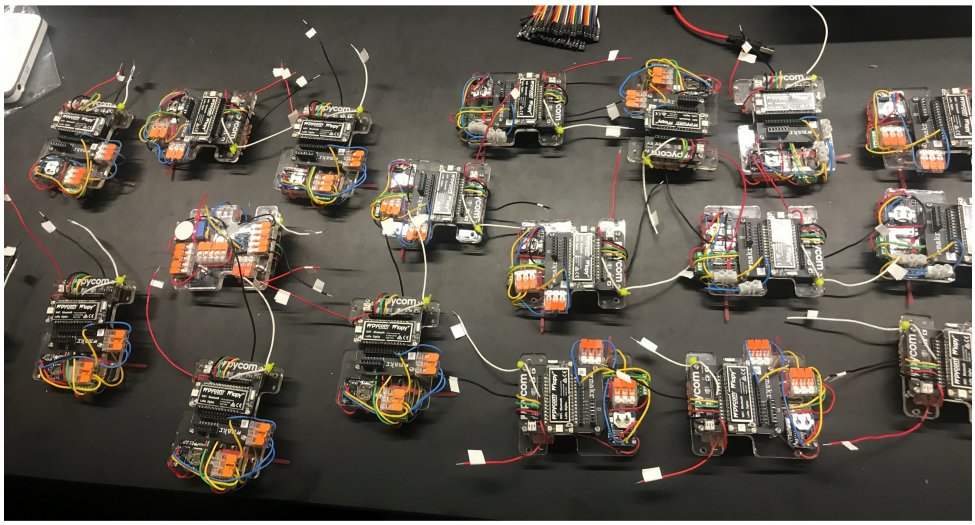

Figure A.5: The fluxbot electronics systems, wired and installed on the custom-cut acrylic mounting board.

To connect the electronics system to its external battery, we threaded a USB cable (with exposed ground and power wires) through a hole in the backside of the chamber body. This hole was fitted with a weather proof, air tight through-port gland, which we finger-tightened around the USB cable once in place. The exposed ground and power wires were snapped into the negative and positive lever-nut connections, respectively. When powering on in the field, we plugged the USB end, extending from the back of the chamber body, into the V44 battery, which was also plugged into a 6W solar charger. We placed the battery itself into a custom-sewn waterproof 'raincoat', and affixed this protected battery to the outside of the chamber with heavy-duty Velcro (Fig. A.6).

We set the (battery-powered) RTCs to the local time at our field site (Laikipia, Kenya) prior to installation in the electronics system. We also manually calibrated each CO_2 sensor, using its accompanying calibration software , to known concentrations of

Figure A.6: Red arrows pointing to the USB connection through the through-port gland (left) connecting the electronics (middle) to the external, waterproofed battery (right). The round PVC collar used for paired CIRAS data collection is visible in the right panel.

atmospheric CO_2. We accomplished this calibration by sealing each sensor inside the chamber of a PP-Systems SRC-2 soil carbon flux chamber (with a CIRAS gas exchange system) using an air-tight rubber seal, such that the CO_2 concentration within the chamber equilibrated and was set as the calibration value for the sensor sealed inside.

A.2.4 Software

The software for the fluxbots was written by co-author Vincent Benenati, in MicroPython. The fluxbots are programmed to operate continuously as long as they are powered on, on an hourly schedule: the first 55 minutes with the linear actuator fully extended (90 percent of the linear range of the actuator) such that the fluxbot lid is open to just under 45 degrees, and the last five minutes with the linear actuator fully contracted (zero percent of its linear range) such that the fluxbot lid is fully closed and the chamber sealed to allow buildup of CO_2 over this five minute period.

The program also controls the data collection and storage schedule: at minutes 18, 36,

and 54, the fluxbot collects and records (to the microSD card housed on the expansion board) three seconds of CO_2 content, temperature, pressure, and humidity of the ambient air while the lid is in the open position. After the lid closes, from minute 55 to the end of the hour, the fluxbot records CO_2, temperature, pressure, and humidity once per second for the entire five-minute period during which the chamber is closed. Associated timestamps are collected for any measurement recorded to the microSD card.

