DISSERTATION

ENVIRONMENTAL CHANGE IMPACTS ON CARBON AND NITROGEN DYNAMICS IN SOILS AND VEGETATION: FROM GLOBAL SYNTHESIS TO LOCAL CASE STUDIES

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ABSTRACT

ENVIRONMENTAL CHANGE IMPACTS ON CARBON AND NITROGEN DYNAMICS IN SOILS AND VEGETATION: FROM GLOBAL SYNTHESIS TO LOCAL CASE STUDIES

Human-induced changes in the Earth system, known collectively as global environmental changes, are modifying terrestrial ecosystems. Feedbacks between land biogeochemistry (e.g., the cycling of elements) and global change are one of the key uncertainties in global climate models, and thus understanding land (e.g., soils and plants) responses to global change will help us predict future climate. In order to advance understating of how soils and plants respond to global changes, we need to work across scales by synthesizing global findings, using experimental networks, and studying context dependent responses at individual sites. Specifically, this dissertation uses this framework to investigate: (1) the responses of carbon (C) in total soil organic matter (SOM) and its fractions to warming, elevated atmospheric carbon dioxide (CO_2), altered precipitation regimes, and nitrogen (N) fertilization globally using meta-analysis; (2) SOM C and N stoichiometry and distribution in response to nutrient fertilization in globally-distributed grasslands; (3) plant and soil biogeochemical responses to increased precipitation at a mesic grassland; (4) bulk (wet and dry) N deposition in response to proximity to the road in a topographically complex, subalpine forest.

Soil organic matter stores carbon (C) and N and thus helps to control climate and provide energy and nutrients for ecosystem function. Thus, understanding SOM responses to global change will help determine future climate and ecosystem processes. However, SOM is made up of a diverse pool of molecules, and separating SOM into more homogenous functional pools

(e.g., particulate and mineral-associated organic matter [POM and MAOM]) can provide clearer understanding of SOM responses to perturbations. By synthesizing global-scale understanding, Chapter 2 showed that POM and MAOM C responded differently to global changes and these responses depended on experiment length, soil depth, and experiment methodology. By investigating how SOM responses to global change varied across a global distribution of grasslands, Chapter 3 found that addition of macro- and micronutrients modified POM and MAOM C:N, depending on ambient environmental conditions, and consistently reduced SOM C stability. By investigating C and N cycling under altered precipitation at a local scale, Chapter 4 showed that studying SOM fractions provided clearer understanding of the mechanisms underlying grassland biogeochemical responses to increased precipitation. Chapters 2-4 all show the value of investigating soil fractions rather than solely the total SOM pool, as studying these fractions provided unique information and greater functional understanding.

Global changes are not felt equally by all ecosystems. Ecosystems near sources of N deposition may be especially vulnerable to this global change. The fifth chapter of this dissertation, like the fourth chapter, focused on understanding local responses to global change. The vast majority of roadside N deposition studies find increased N deposition adjacent to roadways, but we did not find this, potentially due to the complex topography at our site or insufficient vehicle emissions. This suggests higher roadside N deposition cannot be assumed for all ecosystems.

Altogether this dissertation synthesized and advanced our understanding of global change effects on plant and soil C and N pools and cycling.

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CHAPTER 1: INTRODUCTION

1. Background

Humans have had a profound influence on our environment. Global environmental changes are changes in the Earth system due to increased human activities since the turn of the 20th century. The global changes focused on in this dissertation include those associated with climate change: elevated atmospheric carbon dioxide (CO_2), atmospheric warming, and changing precipitation regimes; and also the elevated deposition of nitrogen (N) into ecosystems. The response of terrestrial biogeochemistry to these global changes represents one of the largest sources of uncertainty in global climate models (IPCC 2021), and thus contributes uncertainty to the predictions of the future climate the Earth will experience. This dissertation addresses this uncertainty by investigating carbon (C) and N dynamics in soils and vegetation across scales. The first two research chapters (chapters 2 and 3) focus on global scale analysis of soil organic matter (SOM) C and N cycling under global changes. The final two research chapters (chapters 4 and 5) focus in on local case studies of SOM and plant C and N under global change (chapter 4) and the contribution of N deposition to an ecosystem that evolved with low N (chapter 5). The two components present in each of these chapters are global changes and terrestrial ecosystem C and N cycling. For the global change aspect of this dissertation, I provide background on global changes generally (section 2), the responses of plants and soils to global changes (section 3.2), and additional information on N deposition (section 4), which is the focus of chapter 5. For the terrestrial biogeochemistry aspect of this dissertation, I introduce SOM C and N cycling and the relevant connections to plants (section 3) and N deposition as an N input to terrestrial ecosystems (section 4). Better understanding of how global change will influence plant and soil C and N cycling will improve our understanding of biogeochemical feedbacks to our climate system.

2. Global environmental change

Global change is occurring because of human activity; the most recent Intergovernmental Panel on Climate Change report states that "It is unequivocal that human influence has warmed the atmosphere, ocean and land since pre-industrial times" (Eyring et al. 2021). Climate change associated global changes all stem from the emission of greenhouse gases (CO_2, methane, and nitrous oxide) into the atmosphere by energy production and land use change. The Earth's energy budget is determined by the balance of incoming radiation from the sun and outgoing radiation, emitted back to space, such that if incoming radiation is greater than outcoming radiation, Earth's atmosphere warms. Because greenhouse gases absorb outgoing radiation and then remit it back towards Earth, they change the energy budget and warm Earth's atmosphere, majorly from CO_2 (Gulev et al. 2021). Because warmer air holds more moisture than cooler air and evaporation increases with atmospheric warming, greenhouse gases have also increased the moisture content of the atmosphere, strengthening the global hydrologic cycle (Trenberth 1998; Gulev et al. 2021). Increased moisture in the atmosphere is expected to lead to increased precipitation globally, but variation in magnitude and frequency at individual locations is much less certain (Lee et al. 2021). Warming, altered precipitation, and elevated CO_2 all stem from anthropogenic greenhouse gas emissions and can each individually influence plant and soil C and N cycling.

Nitrogen deposition, on the other hand, is not driven solely by greenhouse gases, but rather associated with increased emission of N into the atmosphere due to human activities generally. Humans have profoundly changed Earth's N cycle by doubling the amount of reactive (e.g., that interacts biologically) N in the environment (Gruber and Galloway 2008). Increased anthropogenic N input is primarily from agricultural N fertilization and secondarily from the

burning of fossil fuels (Gruber and Galloway 2008). Additional N added to agricultural systems can be lost to the atmosphere via ammonia (NH_3) volatilization and nitrification and denitrification, which are enhanced in these fertilized systems (Galloway et al., 2003). Burning of fossil fuels results in oxidation of organic N, with nitrogen oxides (NOx) emitted to the atmosphere. These emissions (e.g., NH_3 and NOx) are then transported and sometimes transformed in the atmosphere before being deposited as wet or dry N deposition in nearby ecosystems. Nitrogen in deposition can be in oxidized (e.g., nitrate [NO_3^-], nitric acid [HNO_3], nitrogen dioxide [NO_2]), reduced (e.g., NH_3 and ammonium [NH_4^+]), or organic forms, and reduced forms dominate U.S. N deposition (Li et al 2016). Fertilization from N deposition can modify C and N cycling in soils and plants.

Looking to the future, atmospheric CO_2 is expected to continue increasing, causing continued warming and changes in precipitation, that may level off if we take action to reduce our greenhouse gas emissions (Lee et al. 2021). In contrast, N deposition is not expected to increase but rather maintain its current rate, which is approximately three times above pre-industrial levels (Kanakidou et al. 2016). Predicted changes in atmospheric CO_2, warming, and altered precipitation, and enhanced N deposition relative to pre-industrial levels, indicate a need to better understand their effects on terrestrial biogeochemistry.

3. Soil organic matter and responses to global change

3.1. Soil organic matter

Our understanding of SOM formation and cycling has changed drastically in the past few decades. Previously, SOM was thought to form from the "humification" of plant detritus and other organic inputs and to persist because of the chemical complexity of humus molecules that were difficult for microbes to decompose (Lehmann and Kleber 2015). Within the past 15 years,

the soil ecology community has converged on a new understanding of SOM as an "ecosystem property" that relies on interactions of climate, plant properties, soil mineralogy, and microbial community and activity (Schmidt et al. 2011). There are several ways that this new view of SOM has been conceptualized (*e.g.,* Cotrufo et al. 2013; Grandy and Neff 2008; Lehmann and Kleber 2015; Waring et al. 2020). This dissertation follows the two-pathway model of SOM formation (Cotrufo et al., 2015), the mineral efficiency and matrix stabilization (MEMS) framework (Cotrufo et al. 2013), and the *ex vivo* and *in vivo* framework (Liang et al. 2017), which together describe the formation and stabilization of two main pools of SOM; the particulate OM (POM) and the mineral-associated OM (MAOM). Because these two pools have different controls and functions in the soil, they have proven useful in understanding SOM responses to management and disturbance (Grandy and Robertson 2007; Mosier et al., 2021). POM is defined as the functional fraction or pool which is composed of structural plant and microbial material and does not interact with minerals (Cotrufo et al. 2015; Six et al. 2001b). In contrast, MAOM is defined as the functional fraction composed of low molecular weight compounds of plant and microbial origin that are sorbed to mineral surfaces or within very fine aggregates (Kleber et al. 2015; Wagai et al. 2020). The low molecular weight compounds are thought to derive from exo-enzyme modification of OM, known as the *ex vivo* pathway, or through microbial anabolism and subsequent necromass production, known as the *in vivo* pathway (Liang et al. 2017). The *in vivo* pathway is expected to be promoted by high quality litter inputs, described in detail later in this section (Cotrufo et al., 2013). By investigating POM and MAOM, as defined by the above frameworks, we can modify our conceptualizations of SOM responses to global change, in which ecosystem responses shape the responses of SOM pools. The influence of global changes on the plant, microbial, and soil properties that drive SOM formation and loss are reviewed below.

Separating SOM into pools with different characteristics has long been used in soil science to understand this complex and variable group of compounds. This dissertation focuses on physical methods of separating, or fractionating, SOM into pools. This is in opposition to chemical and kinetic fractionations, which consist of reacting soils with chemicals to isolate organic molecules and using soil incubation to define pools with different turnover times, respectively (Paul et al. 1999; Stevenson 1994). Rather, physical methods of separation rely on separating soils by size or density, before or after dispersion of soil aggregates (Leuthold et al. 2022). We associate these physically defined fractions with different functional pools, explained above, in the soil. Operationally, POM is defined as the light (< 1.6-1.85 g cm^{-3}) and large (> 53 µm) fraction, as it is not associated with minerals, which are heavy, and has undergone minimal depolymerization (Cambardella and Elliot 1992; Lavallee et al. 2019; Leuthold et al. 2022). However, because POM stabilization is dependent on its relative accessibility to microbes, POM can be further separated into free and occluded POM (fPOM and oPOM), where free POM is not within aggregates and oPOM is occluded in aggregates. Operationally, these are determined by separation before (fPOM) and after (oPOM) dispersion during the fractionation procedure. MAOM, in opposition to POM, is generally defined as the heavy (> 1.6-1.85 g cm^{-3}) and small (< 53 µm) fraction, because of its association with silt and clay-sized minerals. In between MAOM and POM is the heavy, coarse OM (hcOM), which is heavy (> 1.6-1.85 g cm^{-3}) and large (> 53 µm), and thus does not fit our general definitions of POM and MAOM. This fraction is still not fully understood but is thought to consist of organic encrusted sand grains or mineral encrusted POM and is generally a small component of the soil C pool (Leuthold et al. 2022; Samson et al. 2020). There is also the dissolved organic matter (DOM) which is defined as

soluble material smaller than 0.45 µm and accounts for 1-2% of the total SOM (Leuthold et al. 2022).

While no fraction defined above represents a fully homogenous pool of SOM, each is associated with functional properties that make them useful for understanding SOM responses to global change. Because, when not occluded in stable aggregates, POM is not associated with minerals, it has on average a faster mean turnover time in the soil than MAOM (Heckman et al. 2022; von Lutzow et al. 2007). Because of its mineral protection, MAOM is generally viewed as the more stable and less vulnerable to disturbance pool of organic matter (Cotrufo & Lavallee, 2022). However, MAOM can also saturate, depending on the availability of mineral surfaces for sorption (Stewart et al. 2008). This means that the potential to store C and N in MAOM is physically defined, whereas POM could build up without mineral limitations, although most soils are far from saturation (Cotrufo et al. 2019; Georgiou et al. 2022). POM is thought to be more important for providing C and nutrients for microbial energy use and plant growth, as it more physically accessible for microbial use in the absence of temperature, moisture, and oxygen limitations (Bailey et al. 2019; Keiluweit et al. 2017; Lavallee et al. 2020; Vaughn and Torn 2019). Although, MAOM may be an important plant N source in the rhizosphere (Daly et al. 2021; Jilling et al. 2018). Given its greater relative amount of N-rich microbial compounds, MAOM generally has a lower C:N ratio than POM, which means microbes could use MAOM C more efficiently (with less loss to respiration) if MAOM was destabilized (Cotrufo et al. 2019; Lavallee et al. 2020).

Litter quality is also expected to be differently associated with fractions. For this dissertation I define higher quality litters as those with greater content of N and soluble materials and lower C:N and structural material (lignin and cellulose). Higher litter quality is expected to promote

microbial anabolism and form MAOM through the *in vivo* necromass sorption pathway (Cotrufo et al. 2013; Liang et al. 2017), as well as through direct sorption of low molecular weight or N-rich compounds (Islam et al., 2023; Possinger et al., 2020). Dissolved organic matter, albeit a small pool for any given fractionation, represents the OM available to microbes and the organic source for the *ex vivo* MAOM formation pathway (Liang et al. 2017). Heavy, coarse OM has been shown to be an important location for C and N accumulation in agricultural soils (Samson et al. 2020). Overall, these distinct properties of different fractions can be used to understand the response of SOM dynamics to global change.

3.2. Global change effects on plants and soils

3.2.1. Warming

Atmospheric warming driven by human activity is associated with the fear of a carbon-climate feedback, where the warming atmosphere could result in increased soil microbial activity and respiration of C to the atmosphere. This, in turn, will further enhance the warming effect and promote a positive feedback loop of soil C loss (Luo 2007). However, this fear is largely not supported by meta-analyses, which find no change in soil C, likely due to increases in both inputs (i.e., plant growth and microbial biomass carbon) and outputs (i.e., respiration and leaching; Lu et al. 2013; Van Gestel et al. 2018; Zhang et al. 2015). However, this response is dependent on time and soil depth, with greater soil C losses seen over the short term and variable responses at depth (Hicks Pries et al. 2017; Melillo et al. 2002; Soong et al. 2021; Walker et al. 2020). Due to the different controls of POM *versus* MAOM, we might expect these to respond differently to warming. Temperature sensitivity of decomposition increases with increasing molecular complexity and warming increases enzymatic activity, which can increase decomposition when substrates are not physically or chemically protected (Davidson and

Janssens 2006). Both of these metrics could lead to greater decomposition of POM with warming, given POM's comparatively complex molecular structure and lack of protection. In addition, in high latitude ecosystems, POM is largely protected by temperature limitation of decomposition, which warming would mitigate (Vaughn and Torn 2019). Responses of plants to warming may also particularly influence POM. Increased growing season length and species composition changes are two relatively consistent responses of ecosystems to warming (Luo 2007). Both of these responses may promote more structural input into the soil, through longer timespans of plant input (from the increased growing season) and woody plant encroachment in high latitude systems (Myers-Smith et al. 2011). Because structural input is thought to contribute to the POM pool, it may be particularly responsive, although plants input carbon into the soil in both structural and soluble forms and increased plant inputs have been found to increase both POC and MAOC content (Zhang et al. 2022a). Inorganic N availability has also been found to increase with warming, which often is cited as the driver of increased plant growth under warming and may allow for specific increases of relatively N-rich MAOM (Bai et al. 2013). Evaluating the responses of POM and MAOM to warming separately could provide insight on which ecosystems and what ecosystem functions (e.g., C stability or nutrient provisioning) may be particularly susceptible to warming.

3.2.2. Elevated carbon dioxide

Because CO_2 is the substrate of photosynthetic C uptake, it was originally expected that elevated atmospheric CO_2 (eCO_2) would increase soil C stocks (IPCC, 2001). However, like warming, eCO_2 comes with a big climate concern; N availability may not be able to keep pace with plant growth, mitigating the negative feedback of plant growth, and subsequent soil C storage, to climate change (Luo et al. 2004; Reich et al. 2006). A meta-analysis combining four

previous meta-analyses confirmed this concern, and only found increased soil C with concurrent addition of N, but not with eCO_2 alone (Hungate et al. 2009). However, POM is less N-rich than MAOM, suggesting it may increase under eCO_2 due to less N limitation. Indeed, eCO_2 promotes more lignin-rich and N-poor plant tissue, which is likely to contribute to the POM pool (Cotrufo et al. 1998; Norby et al. 2001). However, eCO_2 generally strongly increases soil heterotrophic and autotrophic respiration, likely driving non-responses of the total soil C pool (Zhou et al. 2016). Determining the responses of POM and MAOM to eCO_2 may be able to help clarify N-dependent soil C responses.

3.2.3. Altered precipitation regimes

Global increases in precipitation are expected with climate change but how precipitation will vary spatially is uncertain. Precipitation increases are expected in high latitudes and the wet tropics, while decreases are expected in the subtropics, with greater seasonal amplification across the globe (e.g., wetter wet seasons and drier dry seasons; Lee et al., 2021). Because of these uneven responses, understanding how SOM will respond to increased, decreased, and variable precipitation will be an important part of predicting future C and N cycling. Increased and decreased precipitation generally speed up and slow down C cycling, respectively, with little change in the soil C pool (Song et al. 2019; Yue et al. 2017). Generalizations with changes in precipitation are difficult though, due to variable responses depending on ecosystem type (e.g., mesic, xeric, hydric), soil texture, and extremity of precipitation change (Knapp et al. 2008; Knapp et al. 2017; Zhou et al. 2018). In particular, there is a double asymmetry for microbial biomass and plant production responses to altered precipitation: under moderate changes in precipitation, they respond more to increased than decreased precipitation, but at extreme alterations, they respond more to decreased than increased precipitation (Knapp et al. 2017; Zhou

et al. 2018). Thus, with moderate changes to climate we could expect higher plant and microbial inputs with increased precipitation, potentially increasing MAOM through greater microbial processing of plant inputs. Whereas, with extreme events we might expect decreased precipitation to reduce plant and microbial inputs, potentially reducing both SOM pools. Changes in microbial community could also influence SOM responses to altered precipitation. It has been thought that fungi are more resistant to drought due to their capacity for osmoregulation and ability to reach distant resources with their hyphae (Schimel et al. 2007), but this is not a consistent response (Zhou et al. 2018). The high number of influential covariates in altered precipitation experiments make it difficult to make generalizations about potential SOM responses. Evaluating POM and MAOM responses to altered precipitation could help contextualize these varied responses. Notably, the general finding of no response of SOM to altered precipitation could be due to opposing responses of POM and MAOM. Greater POM decomposition and microbial input to MAOM with release of water limitation, could restructure the SOM pool (He et al. 2012). Changes in the relative amount of POM and MAOM in the SOM pool under altered precipitation regimes could modify SOM turnover times and functionality.

3.2.4. Nitrogen fertilization

Nitrogen fertilization, either to mimic N deposition or for agronomic management, has generally been found to increase C in SOM (Lu et al. 2011; Yue et al. 2016), although there are notable exceptions to this (Boot et al. 2016; Mack et al. 2004). Importantly, multiple authors have noted that experimental additions of N are much higher than that from N deposition in natural ecosystems, which could negatively influence microbial activity and promote soil C storage that we would not expect in reality (Averill et al. 2018; Song et al. 2019; Treseder 2008; Wilcots et al. 2022; Yue et al. 2016; Zhang et al. 2018b). However, these responses to high N

fertilization could be informative for studies where N fertilization is added for management (e.g., in agriculture). There has been much work characterizing plant and microbial responses to N fertilization that could lead to unique responses of POM and MAOM. Addition of N consistently increases plant biomass and has a stronger influence on aboveground than belowground biomass (Xia and Wan 2008). We expect belowground biomass to be used efficiently by microbes and contribute to the MAOM pool (Sokol et al. 2019). So, increased relative aboveground biomass with N addition may contribute more so to POM via the physical transfer pathway (Cotrufo et al. 2015). Decreases in heterotrophic respiration and enzymatic activity with N addition, would also promote POM buildup, and decreases in microbial biomass may reduce input to MAOM (Frey et al. 2004; Treseder 2008; Zhang et al. 2018b). However, these potentially disparate responses of POM and MAOM may be dependent on whether soil pH also changes with N fertilization. Averill and Waring (2018) provided the carbon, acidity, and mineral protection (CAMP) hypothesis, which suggests that N addition without a change in soil pH will release microbial N limitation and allow for efficient C use which they expect to form MAOM through the *ex vivo* pathway. In contrast, if pH changes with N addition, they expect reduced microbial decomposition and assimilation to cause reduced POM decomposition and MAOM formation (Averill and Waring 2018). The CAMP hypothesis has mixed support. The effect of N addition on microbial carbon use efficiency (CUE) is dependent on CUE measurement method and may be due to weakened mineral interactions, rather than reduced microbial N limitation (Feng et al. 2022; Hu et al. 2022). Thus, greater CUE under N addition with no change in soil pH may not support MAOM formation, as suggested by Averill and Waring (2018). In a subtropical forest, N addition did acidify the soil, likely driving reduced microbial biomass and increased C in POM and decreased C in MAOM, strongly supporting the CAMP hypothesis (Chen et al. 2020). Yet,

in a temperate grassland, C limitation, rather than acidification, drove reduced microbial activity with N addition (Ning et al. 2021). A meta-analysis found that light fractions (considered POM) responded more strongly to N addition than heavy fractions (considered MAOM), although both responded positively, potentially suggesting reduced decomposition of both POM and MAOM or a non-microbial pathway of MAOM formation with N addition (Chen et al. 2018b). Further work explicitly focusing on how the responses of POM and MAOM to N addition vary with climate and soil factors should help elucidate these disparate responses.

3.2.5. Multiple global changes

In reality, none of these global changes occur in isolation, so understanding their combined effect on SOM will be important for future C and N cycling. Unfortunately, given the large amount of time and financial resources needed to run global change experiments with multiple factors, we have limited data from studies of multiple global changes. While two-factor global change studies may have additive effects, three- and four-factor studies have demonstrated non-linear (e.g., synergistic or antagonistic) responses to global changes (Larsen et al. 2011; Reich et al. 2020; Shaw et al. 2002; Song et al. 2019; Yue et al. 2017). This suggests that it will be difficult to predict responses of combined drivers from single global change experiments. A four factor experiment in a temperate grassland confirmed the multiple limitation hypothesis, which suggests that adding limiting resources in tandem (e.g., energy in the forms of heat and C, and N) should promote ecosystem processes (Reich et al. 2020). Reich et al. (2020) found that combinations of warming, eCO_2 and N fertilization increased net primary production more so than respiration, which increased plant biomass. Greater plant biomass might contribute to greater plant inputs which has been shown to increase both MAOM and POM C (Zhang et al. 2022a). Reich et al. (2020) also found support for the CO_2-induced water savings hypothesis, in

which higher CO_2 allows for less water loss during photosynthesis. Reduced negative effects of reduced precipitation with elevated CO_2 could allow for continued plant growth with altered precipitation which could sustain POM and MAOM pools. However, without more data from disparate ecosystems, it will be difficult to predict the influence of multiple global change factors on POM and MAOM.

3.3 Research needs for SOM responses to global change

Given our relatively new understanding of SOM formation, there is a need to evaluate POM and MAOM responses to global changes. Specifically, we require more understanding about: (1) whether separating soils into POM and MAOM is useful for understanding global changes; (2) whether there are predictable patterns of responses of POM and MAOM to global change at the global scale; (3) whether changes in POM and MAOM under global change can be related to plant and microbial drivers. By investigating these three lines of questioning, we can determine whether, in relation to global change, fractionation is a worthwhile use of time and resources, POM and MAOM have predictable responses and controls that could be integrated into models, and POM and MAOM provide mechanistic understanding. While there is certainly evidence supporting POM and MAOM as worthwhile, predictable, and mechanistic (Cotrufo et al. 2019; Haddix et al. 2016; Robertson et al. 2019), we lack global, synthesized understanding and knowledge of local scale drivers of POM and MAOM responses to global change.

4. Nitrogen deposition and human activities

The deposition of N was first recognized as a major ecological threat in the United States in the 1980s, as studies in the northeast U.S. found that acid rain N contributed more N than needed for plant demand, with concern for N losses through leaching and nitrous oxide emission (Aber

1998; Aber et al. 1989). Despite reduced N emissions in the U.S., N deposition is still elevated relative to pre-industrial times and is projected to continue growing in other areas of the world, such as southeast Asia (Kanakidou et al. 2016; Li et al. 2016). Multiple N deposition monitoring networks have been setup to monitor U.S. N deposition and similar networks exist in other countries (Zhang et al. 2021). These networks have been extremely useful in documenting reduced N deposition in the U.S. over time and also a change in the character of N deposition (e.g., oxidized vs reduced N). Since the 1980s oxidized forms of N deposition have decreased while reduced forms of N deposition have increased, making NH_4^+ and NH_3 the dominant forms of inorganic N deposition in the U.S. (Li et al. 2016). Deposition of organic N is less studied but estimates suggest approximately a third of N deposition, on average, is from organic N sources (Neff et al. 2002). Monitoring both the amount and type of N deposition is crucial, as different forms of N can have unique impacts on ecosystem processes like plant and soil carbon storage (Lu et al. 2011; Yue et al. 2016).

Nitrogen deposition networks focus on monitoring regional trends but likely miss hotspots of N deposition from power plants and urban activities (Elliott et al. 2009; Wetherbee et al. 2019). Thus, there is a need to study fine scale N deposition and the influence of local sources on nearby ecosystems. Achieving this fine scale understanding will allow for policy to manage the causes of N deposition to reduce human influence on ecosystems. Like with SOM responses to global change, we lack context-dependent local understanding of N deposition in many systems.

5. The focus of this dissertation

Given the influential effects of global change on terrestrial ecosystems and the relevance of land responses to future climate, this dissertation seeks to improve our understanding of the responses of soil and plant C and N cycling to global changes. I investigate these questions

across scales. To address the lack of global data on POM and MAOM responses to global change, I conduct both a meta-analysis (Chapter 2) and collaborate with a global network of grassland nutrient addition experiments (Chapter 3). These studies fill research gaps about the relevance of studying and the general and context-dependent responses of POM and MAOM to global change. To determine local scale drivers of terrestrial ecosystem responses to global change, I investigate plant and soil biogeochemical responses to an altered precipitation experiment (Chapter 4) and determine the importance of proximity to high traffic roads for N deposition in a subalpine forest ecosystem (Chapter 5). These fill research gaps of whether POM and MAOM responses to global change can be connected to plant responses and the importance of highly local N sources for N deposition in ecosystems. Altogether, this dissertation improves our understanding of how soil and plant C and N cycling will be influenced by global environmental change by investigating this topic at both global and local scales.

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CHAPTER 2: SOIL ORGANIC CARBON RESPONSE TO GLOBAL ENVIRONMENTAL CHANGE DEPENDS ON ITS DISTRIBUTION BETWEEN MINERAL-ASSOCIATED AND PARTICULATE MATTER: A META-ANALYSIS[1]

1. Introduction

Soil organic carbon (SOC) accrual has the potential to play a key role in mitigating the climate crisis (Bossio et al. 2020), but SOC is also vulnerable to loss (Sanderman et al. 2017). Global environmental changes, such as increased nutrient pollution, elevated atmospheric carbon dioxide (CO_2), warming, and changing precipitation regimes, have the potential to change SOC storage with feedbacks to climate change (Pendall et al. 2008). Beyond its role in climate regulation, SOC is also important for ecosystem health and functioning, providing nutrients and energy for plant growth and soil organisms, among many other functions (Milne et al. 2015). Understanding effects of global changes on SOC is crucial for accurate model predictions of carbon-climate feedbacks and to inform better ecosystem management to mitigate negative impacts of global changes.