Upon booting up, the program runs through all of its functions to ensure that every piece of hardware is in place and operational. The small LED light on the LoPy 4 (which is visible through the lid of the fluxbot) blinks during this process to indicate that the system is checking the status of each component; each component is assigned an LED color (Table A.1). If the program encounters an error at any component (e.g. the microSD card is not mounted), it will pause the bootup procedure and the LED will continue blinking in the associated color until the error is resolved (e.g. the microSD card is inserted into its mount on the LoPy4). If no errors are encountered, the program will complete the bootup procedure and the LED will begin blinking white every five seconds, indicating that the fluxbot is awaiting the first minute of the next hour to begin its hourly measurement cycles.

At the start of the first hour, the LED will switch to blinking cyan blue every five seconds, indicating the fluxbot has commenced normal scheduled operation. The fluxbot will blink cyan blue throughout its 55 minute "open" period each hour, except at 18, 36, and 54 minutes; at these times, the LED will rapidly blink fuchsia (which indicates measurement recording is occurring) while the fluxbot takes its scheduled ambient measurements. At 55 minutes when the lid of the fluxbot closes, the LED will again blink fuchsia for the final five minutes of the hour, again indicating that the fluxbot is conducting and recording measurements, this time inside the chamber volume.

All data are stored on the microSD card stored on the LoPy4's expansion board. In

Table A.1: Color-coded LED light system to indicate fluxbot status.

red	CO_2 sensor connection error
yellow	BME280 or RTC connection error
dark purple	SD card error
white	boot-up success: waiting for first cycle
cyan	normal operation, not measuring
fuchsia	measurement and recording mode

addition to this data file, an additional data file for each fluxbot is created and stored automatically that logs the dates and times of any boot-ups the fluxbot experiences (e.g. from initial installation in the field, removal/replacement of the microSD card for data collection purposes, powering back up after power loss, *in situ* repairs that required removal of the power source, etc.).

A.2.5 Installation

We chose to install the fluxbots in a large-scale, long-term ecological experiment: the Kenya Long-term Exclosure Experiment (KLEE, established in 1995 to investigate the impacts of wildlife loss on savanna structure and function), located at Mpala Research Centre and Conservancy, in Laikipia, Kenya (Fig. A.7).

Aspects of this experimental site make it perfect to test such a network of autonomous soil carbon flux sensing robots. For example, it is highly homogenous on a coarse scale, with little to no elevation change and one dominant tree species. However, it is also highly heterogeneous on a finer grain, in a systematic and predictable way (Pringle and Tarnita 2017). There are large wildlife species like elephants and lions, making manual sampling at night (especially across the entire expanse of the large experiment) risky and logistically complicated. The possibility of equipment damage from curious wildlife or domestic cattle is also high, also making the use expensive commercial autonomous sensors risky. The region experiences two wet and two dry seasons annually; due to