Many studies have summarized SOC response to different global changes and often find little to no SOC response (Lu et al. 2013; Yue et al. 2017; Zhang 2015) or that responses are variable and strongly dependent on environmental parameters (i.e., moderating effects; De Graaff et al. 2006; Hungate et al. 2009; Lu et al. 2011; Sillen and Dieleman 2012). Contrasting or limited SOC responses to global changes in individual studies may result from opposing mechanisms of formation and loss of SOC component fractions. Because different components of bulk SOC can

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form and persist through different mechanisms, measuring bulk SOC may not be sensitive enough for revealing the mechanistic drivers of overall SOC responses (Cotrufo et al. 2019; Lavallee et al. 2020), and may even be misleading because different fractions of SOC often exhibit contrasting responses to global changes (Chen 2020; De Feudis et al. 2019; Hofmockel et al. 2011; Neff et al. 2002). Thus, there is an urgent need to understand how major SOC fractions respond to global changes, as their unique responses have implications for the underlying drivers of SOC change.

Separating SOC into mineral-associated organic carbon (MAOC) and particulate organic carbon (POC; Cambardella and Elliott 1992) is a simple, efficient method that provides significantly more mechanistic information than bulk SOC alone (Cotrufo et al. 2019; Lavallee et al. 2020; Poeplau et al. 2018). MAOC is defined as the SOC fraction associated with silt- and clay-sized minerals; it is largely made of microbially-derived and low molecular weight compounds (Kleber et al., 2015). In contrast, POC is made mostly of structural plant (i.e. lignin) and fungal materials (Six et al. 2001). Thus, POC is expected to be higher under high structural plant inputs and low microbial decomposition rates, while MAOC is higher under efficient microbial transformation of labile plant inputs in soils with high matrix capacity (Castellano et al. 2015; Cotrufo et al. 2013). Therefore, following the fate of POC and MAOC under global changes can inform on the specific impacts on plant inputs or microbial transformations better than the bulk SOC, whose response is a weighted average of those of POC and MAOC.

Individual studies that separate these fractions find contrasting results and are difficult to generalize. Some studies find increased POC and decreased MAOC in response to N fertilization (Chen 2020; Fang et al. 2014), while others find the opposite (Cenini et al. 2015; Cusack et al. 2011; Neff et al. 2002), or the same effects on both (Borges et al. 2019). These inconsistencies

are found in response to other global changes as well, with some studies finding positive responses of MAOC to elevated CO_2 (Jastrow *et al.*, 2000) and warming (He *et al.*, 2012; Poeplau *et al.*, 2017) and others finding negative or no responses of MAOC and positive responses of POC to elevated CO_2 (Hofmockel *et al.*, 2011), warming (Cheng *et al.*, 2011), and increased precipitation (Song *et al.*, 2012). Yet other studies find no response of either fraction to precipitation (He *et al.*, 2012), or increases in both fractions under elevated CO_2 (Hoosbeek and Scarascia-Mugnozza, 2009), although there is less literature evaluating the response of SOC fractions to these global changes than to N fertilization.

Global changes may modify POC and MAOC by altering either plant input or microbial decomposition, or their interaction. Global changes that increase plant input to the soil such as N fertilization or elevated CO_2 could increase SOC storage, particularly in the plant-derived POC pool (Table 2.1). However, global changes that increase microbial activity will likely accelerate POC decomposition, as microbes process available C sources, but may also stimulate MAOC formation from mineral-association of microbial products. This will likely lead to an overall decrease in total SOC, as soil microbes are unable to transform POC to microbial products with 100% efficiency (carbon use efficiency ≈ 30% in natural systems; Sinsabaugh *et al.,* 2013), so potential MAOC increases would not make up for POC losses (Table 2.1). The relative strength of global change effects will be moderated by edaphic factors such as climate, vegetative cover, soil type, etc. For example, global changes have unique effects in specific climates, such that we might expect stronger warming effects on POC in colder climates where POC is preserved through microbial temperature limitation (Zhang *et al.*, 2015; Vaughn and Torn, 2019), or greater elevated CO_2 effects on MAOC in moist soils where drought would not limit microbial growth (Garten et al. 2009). Additionally, both soil properties and land cover types can exhibit

first order effects on the distributions of POC and MAOC, which may make MAOC more sensitive to all global changes in clay-rich, deep or cropland soils, where MAOC is generally dominant (Cambardella and Elliott, 1992; Schrumpf *et al.*, 2013; Cotrufo *et al.*, 2019; Lugato *et al.*, 2021). Environmental properties may also uniquely modify responses to specific global changes. Different N fertilizer types acidify the soil to different extents (Tian and Niu 2015), which should moderate N fertilizer impacts on MAOC and POC because acidification can inhibit microbial activity, slowing POC decomposition and MAOC formation (Averill and Waring 2018). Additionally, N availability often moderates the responses of soil C to elevated CO_2 (Hungate et al. 2009), and amount and type of warming may modify soil, microbial, and plant C pools (Lu et al. 2013), likely influencing MAOC and POC responses as well.

To summarize the current knowledge and explore the effects of environmental properties on the sensitivity of MAOC and POC to global changes, we performed a systematic meta-analysis on N fertilization, elevated CO_2, warming, increased precipitation, and their combined effects. Overall, we expect POC to be more responsive than MAOC, because it is formed directly from plant inputs and is more readily accessible for decomposition by microbes. We expect the responses of POC and MAOC to be moderated by variation in the environmental factors (i.e. moderators) that drive the specific formation and persistence of each fraction (Table 2.1). Our study reveals the importance of separately considering responses of MAOC and POC to improve understanding of the mechanistic drivers of changes in SOC storage under global change.

Table 2.1. Hypotheses for responses of total soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC) to global changes with expected drivers and moderating influences. Positive, negative, and no response to global changes are indicated by "↑", "↓", and "–", respectively. Key moderators expected to be important for all global change factors are in the "All global changes" row.

Global change factor	Expected responses	Expected drivers	Expected key moderators
Nitrogen fertilization	↑SOC ↑MAOC ↑POC	Higher quantity and quality of plant input, more efficient microbial C use	Soil pH, N fertilizer type
Elevated CO_2	↑SOC –MAOC ↑POC	Higher quantity but lower quality plant input and greater root input, possible N limitation of efficient microbial C use	N availability (N-fixers, soil N, N fertilization)
Warming	↓SOC ↑MAOC ↓POC	Microbial activity increased in excess of plant inputs	Degree of warming, type of warming
Increased precipitation	↓SOC ↑MAOC ↓POC	Microbial activity increased in excess of plant inputs, increased DOC and subsequent association with mineral surfaces	
All global changes			Climate (MAP, MAT, latitude), land cover type, soil properties (depth sampled, soil texture), experiment length, plant inputs (above- and below-ground biomass)

2. Methods

2.1. Study Selection

We conducted a systematic meta-analysis of SOC pool responses to global environmental changes using two databases, the Web of Science Core Collection and the ProQuest Agricultural and Environmental Science Database. Search terms used Boolean combinations of SOC terms and global change terms (Appendix 1, Supplementary Table 1.1), to target studies that employed both a global change experiment and SOC fractionation. Studies were limited to those in English. We considered N fertilization, warming, elevated CO_2, and increased precipitation as global

environmental changes, in line with previous meta-analyses assessing global changes (Song et al. 2019; Yue et al. 2017). The lack of studies on the effects of decreased precipitation (i.e., drought) on MAOC and POC precluded us from incorporating this factor in our study. Studies that addressed multiple global change factors were retrieved from searches for individual global change factors. We included any soil depth, study environment (greenhouse, field, laboratory), and land cover type (cropland, grasslands, forests) to maximize the amount of data. We acknowledge that N fertilization is often integral to cropland management. However, we refer to N fertilization as a global change also in croplands since we evaluate SOC pool responses with and without N fertilization, such that the premise is the same, regardless of the system. With this search criteria, we retrieved 3,378 studies, which we then filtered in two ways. First, we analyzed abstracts of all studies and kept those that met the following criteria: (1) they were primary literature (not reviews, meta-analyses, or book chapters), (2) they appeared to measure SOC fractions that were physically separated as, or could be combined to, MAOC and POC, as explained below, (3) they measured the response of POC and/or MAOC and SOC to a global change factor with a control that was not treated with that global change factor, and (4) they were not duplicate studies. Following this initial study filtering we were left with 168 total studies which included data for 216 individual global change experiments (since some studies addressed multiple global change factors).

Following initial filtering of the abstracts, we downloaded full PDFs for all studies and performed a second filtering based on whether they met our quality criteria and MAOC and POC definitions, as follows. To be included in this study, SOC, MAOC, and/or POC needed to be measured for at least three replicates, to provide quality and robustness to our dataset. Soil had to be sieved to 2 mm or less and dispersed using either sonication, sodium hexametaphosphate, or

shaking with glass beads such that aggregates would be adequately broken down. To be considered MAOC, the dispersed soil fraction had to be smaller than 50-63 μm, if separated by size, or heavier than 1.6-1.85 g cm^{-3}, if separated by density (Lavallee et al. 2020; Sollins et al. 2009). POC was defined as the complement to MAOC, such that dispersed soil fractions larger than 50-63 μm and lighter than 1.6-1.85 g cm^{-3} were considered POC (Lavallee et al. 2020; Sollins et al. 2009). The method used to separate POC and MAOC may slightly affect their properties, since the heavy, coarse C fraction is considered POC when separating by size, and MAOC when separating by density. Yet, this is a minor fraction of SOC, thus when methods were compared, POC and MAOC fractions derived from size or density separation had very similar C contents and turnover times across soil textures (Poeplau et al. 2018). Additionally, MAOC generally has greater microbially derived compounds and lower C:N than POC (von Lützow et al. 2007; Williams et al. 2018), regardless of separation approach. To determine its influence in our dataset, we tested for the effect of fractionation method in our analyses, as described below. We acknowledge that typical aggregate dispersion methods will not necessarily breakdown highly stable silt- and clay-sized aggregates, but that these have similar effects of protection as mineral association and likely contain mineral-associated C (Chenu and Plante 2006; Lavallee et al. 2020; Virto et al. 2008; Virto et al. 2010; von Lutzow et al. 2007). Additionally, small reciprocal contaminations of POC and MAOC may occur, however their effects should be negligible. For more complex fractionations that still employed cutoffs for size and density defined above, fractions were summed to total MAOC and POC pools based on the above definitions. Heavy, coarse SOC fractions (i.e. >1.6 g cm^{-3} and > 50-63 μm; typically < 10% of total SOC) were added to the POC pool, due to their relatively fast turnover times (Poeplau et al. 2018) that suggest lack of stabilization via mineral association.

We extracted data from tables using Tabula software (https://tabula.technology/) or from figures using the metaDigitize package in R (Pick et al. 2018; R Core Team 2019). If crucial data were missing, we contacted authors and if no response was provided by May 2020 (after 2-3 attempts at contact) papers were removed from the analysis. If error type (standard deviation *versus* standard error) was not presented with the data, and the author did not respond, we assumed error type to be standard error, for more conservative error estimates. Following the second filtering, we were left with a final count of 605 observations from 98 total studies across the world ranging in publication years from 1994 to 2019, with 428 observations and 52 studies focusing on N fertilization, 42 observations and 15 studies on warming, 42 observations and 24 studies on elevated CO_2, 18 observations and 5 studies on increased precipitation, and 57 observations and 17 studies that addressed multiple global change factors (Figure 2.1; Appendix 1, Supplementary Table 1.2). For these final studies, we extracted data on soil organic matter C (SOC, MAOC, and POC; g C kg $soil^{-1}$), global change factors (years under factor, level of factor, etc.), and environmental variables (soil type and depth, plant biomass, climate, etc.; for complete dataset see link below). Some data of interest, notably data for microbial activity and biomass, were not reported frequently enough to extract. Where only total soil C was reported and soil pH was below 7 (Wang *et al.*, 2015), we assumed inorganic carbon was negligible, and thus recorded total soil C as SOC. Two studies with pH above 7 did not clearly indicate separation of inorganic and organic C but these were not influential in our study, according to the sensitivity analysis described below. We only included reported fraction data and did not calculate fractions by difference (e.g. SOC-POC = MAOC), as this could produce errors due to incomplete or over-recovery during fractionation. Using only reported data led to different samples sizes for SOC,

MAOC, and POC. The full dataset is freely available on the Environmental Data Initiative (Rocci et al., 2021).

Meta-analytical analysis was only performed when 3 or more studies were available, allowing us to statistically assess the following global changes: N fertilization, warming, elevated CO_2, increased precipitation, N fertilization × elevated CO_2, warming × elevated CO_2, and warming × increased precipitation. When a global change factor was assessed in a multi-factor study, data was used for each factor individually, as well as in the multiple factor analysis. Multiple levels of a global change factor, or studies sampled at multiple time points or under different vegetation, were counted as individual observations. Statistical methods to address this non-independence are discussed in Section 1.3.