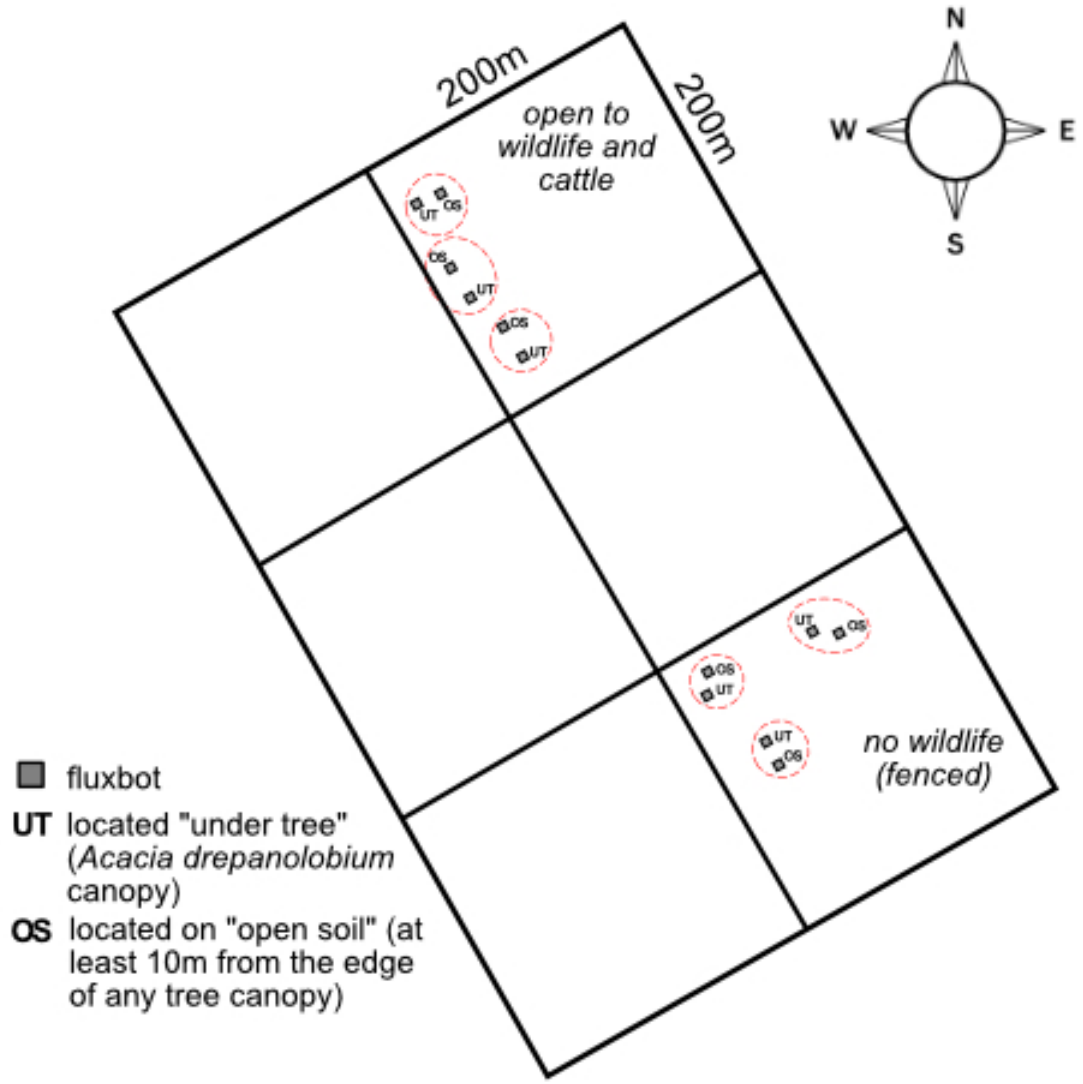

Figure A.7: Birds-eye map of the KLEE experimental design, including the location of each of the 12 fluxbots installed for this study.

the high clay content of the soil (approximately 50 percent) (DeCarlo and Caylor 2019), it is impossible to travel to the experimental area for manual data collection in wet weather, making manual data collection infeasible in these times of year. In addition such sustained, heavy rainfall could also prove hazardous for the range of available expensive commercial sensors that could be installed there over a wet season.

In short, the features described here make the KLEE the perfect site to test the

installation of a network of inexpensive, DIY fluxbots. We installed the 12 fluxbots in two groups of six, across two of its experimental wildlife manipulations: all wildlife present plus cattle grazing; and total exclusion (via electric fencing) of all large mammalian herbivores (e.g. larger than rodents) both wild and domestic (Young et al. 1998). Within each of these two experimental treatments, we separated the fluxbots into groups of two, with two each on the following landscape features: beneath the canopy of the ecologically dominant, nitrogen-fixing tree species *Acacia drepanolobium*, and on open soil patches more than 10m from a canopy edge (Fig A.7).