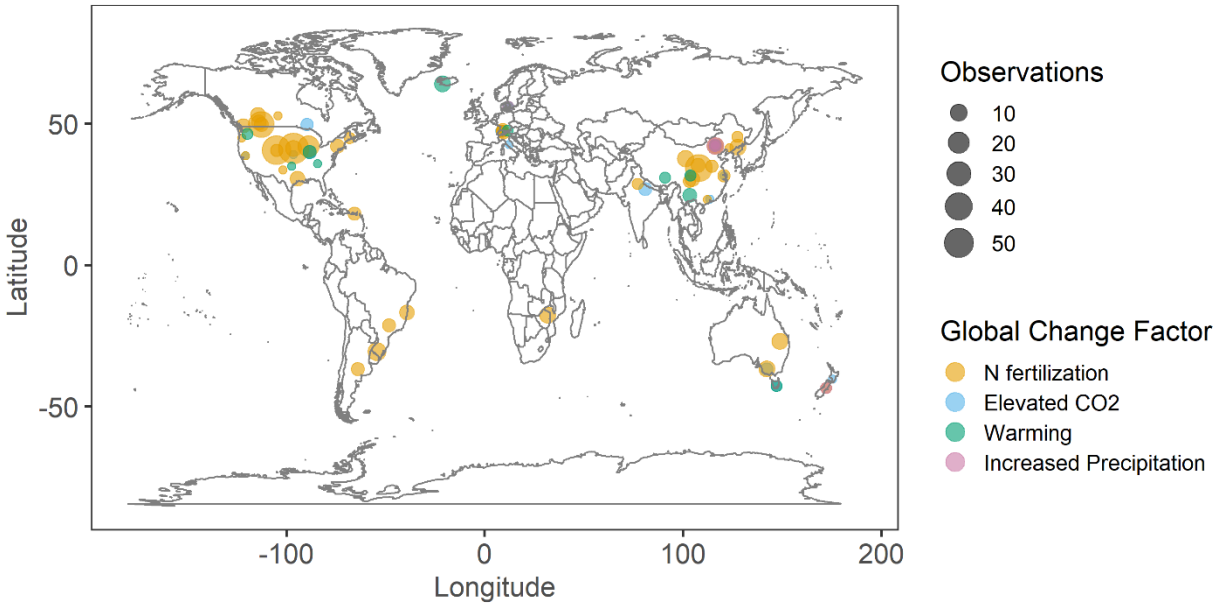

Figure 2.1. Locations for observations used in meta-analysis of the response of soil organic carbon, mineral-associated organic carbon, and particulate organic carbon to global environmental changes. Size of points depicts the number of observations and color depicts the global change factor imposed.

1.2 Data Analyses

We chose C concentration as the response variable for bulk soils (g C in soil organic matter kg $soil^{-1}$), POC (g C in particulate organic matter kg $soil^{-1}$), and MAOC (g C in mineral associated organic matter kg $soil^{-1}$) rather than C stock, which is more commonly used in meta-analysis, because 50% of the studies did not report bulk density values. We chose to avoid imputing bulk density values using a pedotransfer function because these have been shown to introduce considerable bias to bulk density estimates (Kaur et al. 2002). Further, removing studies with missing bulk density values would have limited our number of studies. Where stocks were reported, bulk density was almost always measured, so converting from stocks to concentrations was possible. When only stock values were presented and bulk density values were not provided (n=13 observations; < 3% of N fertilization and warming, 0% for elevated CO_2 and increased precipitation), effect sizes were calculated on stocks, which would provide the same effect size as for concentration, assuming no change in bulk density between the global change factor and the control. Stocks were converted to concentrations using the following equation (rearranged from Guo and Gifford, 2002):

$$C_c = C_s / (B_d \times d \times cf) \quad \text{(Eq 1)}$$

Where C_c is the concentration of C in g C kg $soil^{-1}$, C_s is the C stock, B_d is the bulk density in kg m^{-3}, d is the depth of the soil sampling in m, and cf is a conversion factor depending on units of C_s. For example, if C_s was in units of Mg C ha^{-1}, cf would be 100, to convert Mg to g (multiply by 10^6) and ha to m^2 (divide by 10^4).

For a small number of studies (< 10% of N fertilization, < 5% of warming, 0% of elevated CO_2 and increased precipitation) error values were not reported. For these studies, error was imputed using the equation:

$$\widetilde{SD}_j = \bar{X}_j \left(\frac{\sum_i^K SD_i}{\sum_i^K \bar{X}_i} \right) \quad \text{(Eq 2)}$$

Where $\bar{X}_j$ is the observed mean of the study missing its standard deviation, K is the number of studies with complete information, and $\bar{X}_i$ and SD_i are the mean and standard deviation, respectively, for the i^{th} study with complete information.

To ensure normality, we used the log response ratio (LRR) of each study (Hedges et al. 1999) as the measure of effect size:

$$LRR = \ln(\bar{X}_{trt}) - \ln(\bar{X}_{cntrl}) \quad \text{(Eq 3)}$$

Where, $\bar{X}_{trt}$ is the mean value of the global change factor (i.e. N fertilization) and $\bar{X}_{cntrl}$ is the mean value of the control. We then back transformed these values to mean percent change (MPC) to ease interpretation using the below equation:

$$MPC\ (\%) = (e^{LRR} - 1) * 100\% \quad \text{(Eq 4)}$$

1.3 Meta-analytical statistics

We fit overall models for SOC, MAOC, and POC for each global change factor. Overall effect sizes were considered significant if the 95% confidence interval did not overlap zero. To account for non-independence of effect sizes from the same experiment, either over time or due to multiple levels of a global change factor, we fit a modified three level meta-analytical model as described by Assink and Wibbelink (2016) using the metafor package in R (R Core Team 2019; Viechtbauer 2010). This model allows for maximum retention of data with adequate accounting for non-independent responses, without the requirement of individual study covariances, which are rarely reported. Our model accounts for three levels of variability; (1) sampling variance of each effect size, (2) variance between effect sizes extracted from the same

study nested within the same experiment, and (3) variance between effect sizes of experiments. To ensure this model was appropriate, as opposed to a univariate model that only accounts for the first level of variability, we performed two log-likelihood-ratio tests comparing the full model (with all three levels) to reduced models omitting either within-experiment or between-experiment variance. We found that for the majority of our studies, the test indicated that the variance components (within or between) were significantly greater than zero. In addition, sampling variance of each effect size accounted for less than 75% (percent allocated to sampling variance = 1.2-45.3%) for all models, indicating the need to account for the other forms of variance (Hunter and Schmidt 1990).

We found significant between- and within-study variance, so it was logical to proceed with moderator analysis. Because we used a restricted maximum likelihood estimation method, we could not compare models with and without moderators using traditional log-likelihood ratio tests. Rather, we used an omnibus test to test whether there was a significant moderating effect, where at least one level of the moderator had a regression coefficient significantly different from zero, as compared to the null hypothesis of all regression coefficients being equivalent to zero (Assink and Wibbelink 2016). We used this test for both continuous and categorical moderators to determine whether responses of the soil fractions to global changes were dependent on variation in the level of the moderator. For continuous variables, we assessed the relationship between variation in the moderator and LRR, rather than the MPC, because the transformation to MPC could make the regression difficult to interpret. We tested some moderators that required categorization by us, and used previous meta-analyses, our data structure, and scientific knowledge for categorization and the method of Assink and Wibbelink (2016) for binning.

We tested key moderators for specific global changes as outlined in our hypotheses (Table 2.1). In addition, we tested soil (texture, depth sampled), climate (mean annual temperature [MAT], mean annual precipitation [MAP], latitude), and vegetation (above- and belowground biomass) variables, experiment length, and fractionation method, for all global change factors. Experiment length and soil, climate, and vegetation variables are known to be important drivers of SOM formation and stabilization (Cotrufo et al. 2019; Wiesmeier et al. 2019) and fractionation method is a key methodological consideration of our study (Appendix 1, Supplementary Table 1.3 and 1.4). For soil depth, we separated samples into surface (< 10 cm), subsoil (10-30 cm), and deep soil (> 30 cm). Our data was skewed towards shallow depths, which necessitated a very shallow grouping of 0-10 cm, whereas 30 cm was chosen based on previous meta-analyses (Guo and Gifford 2002). For elevated CO_2, there was only one study in the deep soil group, so the sub- and deep soil groups were combined as "deeper" soil, and for warming, there were no deep soil studies. Additionally, we created a "profile" group (< 10 cm to > 10 cm) comprising data from studies that did not distinguish among our defined soil layers, and rather, separated the soil samples at a depth other than 10 or 30 cm (e.g., 5-15 cm or 0-40 cm). Experiment length was split into ≤ 5, > 5, and > 10 years, where applicable, in line with other meta-analyses (Bai et al. 2019; Johnson and Curtis 2001). Nitrogen fertilizer type was split into organic (urea, compost, manure), inorganic (nitrate, ammonium, ammonium nitrate) and combined, based on the definitions of organic and inorganic N.

We began by testing each moderator individually with each global change-fraction pairing and performed pairwise comparisons of each level of the moderator. Then, to determine how robust these relationships were, we added all significant moderators for a given global change-fraction pair into one model as suggested in Assink and Wibbelink (2016). If the

moderators were still significant following the multiple moderator test, we considered those relationships robust (Assink and Wibbelink 2016).

To evaluate publication bias for overall responses, we used Egger's regression test modified for multivariate meta-analysis (Habeck and Schultz 2015). This test evaluates the intercept of a regression of standardized effect sizes against inverse standard error. A significant ($p < 0.1$), positive intercept indicates that larger effect sizes are associated with studies with lower precision, implying publication bias (Egger et al. 1997). To evaluate publication bias further, we visually assessed histograms for skew towards very positive or very negative effects. Positive skew indicated that there was a tendency toward large effect sizes, suggesting publication bias (Basche and DeLonge 2019).

To determine how robust the overall responses were, we performed a sensitivity analysis. We used a jackknife procedure, which removed individual studies from each global change factor-SOC pool pair and recalculated the mean effect sizes to determine the influence of the removed study (Basche and DeLonge 2019). If the removal of the study changed the significance of the overall effect size, it was considered influential. When reporting the results of this sensitivity analysis, we determine robustness of the overall dataset based on the number of studies that modified the significance, but still retained all of the data in our analysis.

3. Results

3.1. Data Availability

The majority of studies used in this meta-analysis evaluated the response of SOC fractions to N fertilization (53%), with most other studies evaluating elevated CO_2 (24%) or warming (15%). The remaining 8% of studies were split between increased precipitation, N fertilization × elevated CO_2, warming × elevated CO_2, and warming × increased precipitation.

Data for combined global changes can be found in the Appendix 1 (Appendix 1, Supplementary Figure 1.5 and 1.6). There were not enough studies to evaluate any other combination of global change factors, including interactive effects. In addition, the majority of the studies were carried out in temperate and subtropical ecosystems (Figure 2.1), as is common for meta-analyses of ecological studies (e.g. Basche et al. 2014; Guo and Gifford 2002; Poeplau and Don 2015). Lastly, inconsistent reporting of covariates and lack of certain measurements prevented us from fully evaluating all moderators that we considered important drivers of MAOC and POC dynamics. Most notably, there was a dearth of information on plant and microbial inputs, as represented by aboveground and belowground plant biomass and microbial biomass, and soil CO_2 and methane emissions.

3.2. Nitrogen Fertilization

There was a significant positive effect of N fertilization on all SOC fractions, but the overall mean effect size was larger for POC (MPC = 13.17%; CI = 5.11 – 21.73%) than MAOC (MPC = 4.49%; CI = 0.327 – 8.82%) or total SOC (MPC = 5.64%; CI = 3.00 – 8.37%; Figure 2.2). Egger's test indicated publication bias for SOC (int = 0.044, p = 0.001), MAOC (int = 0.042, p = 0.054), and POC (int = 0.153, $p < 0.001$) in response to N fertilization. Histograms for each of the N fertilization-SOC pool pairings were slightly positively skewed (Appendix 1, Supplementary Figure 1.1). Though these findings indicate that the effect size may be overestimated, the relatively small intercept estimates and minimal skew indicate that this overestimation may be minimal. The sensitivity analysis indicated these results were generally robust, although removal of two specific studies caused the MAOC CI to overlap with zero (Appendix 1, Supplementary Figure 1.2).

Soil depth sampled was a significant moderator for the response of SOC ($p < 0.001$) and POC ($p < 0.001$) to N fertilization but not for MAOC ($p = 0.559$). The response of POC switched from positive to negative with depth and the positive response of SOC became insignificant with depth (Figure 2.2). We could not explicitly test the moderating effect of N application method (e.g., surface-applied vs. banded) because it was not consistently reported. Where reported, N fertilizers were most often surface-applied, and in the few studies that used subsurface banding of N fertilizers, N was most often added to the top 10 cm of the soil. Thus, N application method was unlikely to drive variation in responses between our studies. Experiment length was 9.8 years on average and was a significant moderator for SOC ($p = 0.003$) and POC ($p = 0.028$) response to N fertilization but not for MAOC ($p = 0.297$). Experiments 5-10 years long and > 10 years had significantly greater positive MPC in SOC under N fertilization as compared to experiments ≤ 5 years long (Figure 2.2). Similarly, experiments > 10 years long had a significantly greater positive MPC in POC under N fertilization as compared to those 5-10 years long and ≤ 5 years long (Figure 2.2). Belowground biomass had a significant positive relationship with SOC LRR (coefficient = 0.118; $p = 0.008$) but not with POC (coefficient = 0.007; $p = 0.935$) or MAOC (coefficient = 0.067; $p = 0.692$) LRR (Appendix 1, Supplementary Figure 1.3). Relationships between POC MPC and soil depth sampled and experiment length were robust when combined in a multiple moderator model (Appendix 1, Supplementary Table 1.4). However, for SOC, only belowground biomass remained significant in the multiple moderator model, indicating that the relationships with soil depth sampled and experiment length should be regarded with caution (Appendix 1, Supplementary Table 1.4).

Although the overall moderating effect of land cover type, experiment type, and N fertilizer type were not significant, pairwise comparisons revealed significant differences

between some levels of these moderators (Figure 2.2). The MPCs in SOC under N fertilization in cropland and forest soils were significantly higher than zero but not for grassland soils, whereas the MPCs in POC in cropland and grassland soils were significantly greater than zero but not in forest soils. Similarly, the responses of all SOC fractions from field studies were significantly greater than zero, whereas the responses from lab studies were not. Results for land cover and experiment type may be related to higher observation numbers for croplands and field experiments, respectively. The responses of SOC and POC to inorganic N and organic N were significantly different from zero but the response to combined inorganic and organic N was not. Other moderators that were evaluated but showed no differences were initial or change in soil pH, climate [mean annual temperature (MAT) and mean annual precipitation (MAP)], latitude, fractionation method, and soil texture (% sand, silt, and clay; Appendix 1, Supplementary Table 1.3 and 1.4).