We installed the fluxbots using a rubber hammer to gently tap the bottom edge of each chamber's body 2.5 inches into the soil, such that the protruding portion measured 5x5x5". An extra length of PVC was gently tapped into the soil to the right side of each bot, to serve as a horizontal surface for the solar panel. After 'planting' each body in the soil, the lids were screwed on, and actuators positioned and plugged into the electronics system. The solar panels were horizontally fixed to the extra PVC using silicone caulk, and plugged into the V44 batteries fixed to the side of the chamber body. As the last step to power on each fluxbot, the USB protruding from the back of the chamber was plugged into the battery. Upon powering on, each fluxbot ran through its aforementioned diagnostic tests.

The network of fluxbots remained in the field for 2.5 months (August through mid-October 2019), over a period of time encompassing the end of a dry season and the start of a wet season (though most of the month of August was used as a test month, where we collected data but did not include them in the final dataset; see main text). We manually collected data from each fluxbot every few (3-4) days by removing the fluxbot's microSD card from the lid in its open position (thereby temporarily pausing the fluxbot's operation due to 'missing' hardware), and downloading its data to a Toughbook laptop using a card reader. This process took several minutes at most, and upon replacement of the microSD

card the fluxbot resumed its scheduled activity according to its synchronized RTC.

A.2.6 Allan Variance

We determined a 20-second average transformation for the raw data by calculating the Allan variance, or two-sample variance, of the data at different averaging times. The Allan variance estimates signal instability due to noise (not systematic errors caused by temperature or other environmental change), and is defined by:

$$\tau = 1/(2\tau^2) < (x_{n+2} - 2x_{n+1} + x_n)^2 > \tag{A.1}$$

We averaged raw data over values of τ (the averaging window) with overlap (e.g. a rolling average), and calculated the Allan variance of the dataset for each value of τ ranging from 1 to 120 seconds. By plotting the resulting array (e.g. values of Allan variance corresponding to each τ), we determined that a 20-second averaging interval optimally reduces noise in the raw $_{\text{ppm}}$ data while maintaining high temporal resolution (Fig. A.8).

A.2.7 Comparison with manual data collection

In the month of August 2019, we manually collected daytime soil carbon flux measurements every 1-2 days, using a portable sensor system (PP-Systems CIRAS 3, SRC-2 soil flux chamber attachment). A PVC collar, fitted to the SRC-2 chamber diameter, was installed approximately 15cm from each fluxbot at the same time as fluxbot installation for the purposes of collecting contemporaneous manual data. These measurements were taken opportunistically (e.g. when we were conducting data collection from the fluxbots or troubleshooting at the KLEE plots more generally). Data were collected between 9am and 4pm, on days where no precipitation was actively occurring.

After the completion of the study we used timestamps from the two data streams

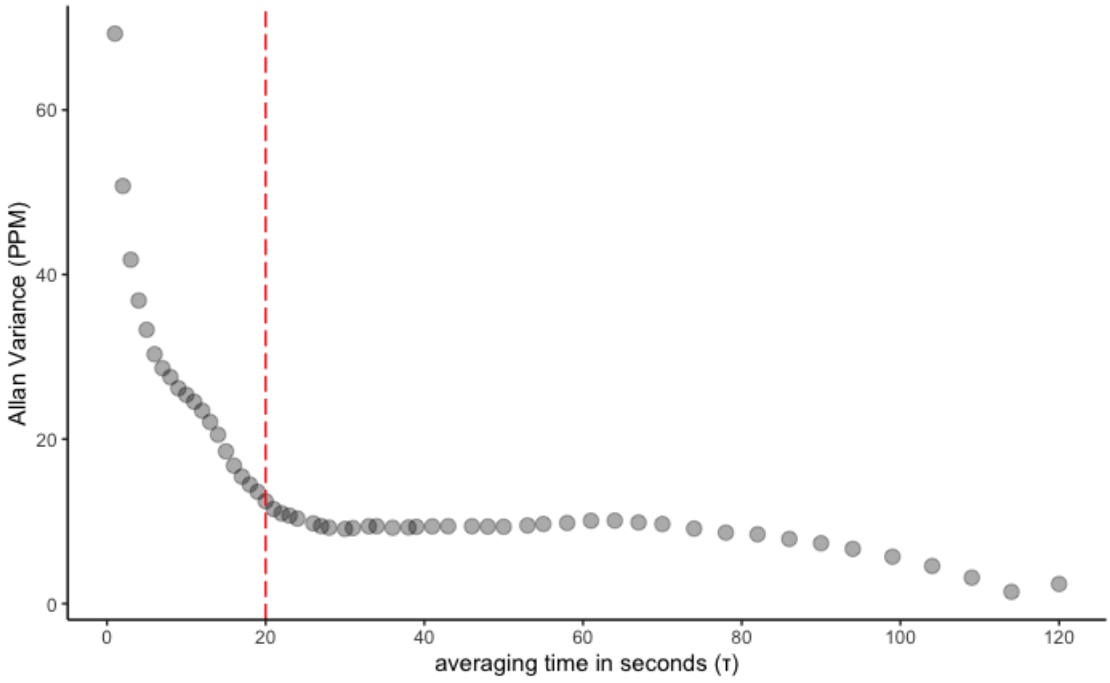

Figure A.8: Allan variance calculation of raw fluxbot data indicating that a 20 second rolling mean transformation of the raw data optimally reduces noise and maintains high temporal resolution.

(fluxbots and CIRAS) to match each manually-collected CIRAS flux measurement to the flux measurement that was collected closest in time by its associated fluxbot. If the elapsed time between the fluxbot measurement and the CIRAS measurement was greater than 25 minutes, the pair was discarded. While these CIRAS data were taken in the period of time prior to fluxbot re-calibration on August 23rd (see main text), for the sake of comparison between methods we analyzed the matched CIRAS/fluxbot dataset separately from the final fluxbot dataset (which did not include the fluxbot data collected before August 23rd).

The mean for manually-collected CIRAS fluxes was 1.69 µmol/m^2/sec, with a median of 1.435 µmol/m^2/sec; the coefficient of variation was 58.1%. The mean for fluxbot-collected fluxes matched with those collected manually with the CIRAS was 7.12 µmol/m^2/sec, with a median of 3.08 µmol/m^2/sec; the coefficient of variation was 148.6%. The corre-

lation between the two methods of data collection was negative, at -0.146.

However, a high level of spatial variability in soil carbon flux is not uncommon particularly in drought-prone dry grasslands (Foti et al. 2016). Manual CIRAS data were collected only when rainfall was not actively occurring, and during daylight hours when the soils may dry swiftly. In this system, the clay-rich vertisol soil forms drying-related cracking morphologies (Somasundaram et al. 2018), with the potential to locally increase soil carbon flux by orders of magnitude; in addition, relatively dry soils demonstrate greater numbers of outliers in carbon flux (DeCarlo and Caylor 2020). As low soil moisture and cracking both contribute to low autocorrelation between even spatially close (less than 15cm) sampling sites (Rochetten, Desjardins, and Pattey 1991), we would expect little fidelity between the paired measurements.

A.2.8 Total cost and parts list

In an effort to make our design accessible to other researchers, we present below an itemized parts list, consisting of all materials used in this study and as such those materials we recommend for replication of the fluxbot design. The total cost of one fluxbot is $361.72 US dollars in materials.

Table A.2: Parts list for a single fluxbot, including purchasing information and quantities per bot, cost per unit item for a single fluxbot, and total cost of materials for a single fluxbot.