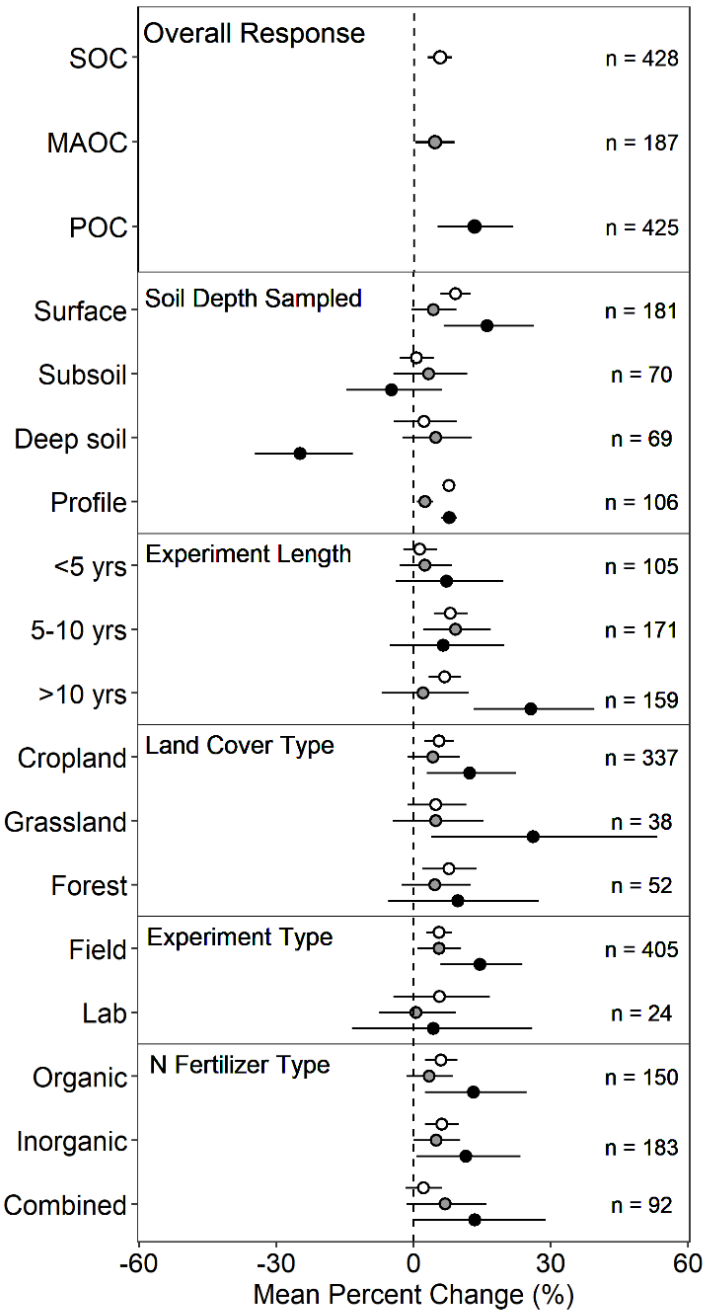

Figure 2.2. Mean responses of carbon concentrations (g C kg soil^{-1}) in total soil organic carbon (SOC, open symbol), mineral-associated organic carbon (MAOC, grey symbol), and particulate organic carbon (POC, black symbol) to nitrogen fertilization as moderated by soil depth sampled, experiment length, land cover type, experiment type, and N fertilizer type using published data with 95% confidence intervals and number of observations. Soil depth is defined as surface (< 10 cm), subsoil (10-30 cm), deep soil (> 30 cm), and profile (< 10 cm to > 10 cm).

3.3. Elevated CO_2

There was a tendency for increased POC under elevated CO_2 (MPC = 5.11%; CI = -0.76–11.32%) but no effect on MAOC (MPC = -0.83%; CI = -6.42 – 6.42%) and total SOC (MPC = -3.68%; CI = -13.85 – 7.69%; Figure 2.3). Egger's test indicated publication bias for POC (int = 0.065, p = 0.032) in response to elevated CO_2 and the histogram was slightly positively skewed (Appendix 1, Supplementary Figure 1.1). Though these findings indicate that the effect size may be overestimated, as for the response to N fertilization, the relatively small intercept estimate, and minimal skew indicate that this overestimation may be minimal. Egger's test did not indicate publication bias for SOC and MAOC in response to elevated CO_2 and histograms displayed normality (Appendix 1, Supplementary Figure 1.1). The sensitivity analysis indicated these results were generally robust, and removal of specific studies caused the POC response to become significantly positive (Appendix 1, Supplementary Figure 1.2).

The response of SOC to elevated CO_2 was moderated by soil depth sampled (p = 0.018) with a significantly smaller and negative response for observations from deeper soil layers than from those not separated by depth (i.e. profile; Figure 2.3). Experiment length was 5.1 years on average and significantly moderated the response of MAOC (p = 0.048) but not SOC (p = 0.614) or POC (p = 0.589). The response of MAOC was positive on average for experiments ≤ 5 years long but negative on average for experiments > 5 years long, although MAOC was not significantly different from zero for either timeframe (Figure 2.3). Additionally, while land cover type was not a significant moderator overall (p = 0.111), the response of POC in grasslands was significantly higher than the response in croplands (Figure 2.3). Other moderators that were evaluated but showed no differences between levels of moderators were measures of N availability (presence of N-fixers, N fertilizer amount, soil N), plant biomass (aboveground and

belowground), latitude, site climate (MAT and MAP), fractionation method, and soil depth and texture (Appendix 1, Supplementary Table 1.3 and 1.4).

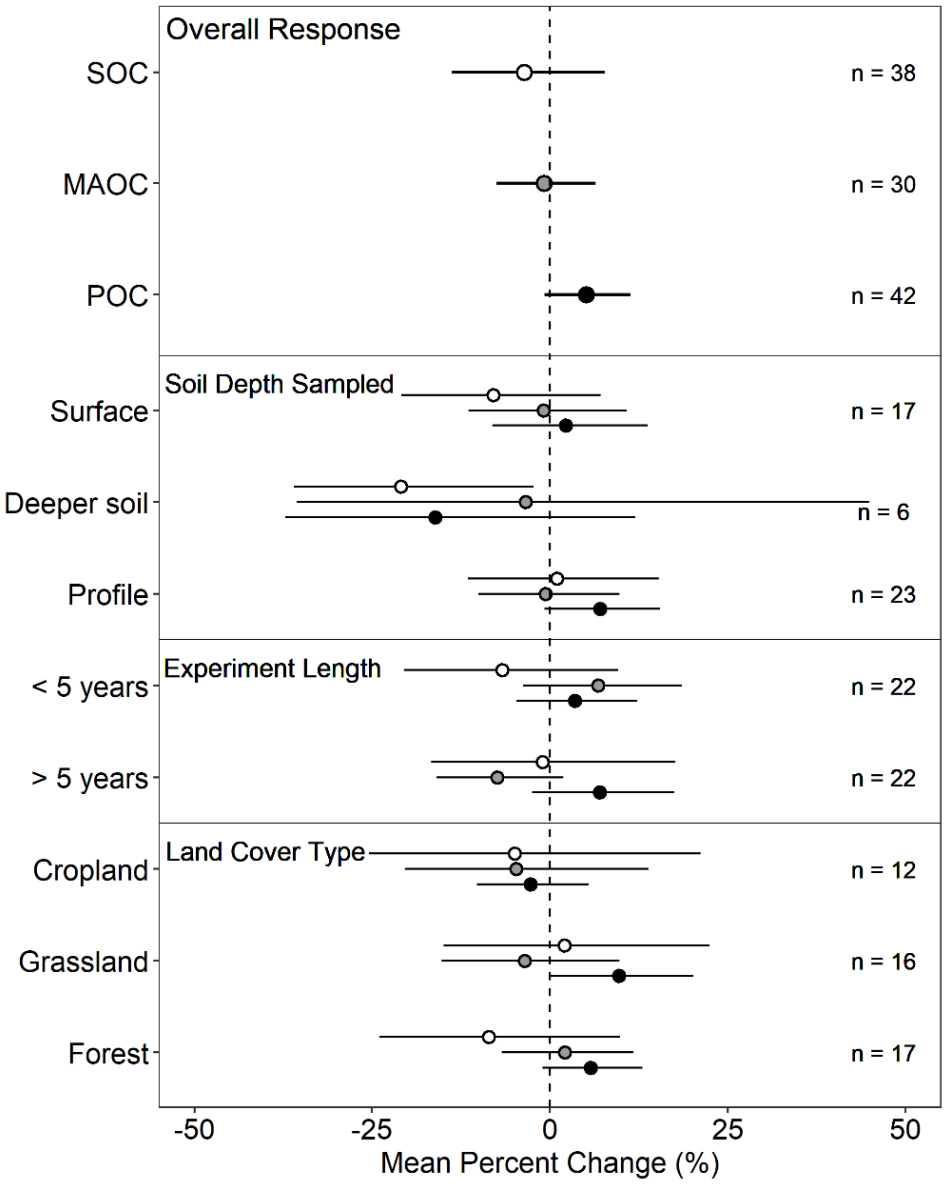

Figure 2.3. Mean responses of carbon concentrations (g C kg soil^{-1}) in total soil organic carbon (SOC, open symbol), mineral-associated organic carbon (MAOC, grey symbol), and particulate organic carbon (POC, black symbol) to elevated CO_2 as moderated by soil depth sampled, experiment length, and land cover type from published studies with 95% confidence intervals and number of observations. Soil depth is defined as surface (< 10 cm), deeper soil (> 10 cm), and profile (< 10 cm to > 10 cm).

3.4. Warming

There was a negative tendency of POC under warming (MPC = -10.05%; CI = -19.91 – 1.03%) but no clear effect on MAOC (MPC = -0.53%; CI = -9.59 – 9.45%) and total SOC (MPC = -1.15%; CI = -9.60 – 8.09%; Figure 2.4). Publication bias, as assessed by Egger's test and histograms, was not indicated for any fraction in response to warming (Appendix 1, Supplementary Figure 1.1). The sensitivity analysis indicated these results were generally robust, but removal of specific studies caused the POC response to become significantly negative (Appendix 1, Supplementary Figure 1.2).

The responses of soil fractions to warming were dependent on the degree of warming and whether air or soil was warmed (Figure 2.5). When soil was warmed there was a significant negative relationship between degree of warming and the LRR of all fractions (Figure 2.5). This relationship remained significantly negative for SOC and POC regardless of removal of influential points (Figure 2.5, inset). When air was warmed, the relationship between degree of warming and POC LRR changed from a negative relationship (coefficient = -0.014; p =0.048) to a positive relationship (coefficient = 0.198; p = 0.071) when influential points from extreme warming observations (> 4°C) in altitude transplant, incubation, and geothermal studies were removed (Figure 2.5, inset). This trend also appeared for SOC and MAOC, although they were not significant (Figure 2.5). There was also a relationship between POC LRR and latitude, whereby there was a negative response of POC at high latitudes, when including all observations (coefficient = -0.013; p = 0.005: Figure 2.6) or just those where air was warmed (coefficient = -0.011; p = 0.094; Figure 2.6, inset). When degree of warming for air or soil, and latitude were combined in multiple moderator models for POC, only degree of soil warming remained significant, regardless of the removal of extreme warming observations (Appendix 1,

Supplementary Table 1.4), suggesting relationships between POC LRR and degree of air warming and latitude should be regarded with caution. Land cover type was a significant moderator for MAOC responses to warming ($p = 0.026$), where there was a negative response on average in grasslands and a positive response on average in forests, although neither of these were significantly different from zero (Figure 2.4). When degree of soil warming and land cover type were combined in a multiple moderator model for MAOC, only degree of soil warming remained significant, suggesting the moderating influence of land cover type should be regarded with caution. Additionally, soil depth sampled had a significant moderating effect on the response of SOC to warming ($p < 0.001$) where the profile response was positive on average, whereas the response at the surface and subsoil were negative on average (Figure 2.4). However, none of these responses were significantly different from zero. When degree of soil warming and soil depth sampled were combined in a multiple moderator model for SOC, neither were significant, suggesting both relationships be regarded with caution. We were unable to evaluate the moderating influence of belowground biomass due to low data availability. Other moderators that were evaluated but showed no differences between levels were experiment length (avg = 4.8 years), aboveground biomass, site climate (MAT and MAP), fractionation method, land cover type, and soil texture (Appendix 1, Supplementary Table 1.3 and 1.4).

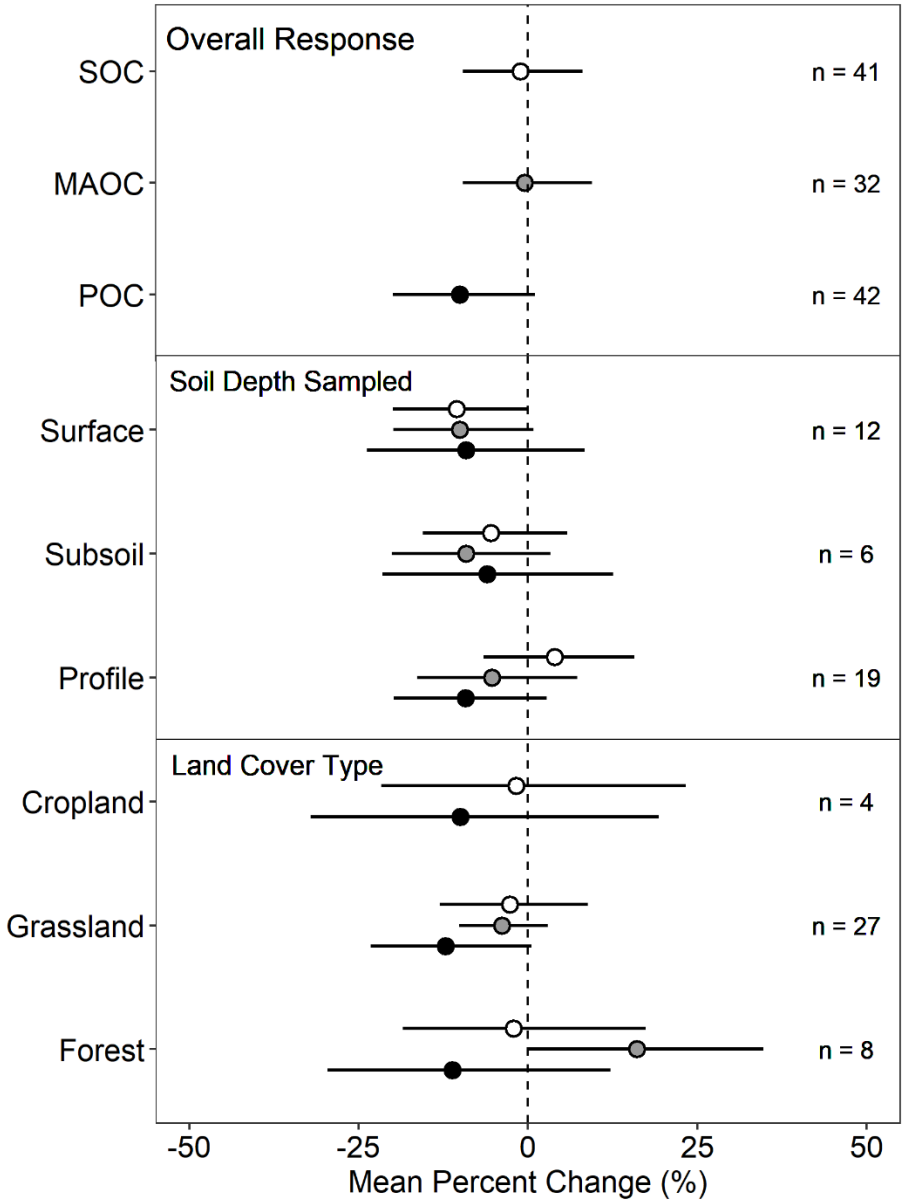

Figure 2.4. Mean responses of carbon concentrations (g C kg soil-1) in total soil organic carbon (SOC, open symbol), mineral-associated organic carbon (MAOC, grey symbol), and particulate organic carbon (POC, black symbol) to warming as moderated by soil depth sampled and land cover type using published data with 95% confidence intervals and number of observations. Soil depth is defined as surface (< 10 cm), subsoil (10-30 cm), and profile (< 10 cm to > 10 cm).

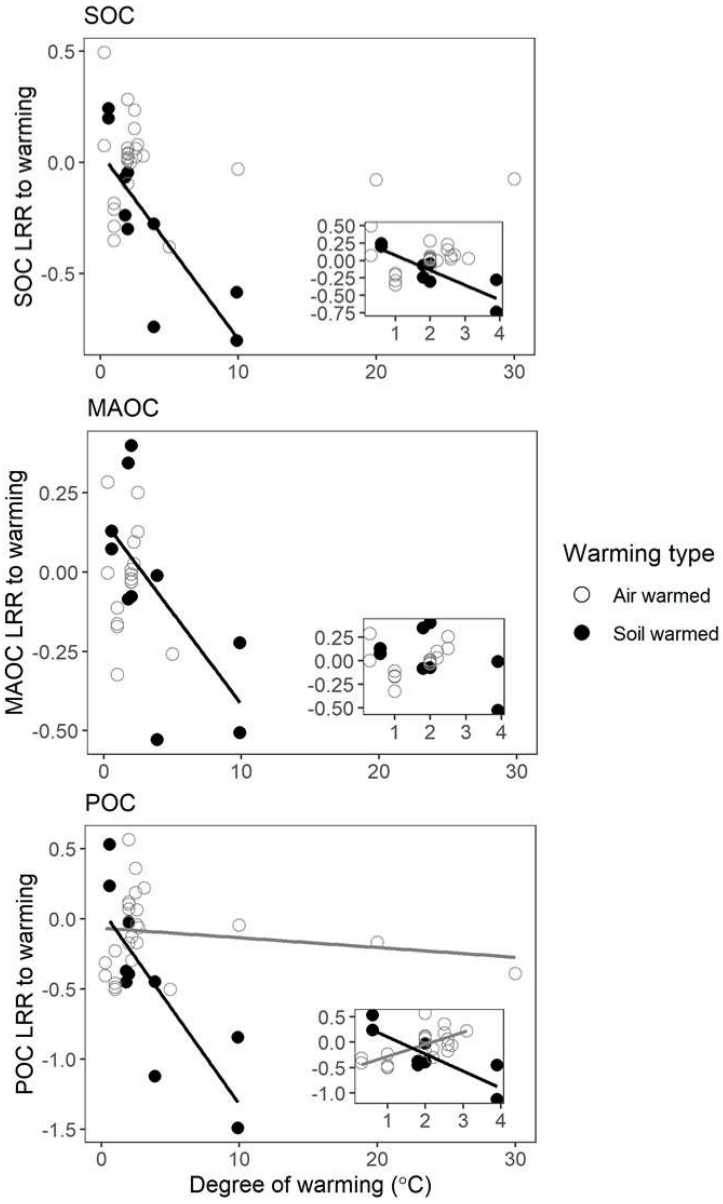

Figure 2.5. Log response ratio (LRR) of SOC, MAOC, and POC to warming regressed against the imposed degree of warming in experiments where air (open) or soil (closed) was warmed. Inset of each plot shows same relationship where extreme warming (> 4 °C) studies were removed. Significant regressions have linear regression lines. Acronyms are as in Figure 2 legend.

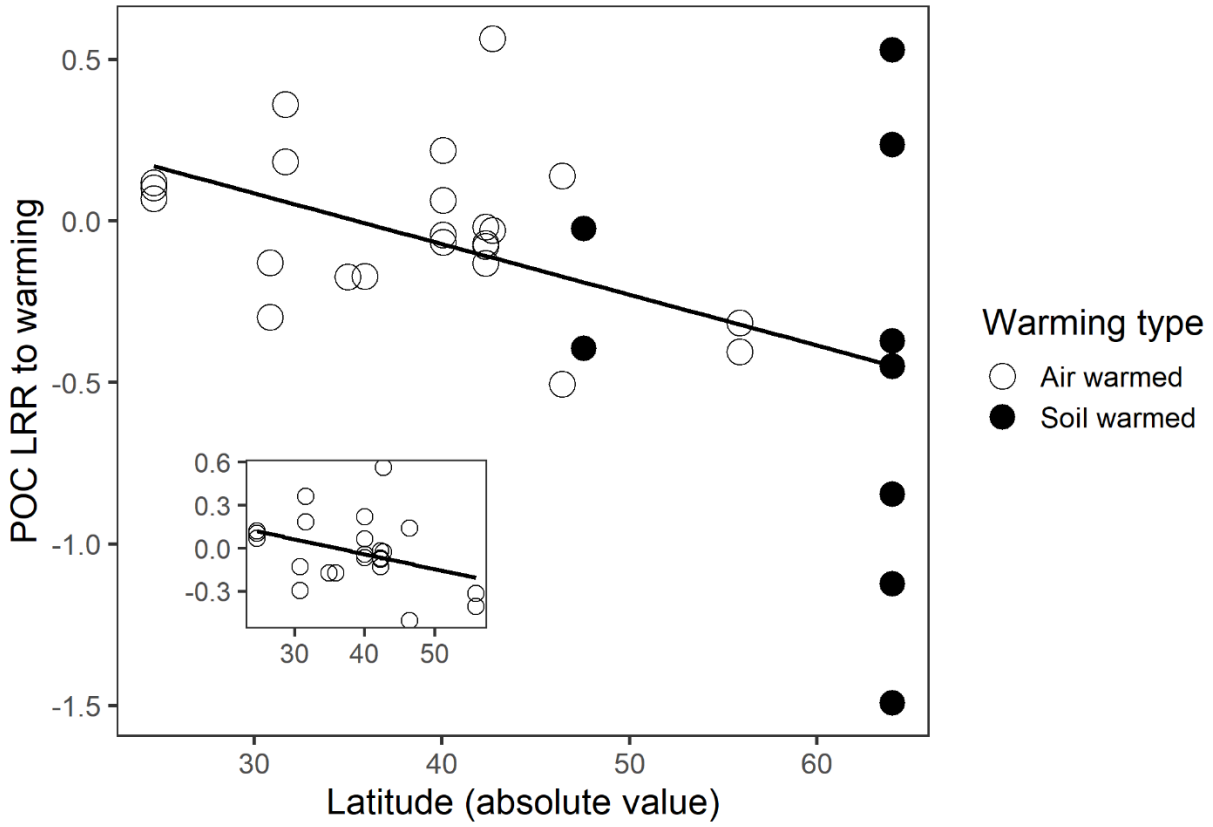

Figure 2.6. Log response ratio (LRR) of particulate organic carbon (POC) to warming regressed against absolute value of latitude where air (open) or soil (closed) was warmed. Inset of the plot shows same relationship where soil warmed points have been removed.

3.5. Precipitation

There were no clear effects of increased precipitation on C content in any soil fraction, although there was a weak negative tendency for POC (MPC = -8.35%; CI = -29.78 – 19.64%) and a positive tendency for MAOC (MPC = 10.21%; CI = -3.04 – 25.28%) and total SOC (MPC = 3.76%; CI = -3.00 – 10.99%; Appendix 1, Supplementary Figure 1.4). Egger's test did not indicate publication bias for any fraction in response to increased precipitation. Histograms were difficult to interpret due to small sample sizes (Appendix 1, Supplementary Figure 1.1). Sensitivity analysis indicated these results were generally robust (Appendix 1, Supplementary Figure 1.2). Experiment length was 6.2 years on average but could not be evaluated as a

categorical moderator due to low study numbers. Experiment length as a continuous variable, fractionation method, soil texture, and MAT did not significantly moderate the responses of SOC, MAOC, or POC (Appendix 1, Supplementary Table 1.3 and 1.4). Due to the combination of low study numbers and unreported data, we were unable to evaluate MAP, above- and belowground biomass, land cover type, and latitude as moderators. While we could not evaluate decreased precipitation with meta-analysis due to low study numbers, the LRRs of MAOC and POC were negative on average with high variability in POC responses (see dataset on the Environmental Data Initiative linked above). Only one of the decreased precipitation studies reported SOC in addition to MAOC and POC and found no response.

4. Discussion

By performing the first systematic meta-analysis of SOC fraction responses to multiple global changes, we found that POC and MAOC behaved in unique ways that could not be ascertained from bulk SOC responses. POC was the most responsive fraction, as predicted (Table 2.1), and responded most strongly to moderators. Because of the generally consistent responses across global changes, we broadly discuss the findings of the meta-analysis within the current understanding of processes controlling POC and MAOC formation and losses and propose possible mechanisms responsible for the observed changes. We then discuss implications of our findings for climate change feedbacks and suggest areas for future investigation. Overall, our results highlight the value of studying the responses of POC and MAOC, rather than bulk SOC, to understand the mechanisms underlying global change effects on SOC and provide guidance for future work.

4.1. Unique responses of POC and MAOC to global changes

It was not surprising to find that POC was the most responsive fraction to global changes, as average experiment lengths for each global change factor were between 4.8 and 9.8 years, well within POC turnover times of years to decades (Kögel-Knabner et al. 2008). We suggest that the heightened POC response is specifically due to the sensitivity of its formation and loss processes to global changes, which is also the reason why it has historically been chosen as a diagnostic fraction (i.e. Cambardella and Elliott 1992). Positive responses of POC to both N fertilization (Figure 2.2) and elevated CO_2 (Figure 2.3) are likely due to them stimulating plant productivity and increased structural plant inputs to soil (Cotrufo et al. 1994; Cotrufo et al. 1998; De Graaff et al. 2006; Nie et al. 2013; Norby et al. 2001; Xia and Wan 2008). Since plants are more N-limited than microbes (Soong et al. 2020a), the increase in plant input under N fertilization is likely to be stronger than that of microbial decomposition, which anyway is generally decreased under N fertilization due to lower microbial biomass and respiration (Treseder, 2008; Zhang *et al.*, 2018) and suppression of lignin-degrading enzyme activity (Frey *et al.*, 2014; Rinkes *et al.*, 2016) under N additions. Other studies report increased soil respiration and C mineralization under N fertilization, likely due to increased belowground C inputs from N fertilization-induced plant growth, but these responses have been found to be weaker than plant responses, leading to increased soil C (Lu *et al.*, 2011). Negative responses of microbial activity to N fertilization are often attributed to reduced soil pH (Averill and Waring, 2018; Chen *et al.*, 2020; Pan *et al.*, 2020) but we do not find that to be an important moderator in our study, suggesting that greater responsiveness of inputs over outputs is the most plausible explanation. Similarly, under elevated CO_2, increased plant inputs may simply outweigh increases in microbial respiration, which have been observed under elevated CO_2 (De Graaff et al. 2006). Our results largely agree with the only other meta-analysis we know of that has assessed SOC fraction responses to N fertilization,

which also found a larger effect size for the light fraction (i.e. POC) than the heavy fraction (i.e. MAOC; Chen et al. 2018). Chen et al. (2018) also suggest that increases in the light fraction are likely due to increased plant input and our data support and further this conclusion, as we include size separation in our study in addition to density separation, and evaluate responses over deeper soil depths.

MAOC was much less responsive than POC overall, likely due to the typically larger pool size in mineral soils and slower turnover time of MAOC (decades to centuries; Kögel-Knabner et al. 2008), which is longer than the average experiment length of any global change factor we evaluated. However, contrary to our expectations (Table 2.1), MAOC response to elevated CO_2 was positive for shorter experiments and negative for longer experiments. Because experiment length was the only significant moderator on MAOC response to elevated CO_2, it is unlikely that this finding was confounded by another moderator we evaluated. Rather, this finding may indicate a moderating influence of a variable we did not explicitly test, such as plant input quality, which is expected to become richer in structural compounds under elevated CO_2 (Ainsworth and Long 2005; Cotrufo et al. 1998), potentially reducing inputs into the MAOC pool over time. Also root exudation, which is initially stimulated under elevated CO_2 (Nie et al. 2013), may increase MAOC in the short term. Overall, we suggest that the MAOC responses to global changes are driven by the net response between its formation and destabilization processes but, unfortunately, we lack explicit data to explore these pathways. For example, we saw a positive MAOC response under N fertilization (Figure 2.2), which has been shown to increase availability and decomposition of high quality plant litter (Knorr et al. 2005; Xia and Wan 2008), likely contributing to MAOC formation via the *in vivo* pathway (Cotrufo et al. 2013; Liang et al. 2017). In addition, increased DOC production and root exudation under N

fertilization (Liu and Greaver 2010; Lu et al. 2011; Zhu et al. 2016) could contribute to MAOC formation via the direct sorption, *ex vivo* pathway of MAOC formation (Liang et al. 2017; Sokol et al. 2019). It is important to note that global changes may destabilize MAOC via redox and solubility reactions, making it available for decomposition, but there is not yet enough available research to investigate this via meta-analysis (Bailey et al. 2019). By evaluating POC and MAOC responses with regard to their formation and loss processes, mechanistic understanding of changes in the total SOC pool can be inferred.