Part	**Qty/bot**	**Cost/bot**	**purchased**	**Catalogue ID**
5”x5” PVC pipe	16”	5.30	Home Depot	205883925

Acrylic roof	5x5”	1.12	Amazon, customized with laser cutter	1/4” acrylic, B07PH1WY8S
Neoprene ring	20”	1.52	McMaster Carr	1566N212
Weather strip	18”	0.79	Amazon	Brand: M-D Building Products, Part number: 78394
Acrylic electronics mounting plate	4.5 x 3”	1.13	Amazon; customized with laser cutter	1/4” acrylic (see above)
linear actuator, L12-R Micro with limit switches, 50mm extension, 100:1 gear ratio, 6V	1	60.00	Actuonix	Brand: Actuonix Motional Device Inc., Product code: L12-R
Pycom LTD Expansion Board 3.0	1	22.50	Pycom	UNIVERSAL EXPANSION BOARD PYCOM
Pycom LoPy4	1	49.95	Pycom	LoPy4
CozIR-LP Miniature 5,000ppm CO2 sensor	1	99.00	CO2meter.com	Brand: CozIR, GC-0027
RTC board	1	5.20	Adafruit	PCF8523
BME board	1	9.95	Adafruit	Brand: Bosch, BME280
Zip tie	3	0.12	Misc.	N/A
Lever nut connectors: 3-port	3	1.50	Amazon	Brand: Wago, 221-413
Lever nut connectors: 5-port	3	1.50	Amazon	Brand: Wago, 221-415

Battery and solar power kit	1	87.20	Voltaic Systems	P106-K
Duct tape	24”	0.11	Misc.	N/A
Cable thru-port gland, 3-6.5mm adjustable	1	0.40	Amazon	Brand: DGZZI, size: PG70
1/4” cable cover PET braided	18”	0.50	Amazon	Brand: Alex Tech, Model number: 4330220706
Mounting plate bolts	4	0.57	McMaster Carr	92095A474
Mounting plate rubber washers	4	0.35	McMaster Carr	90133A005
Mounting plate spacers	4	0.51	McMaster Carr	90176A151
Mounting plate nuts	4	0.25	McMaster Carr	94150A325
Expansion board bolts	4	0.49	McMaster Carr	92095A186
Expansion board spacers	4	0.33	McMaster Carr	94669A101
Expansion board nuts	4	0.25	McMaster Carr	94150A325
BME bolts, 12mm length 0.4mm pitch	2	0.16	Amazon	uxcell M2x12mm machine screws pan Phillips cross head screw 304 100pcs, A18111200 ux0285

RTC bolts, 12mm length 0.4mm pitch	2	0.16	Amazon	uxcell M2x12mm machine screws pan Phillips cross head screw 304 100pcs, A18111200 ux0285
BME nuts	2	0.34	Amazon	uxcell Hex Nuts, M2x0.4mm metric course thread hexagon nut 304, 50pcs, a18082200 ux0391
RTC nuts	2	0.34	Amazon	uxcell Hex Nuts, M2x0.4mm metric course thread hexagon nut 304, 50pcs, a18082200 ux0391
Actuator bolts	2	0.40	McMaster Carr	98164A444
Actuator upper spacer	1	0.27	McMaster Carr	92510A445
Actuator lower spacer	1	0.27	McMaster Carr	92510A444
Actuator far spacer (both) 2		0.46	McMaster Carr	92510A440
Actuator nut	2	0.16	McMaster Carr	90715A007

Hinge, Everbilt brand 1-1/2 in. x 1-1/2 in. stainless steel narrow utility hinge non-removable pin (2-pack)	2	3.27	Home Depot	204727559 (internet), 1001330665 (store)
Bolts for hinges	8	0.29	McMaster Carr	92010A801
16GB Sandisk microSD card	1	5.99	Amazon	B00FZ VQPBC
Pin pitch changer 1.27mm to 2.54mm	1	1.49	Adafruit	F127T254P06-ND
Screw terminal headers; RTCs	1	1.21	Adafruit	2135
Nylon rip-stop fabric	6x4" and 8x6"	0.22	Fabric Wholesale Direct	Brand: Ottertex, Ottertex nylon ripstop 70 denier (PU coated) - 1.9oz
Velcro	4"	0.84	Amazon	Brand: Velcro, Model number: 90197
22 gauge wire	variable; see wiring schematic	1.30	Amazon	Brand: EX Electronix Express, Part number: 27WK22 SLD25
Total cost per fluxbot:		**$367.71**		