4.2. Influence of moderator variables

Soil depth sampled and experiment length were broadly important moderators, as expected (Table 2.1), urging the need for longer term studies that sample the full soil profile. Soil depth sampled was the strongest predictor of POC response to N fertilization, with positive POC responses at the soil surface and negative responses at depth (Figure 2.2). This pattern may result from N application largely occurring at or in the surface soil, leading to positive POC responses through greater plant input (Xia and Wan 2008), including structural plant input, and reduced lignin-degrading enzyme activity (Rinkes et al. 2016) at the soil surface. In contrast, since N addition occurs at the surface, response to N addition is reduced at depth (Maillard et al. 2015; Stewart et al. 2016; Zhong et al. 2015). This could lead to lower POC in the subsurface due to smaller plant input and greater POC decomposition at depth, where microbes are more C-limited as compared to surface soils (Soong *et al.*, 2020), and increased DOC production under N addition (Lu *et al.*, 2011) may stimulate microbial activity, resulting in higher POC decay. Total SOC responses were consistently related to soil depth for elevated CO_2 (Figure 2.3) and, non-robustly, for N fertilization (Figure 2.2) and warming (Figure 2.4), in line with previous work (Harrison et al. 2011; Iversen et al. 2012; Luo et al. 2020). The contrasting responses of deeper

soil profiles to overall responses found in our study caution against the common assumption that deep soil is resistant to global changes and stress the need for considering deep soil for understanding feedbacks of SOC to climate change.

We interpret many of our findings with the assumption that plant responses are major drivers for soil responses, and we found some direct evidence of this. Our findings of moderating influences of soil depth on SOC fraction responses to N fertilization, albeit not robust, align with the positive relationship between SOC LRR to N fertilization and belowground biomass (Appendix 1, Supplementary Figure 1.3). Nitrogen fertilization has been found to consistently increase root biomass (Xia and Wan 2008), likely leading to increased root input to the SOC pool, particularly in shallower soil depths. We were surprised to find no other significant relationships between above- and belowground biomass and LRRs but this may be due to limited data availability, since biomass data was available for only 6-22% of all observations for a given GC factor-SOC fraction pairing.

It is well known that ecosystems responses may vary over time as thresholds are crossed or species adapt (Melillo et al. 2002; Walker et al. 2020). The positive N fertilization effect on POC was greater for experiments longer than 10 years (Figure 2.2). Overall SOC increases under N fertilization are amplified over time due to increased plant inputs coupled with reduced microbial decomposition (Xu et al. 2020). Further, in forested systems, N addition preferentially supported woody plant growth over herbaceous plant growth (Xia and Wan 2008) and suppressed lignin decomposition over time (Hobbie et al. 2012). These are all pathways that are expected to favor POC, rather than MAOC, buildup. Variation of MAOC responses to elevated CO_2 represent opposing effects, where plant and microbial responses, associated with MAOC accumulation may be favored in the short term but dissipate over time (Section 4.1). Although not robust,

length of study also had a moderating effect on SOC, with a positive SOC response for N fertilization experiments 5-10 years long and >10 years long (Figure 2.2), altogether suggesting the need for long-term global change studies (Luo et al. 2011).

While we found broadly important moderators for N fertilization and elevated CO_2, responses to warming were modified by global change specific moderators. The effect of degree and type of warming (soil vs air) on POC, after removing extreme warming points (Figure 2.5, insets), highlights plant impacts on SOC. When soil warming occurs, microbial decomposition of more complex plant-derived compounds - which are more often found in POC - is promoted (Feng et al. 2008; Pisani et al. 2015). However, when air is warmed, plant growth (Rustad et al. 2001), plant carbon (Lu et al. 2013), and plant inputs (Cheng et al. 2011) increase, which potentially led to the positive relationship between degree of warming and POC LRR when the air is warmed. Divergent responses to air and soil warming also caution against assuming that soil *versus* air warming manipulations produce comparable results, especially when developing or testing models, although air warming was not a robust moderator. This is especially important to note given experimental warming of the air does not effectively warm the full soil profile, as is expected under projected future warming scenarios (Soong et al. 2020b).

Other moderating relationships that were significant on their own were not robust in multiple moderator models, which may be partially due to our limited sample size. Thus, some of these non-robust moderators may deserve further evaluation with a larger dataset and/or targeted mechanistic studies. For example, we were surprised to only find climate (i.e. latitude, MAP, or MAT) influences on POC, but not on SOC or MAOC. The response of POC to warming was negatively related to latitude (Figure 2.6), potentially indicating release of microbial temperature limitation in high latitude systems with warming (Lu et al. 2013; Xue et al. 2016; Zhang 2015).

With a larger and more geographically diverse dataset, evaluating the response of POC and MAOC to climate factors in tandem with global changes may provide insight on global variation in SOC responses to global changes. Similarly, land cover type was never a robust or significant moderator, but we found divergent POC and MAOC responses based on land cover type under N fertilization (Figure 2.2), elevated CO_2 (Figure 2.3), and warming (Figure 2.4), suggesting characterizing the responses of SOC fractions to global change by land cover type may be helpful for identifying global patterns, as we have found previously (Lugato *et al.*, 2021). Lastly, lack of key measurements such as bulk density, plant and microbial biomass, and soil respiration limited how we were able to evaluate our data, and represent important measurements for future research. In general, larger and longer-term datasets, particularly for warming, elevated CO_2, and increased precipitation, will be important for determining whether any additional moderating influences can be used to contextualize SOC, MAOC and POC responses to global environmental changes. Additionally, given that fractions separated by size and density did not respond differently based on fractionation method, assessing MAOC and POC derived from these fractionation types together may be an effective way to increase data availability.

4.3. Implications

Beyond improving mechanistic understanding, evaluating responses of MAOC and POC to global changes can provide insight on functionality and stability of SOC under global change. Larger increases in POC (+13.17%) as compared to MAOC (+4.49%) under N fertilization represent a shift in the character of SOC, meaning that even though the total SOC pool increases, a higher proportion of it will be more sensitive to other global changes such as warming, which our study finds reduces POC (-10.05%). At the same time, POC buildup under N fertilization can be viewed as increasing the amount of SOC that is readily available to support ecosystem

functions such as nutrient cycling and plant growth, especially important in agroecosystems, where N fertilization is common. Loss of POC under warming indicates the sensitivity of this pool and aligns with findings of higher SOC loss with warming in places where POC is preserved by temperature limitation (Vaughn and Torn, 2016). Increased POC under elevated CO_2 (+5.11%) can help explain inconsistencies in past findings. SOC response to elevated CO_2 has been shown to depend on N availability (Hungate *et al.*, 2009) but POC requires less N for formation than MAOC (Cotrufo *et al.*, 2019), potentially providing a path of soil C storage under elevated CO_2 that is not limited by N availability (Luo *et al.*, 2004). Our analysis suggests that increased precipitation has no effects on SOC pools but we stress the limited data availability and encourage the study of SOC responses to changing precipitation regimes, which are expected under climate change (Knapp *et al.*, 2008). While simply adding the responses of each fraction together paints a positive picture of global change effects on soil C, we must be wary of largely unrealistic additions of N (Song *et al.*, 2019) that may be less effective in natural systems, as well as non-additive effects when these global changes occur in tandem (Reich *et al.*, 2020). However, our results generally suggest that global changes are most likely to influence less stable forms of SOC (e.g., POC), which, in the case of warming, may indicate POC-dominated ecosystems are particularly susceptible to SOC loss.

4.4. Looking forward

Our findings support a more nuanced approach to understanding the effects of global changes on soil C where POC and MAOC are evaluated separately. POC was the most responsive fraction to global change, likely due to most studies in this analysis having a relatively short duration (5-10 years), whereas MAOC was generally less responsive. Our study suggests several areas for future work. The lack of observed MAOC responses to global change,

as well as the moderating effect of experiment length, highlights the need for continued, long-term studies. Moderating influences of soil depth and air *versus* soil warming suggest the need to carefully consider methodology of global change experiments when evaluating responses of soil C. Limited data from the tropics and high latitudes, and from combined global change experiments, needs to be expanded to provide a fuller picture of global SOC responses under projected environmental change. Lack of data availability may be partially addressed through global scale modeling efforts, which would require integration of soil C fraction data, rather than solely total soil C, into soil databases (as is done by the International Soil Radiocarbon Database [ISRaD]; Lawrence *et al.,* 2020). Overall, our study shows that investigating responses of SOC fractions to global changes provides additional and non-additive information compared to total SOC responses alone, highlighting important avenues for future work to determine what role soil C will play in mitigating the climate crisis.

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CHAPTER 3: IMPACTS OF NUTRIENT ADDITION ON SOIL CARBON AND NITOGEN STOICHIOMETRY AND STABILITY IN GLOBALLY-DISTRIBUTED GRASSLANDS[2]

1. Introduction

Global environmental change is expected to greatly change nutrient availability to ecosystems, through warming induced mineralization of nitrogen (N) and phosphorus (P) (Bai et al. 2013, Shaw and Cleveland 2020), anthropogenically-enhanced atmospheric deposition of N, P, and potassium (K; Van Langenhove et al. 2020), and direct fertilizer application to managed systems. These changes to nutrient supply may alter soil organic matter (SOM), a key component of ecosystems which provides nutrients for plant growth and climate mitigation by storing carbon (C; Bossio et al., 2020). Grasslands are crucial areas for SOM storage, covering 30-40% of the global land surface (Reynolds, 2005; Petri et al., 2009) and storing 16% of global soil C (Anderson 1991). Previous research has revealed that grassland SOM C is affected by nutrient availability, depending on environmental conditions (Crowther et al. 2019, Keller et al. 2021, Yue et al. 2017). However, investigating SOM C and N coupling and distribution between functionally different pools of SOM can provide new insight for soil responses to global change, such as model constraints for microbial metabolism or vulnerability of soil C to warming (Buchkowski et al., 2019; Rocci et al., 2021). By investigating SOM C and N stoichiometry and the distribution of C and N between SOM fractions differing in their stability, we can better understand how changes in nutrient availability may modify future grassland biogeochemistry.

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Experiments that investigate responses of SOM to changes in nutrient availability are often carried out as nutrient addition experiments. Nutrient addition has been shown to influence total soil C and N in grassland systems, with directionality dependent on nutrient identity, climate and edaphic characteristics, and land management (e.g., increased with N: Ziter and MacDougall 2013, Cenini et al. 2015, Seabloom et al. 2021b; no change with N or P: Chen et al. 2019, Crowther et al. 2019, Luo et al. 2020, Song et al. 2014, Mazzorato et al. 2022; reduced with N and P: Luo et al. 2020; increased with NPK: Cenini et al. 2015, Crowther et al. 2019, Seabloom et al. 2021b). However, understanding whether soil C and N responses are coupled can provide unique information beyond the independent response of C or N. Variation in SOM C:N represents changes in the nutrient requirement for C storage (Cotrufo et al., 2019), can be indicative of mechanistic changes, and its inclusion in soil biogeochemistry models reduces uncertainty in element cycling (Buchkowski et al. 2019). There is limited work explicitly investigating response of SOM C:N to nutrient addition, but both global and continental scale studies of chronic nutrient addition in grasslands suggest C and N are coupled, with no change in C:N in response to N and P addition (Keller et al. 2021), or their combined addition with K (Seabloom et al. 2021a). However, SOM is a highly complex and heterogenous pool (Christensen 2001), and separating it into components may provide more mechanistic insight regarding soil C:N responses to nutrient additions.

Soil organic matter is made of a high diversity of molecules and physical structures, but one way to reduce this complexity is to characterize SOM into a small number of distinct pools with contrasting physical-chemical characteristics and functionalities. One example of such a separation is to distinguish particulate organic matter (POM) and mineral-associated organic matter (MAOM), which are two fractions whose formation and persistence are thought to be

controlled by different mechanisms (Cambardella and Elliott 1992, Cotrufo et al. 2019, Heckman, Hicks Pries, et al. 2022, Lavallee et al. 2020). Particulate organic matter is thought to form from the fragmentation of structural plant and microbial compounds (Cotrufo et al. 2015, Six et al. 2001), and unless it is occluded in stable aggregates, it lacks physical protection. MAOM is thought to form from microbial necromass and dissolved organic matter (DOM) sorption to minerals or organic matter occlusion in very fine (<53 μm) aggregates (Kleber et al. 2015, Liang et al. 2017, Wagai et al. 2020). Therefore, is it thought to be less accessible to further microbial decomposition. POM is lost through enzymatic degradation, the rate of which depends on POM chemistry and microbial community and activity, which may be inhibited or limited by temperature, moisture, or nutrient availability (Bailey et al. 2019; Keiluweit et al., 2017; Vaughn and Torn, 2019; Ochoa-Hueso et al., 2020). In contrast, MAOM is expected to be lost via desorption (Bailey et al. 2019), leading to a higher mean residence time for MAOM than POM (Kögel-Knabner et al. 2008, von Lützow et al. 2007), when POM is not occluded in aggregates (Heckman, Hicks Pries, et al. 2022).

Because of these different formation and loss processes, changes in C:N stoichiometry of POM and MAOM may be indicative of changes in the mechanisms underlying SOM C:N change. For example, POM C:N may vary due to changes in plant input C:N stoichiometry (Buchkowski et al. 2019). In contrast, MAOM C:N may vary due to changes in C:N stoichiometry of DOM, given the direct sorption pathway of MAOM formation (i.e., ex vivo, *sensu* Liang et al. 2017), or through changes in the microbial community, given the microbial anabolism pathway of MAOM formation (i.e., in vivo, *sensu* Liang et al. 2017). There is limited work on drivers of MAOM desorption but recent work contrasts in suggesting organic N may be preferentially desorbed in the rhizosphere (Jilling et al. 2018) and that N-rich molecules are

strongly sorbed to minerals (Possinger et al., 2020), making it difficult to hypothesize about changes in MAOM C:N. Because of these more specific controls of MAOM and POM, investigating their C:N stoichiometry can inform the overall response of SOM C:N to nutrient addition. For example, it has been shown that N addition reduces plant C:N (e.g., Han et al. 2014, Rueth and Baron 2002) and P addition desorbs C compounds (Spohn and Schleuss 2019), potentially resulting in decreased POM C:N and MAOM C:N, respectively. Thus, C:N stoichiometry of SOM fractions could be a powerful tool for better characterizing grassland soil biogeochemistry in response to greater nutrient availability.

While changes in C:N can indicate changes in the mechanisms driving nutrient addition responses, investigating the distribution of C and N between SOM fractions can provide additional characterization of SOM responses. Specifically, changes in the distribution of C and N between MAOM and POM can indicate changes in SOM stability/vulnerability (i.e., more MAOM = greater resistance to disturbance and global change; Grandy and Robertson 2007, Rocci et al. 2021), turnover time (i.e., more MAOM = longer turnover time; Kögel-Knabner et al. 2008), N requirement for C storage (i.e., more MAOM = higher N requirement; Cotrufo et al. 2019) and dominant drivers of formation and loss. Thus, we interpret greater C and N storage in POM, relative to MAOM, as less stable total C and N pools. Previous work supports disparate responses of POM and MAOM to nutrient addition, and, importantly, that these responses are modified by environmental properties. For example, at both a temperate grassland with sandy soil (Cenini et al. 2015) and an alpine grassland with clay loam soil (Chen et al. 2019), N addition promoted relative C storage in MAOM. In contrast, at a semiarid grassland with sandy soil, N addition promoted increased POM C and N (Song et al. 2014). These results point to the likelihood of climatic and edaphic control of SOM responses to nutrient addition, which likely

align with the formation and loss pathways associated with POM and MAOM. For example, addition of a specific nutrient in climates where that nutrient is generally limiting (e.g., cool climates have greater N limitation; Fay et al. 2015) or a combination of nutrients (NPK) in a variety of climates (Fay et al., 2015), may more effectively release plant growth limitation, and consequently increase structural input into the POM pool. In contrast, wetter climates may support loss of POM C and N with addition of multiple nutrients (NPK), due to concurrent release of water and nutrient limitation to decomposition (Ochoa-Hueso et al. 2020). In clay-rich soils, greater availability of sorption sites may allow for sorption of increased DOM (Lu et al. 2011b, Schleuss et al. 2019) and microbial necromass (Averill and Waring 2018) following nutrient addition, promoting increased MAOM-C and -N. However, it is difficult to compare responses of MAOM and POM in studies from different sites with variable methodologies that measure different covariates, preventing clear attribution of variability to environmental, rather than methodological, differences.

The Nutrient Network [NutNet (Borer et al. 2014)] is a global experiment with identical nutrient addition treatments replicated in grasslands experiencing many different edaphic and climatic conditions, allowing for assessment of the influence of site conditions on the response of grassland SOM to addition of N, P, and K plus micronutrients ($K_{+\mu}$). Previous NutNet studies suggest $K_{+\mu}$ availability is important in determining soil C and N, finding increased soil C under $K_{+\mu}$ and $NPK_{+\mu}$ additions (Crowther et al. 2019, Seabloom et al. 2021a) and increased C in the light fraction (conceptually similar to POM) under $NPK_{+\mu}$ addition (Lin et al. 2019). However, studies that examine soil C fractions at NutNet sites that did not include the $K_{+\mu}$ treatments (Riggs et al., 2015; Keller et al., 2021) have not detected effects of nutrient addition on total soil C. For example, a short term (3-5 years of nutrient addition) study of NutNet sites in the central

plains of the US found increased C in macroaggregates under N addition, with no change in total soil C (Riggs et al. 2015). In contrast, a longer-term continental-scale study (9-10 years) of N and P addition, inclusive of sites involved in the short-term study, found no effect of nutrient addition on C or N in SOM or its fractions (Keller et al., 2021). Thus, there is uncertainty around fraction responses to nutrient addition.

To this end, we investigated C and N stoichiometry and distribution in SOM, MAOM, and POM in response to six or more years of N, P, $K_{+\mu}$, and combined ($NPK_{+\mu}$) nutrient addition at 11 NutNet sites on three continents, varying in climate, soil texture, plant productivity, and soil fertility (Table 3.1). We tested the following hypotheses about responses of SOM-C and -N to greater nutrient availability, and variation in these responses across a wide range of environmental characteristics: (1) Nitrogen in SOM, MAOM, and POM will be more responsive to the addition of N and $NPK_{+\mu}$ than C, reducing C:N. We expect POM C:N stoichiometry to be more responsive to nutrient addition than MAOM C:N stoichiometry. This is because POM C:N will more strongly mirror responses of plant C:N, which has been shown to be sensitive to nutrient addition. Consequently, C and N will be preferentially stored in POM with added nutrients, due to release of plant growth limitation, preferentially increasing POM C and N (N, $K_{+\mu}$, $NPK_{+\mu}$ addition), and desorption of MAOM-C and -N (P addition; Spohn and Schleuss 2019). (2) We expect decoupling of C:N stoichiometry of SOM, MAOM, and POM in productive systems with non-limiting nutrients and climate (high temperature and precipitation). These systems may allow for luxurious N use by plants, likely reducing plant and, subsequently, POM C:N. Additionally, excess N in DOM may be directly sorbed to MAOM, reducing MAOM C:N. In contrast, in nutrient-limited grasslands, we expect nutrients to increase plant productivity, leading to preferential storage of C and N in POM, with no change in

stoichiometry. We expect this to be moderated by soil texture, as sandy soils are limited in their ability to store MAOM C and N. Our study provides insight on differing responses of soil C and N, and SOM fractions, to increased nutrient supply in grasslands spanning gradients of climate, plant productivity, and soil conditions, that can help inform how nutrient availability will regulate soil C and N coupling and stability across grassland environments.

2. Methods

2.1. Study sites and soil collection

This study used archived soil samples from 11 NutNet (Borer et al. 2014) sites spanning three continents (Table 3.1). NutNet is a coordinated, globally-distributed experiment with identical treatments of synthetic nutrient addition in more than 90 grassland sites around the world. NutNet treatments include a control with no nutrients added, and a full factorial combination of N (timed-release urea; $(NH_2)_2CO$), P (triple-super phosphate; $Ca(H_2PO_4)_2$), and $K_{+\mu}$ (potassium sulfate; K_2SO_4), each applied annually at 10 g m^{-2} yr^{-1}. A micronutrient mix (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn) was added at a rate of 100 g m^{-2} to plots fertilized with $K_{+\mu}$ in the first treatment year only, to prevent potential toxicity. For each treatment and the control, 5 x 5 m plots replicated three times were established in a randomized complete block design (Borer et al. 2014).

At each NutNet site, two to three 2.5 cm diameter and 10 cm deep soil cores were collected from each plot before treatments were applied (pre-treatment soils). Soils were collected from the surface with vegetation removed prior to sampling and roots were removed by 2 mm sieving. A depth of 10 cm was chosen to capture the most biologically and chemically active area of the soil. Once treatments were initiated, soils were collected and handled with the

same method every 3-5 years and sent to the University of Minnesota where they were air-dried and archived (post-treatment soils). Because SOM C and N has been shown to be unaltered when air-dried and stored for long periods (Blake et al. 2000), we used post-treatment archived soils for measurement of SOM fractions and used pre-treatment soil data to characterize the ambient environment of each grassland. We analyzed archived post-treatment soil samples from sites that had received a minimum of 6 years of nutrient addition, with the assumption that the treatment had sufficient time to affect SOM pools (Ferreira et al. 2018, Hagedorn et al. 2003), leaving us with soil samples from 25 NutNet sites. We conducted the SOM fractionation (see *Soil Analyses*) on a set of 11 of those sites that represented the widest range of climate, productivity, and soil texture and fertility (e.g., concentrations of soil N, P, and K) of all NutNet grassland sites (Appendix 2, Supplementary Figure 2.1; Table 3.1). Soil texture (% sand) and nutrients (ambient soil N, P, and K) were measured on pre-treatment soils (detailed in the Appendix 2, Supplementary Material A). Only % sand was used as it was the direct complement of % silt+clay. Live plant biomass was estimated annually (except at Cereep, which did not separate live and dead biomass) from clipped samples of live peak aboveground biomass from two 0.1 m^2 quadrats per plot. Samples were dried to constant mass at 60 °C.

We assessed the effects on SOM fractions of addition of N, P, and $K_{+\mu}$ individually, and in combination ($NPK_{+\mu}$), along with control plots, giving a total of 5 treatments. We did not include pairwise factorial combinations of N, P, and $K_{+\mu}$ (e.g., NP, $NK_{+\mu}$, etc.) in our analysis. Treatments were replicated in 3 blocks at each site providing a total of 163 observations at the plot scale, because Elliot Chapparal and Cowichan were each missing archived soils for a single control plot.

Table 3.1. Nutrient Network sites used in analysis of soil organic matter (SOM) responses to nutrient addition. Soil data represent ambient conditions (pre-treatment sampling detailed in Appendix 2, Supplementary Material A). Climate data were extracted from the WorldClim dataset using R statistical software based on latitude and longitude of sites. WRB soil classifications were extracted from the FAO digital coverage using ArcGIS based on latitude and longitude of sites. Years of treatment (YT) pertains to the time the soil used for SOM measurements was collected. MAT, mean annual temperature; MAP, mean annual precipitation; WRB, World Reference Base soil classification; aboveground biomass, mean peak annual biomass in the control plots; N, nitrogen; P, phosphorus; and K, potassium; Y0, pre-treatment measurements made in year 0 of the treatment. For control plot aboveground biomass, values were averaged over Y0 – YT. For WRB, VR, Vertisols; LC, Luvisols, Cambisols; GL, Gleyisols, Histosols, Fluvisols; LP, Leptosols, Regosols; KS, Kastanozems, Solonetz; PH, Phaeozems; AN, Andosols.

Site Name	Country, Continent	Years of treatment (YT)	Soil pH (Y0)	MAT (ºC; Y0)	MAP (mm; Y0)	WRB classification, % sand (Y0)	Aboveground Biomass (g m^{-2}; Y0-YT)	Soil N (%; Y0)	Soil P (ppm; Y0)	Soil K (ppm; Y0)
Burrawan	Australia, Australia	8	5.6	18.4	682	VR 82.2%	238.4	0.084	17.1	72.5
Bogong	Australia, Australia	7	4.5	6.5	1507	LC 63.7%	524.9	0.602	46.0	188.0
Cereep	France, Europe	6	6.1	11	643	LC 73.1%	N/A	0.134	126.0	181.0
Lancaster	England, Europe	8	4.8	8.1	1297	GL 50.2%	120.2	1.070	35.3	106.0
Val Mustair	Switzerland, Europe	8	5.7	0.5	1093	LP 57.6%	219.9	0.576	44.4	108.9
Cedar Point Biological Station	USA, North America	9	6.6	9.2	457	KS 70.2%	177.9	0.113	33.1	285.0
Chichaqua Bottoms	USA, North America	10	6.1	9.1	850	PH 88.3%	395.3	0.061	62.3	93.3

Cowichan	Canada, North America	9	5.6	9.7	807	PH 32.7%	507.8	0.399	38.7	88.3
Elliot Chapparal	USA, North America	8	5.7	16.7	242	LC 54.2%	285.8	0.152	16.8	261.0
Smith Prairie	USA, North America	9	6.1	9.8	614	AN 78.0%	373.7	0.522	75.6	179.0
Spindletop	USA, North America	9	6.4	12.5	1136	LC 29.3%	375.3	0.255	230.0	79.6

2.2 Soil analyses

To separate MAOM and POM, we performed a physical fractionation procedure, modified from Cotrufo et al. (2019). In brief, 10 g of archived soil for each replicate, treatment, and site (n=163), were dispersed in DI water with glass beads (Balesdent 1987) to break up aggregates by shaking at low speed on a reciprocal shaker for 18 hours. For the Lancaster and Cedar Point soils, 5.5 g soil was used, because they were resistant to dispersion and in low availability, respectively. Lancaster soils were also shaken at fast speed for 2 hours and at low speed for 16 hours to increase dispersion. Any remaining aggregates were manually disrupted during wet sieving (below). Following shaking, the slurry was then rinsed onto a 53 μm sieve with the fraction passing through (<53 μm) representing MAOM and the fraction remaining on the sieve (>53 μm) representing POM. We ensured the sum of POM and MAOM mass following fractionation was 100 ± 5% of the starting weight of the soil sample (mean; range = 99.5%; 96.3-104.1%). There are many fractionation approaches to characterize SOM pools but this was chosen as the most appropriate to separate SOM into two meaningful fractions (POM and MAOM) with distinct controls while being an efficient approach for our large sample number (Cotrufo et al. 2019, Poeplau et al. 2018). However, we acknowledge that all SOM fractionation

schemes have limitations, and that this method may allow for inclusion of small-sized structural material (conceptually POM) in our MAOM pool (< 53 µm), as well as some large-sized protected material (conceptually MAOM) in our POM pool (> 53 µm). Nevertheless, we contend that the inclusion of conceptually incorrect material in these pools is small and that our physical pools have average properties expected of their conceptual matches (e.g. higher C and N and lower C:N in MAOM), as has also been shown by Poeplau et al. (2018) for this fractionation method. Following physical fractionation, we measured total C and N individually in SOM (bulk soil), MAOM, and POM (n= 489) on either a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI) or a Costech elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA), as for ambient soil N, above. Samples and standards were crosschecked between instruments to ensure values were comparable. All soils were free of carbonates, as evidenced by effervescence class assessment with 1M HCl (Soil Survey Staff 2014), so total soil C is defined as organic.

2.3 Data analyses

The response variables of interest for this study were C:N stoichiometry in SOM, MAOM, and POM, and the proportion of C and N in MAOM (fMAOM-C = MAOM-C/SOM-C; fMAOM-N = MAOM-N/SOM-N; Appendix 2, Supplementary Table 2.1). These responses are informed by C and N concentration (g of C or N kg^{-1} soil) data, which can be found in the Appendix 2. Because plot-resolved bulk density values were not available for all sites, we were unable to evaluate C and N stocks. We evaluated (1) how SOM C and N stoichiometry and distribution responded to nutrient treatment, (2) how SOM responses to nutrient treatments were modified by site-specific environmental variables, and (3) the relative importance of each predictor variable (combining treatment and environmental variables as predictors in the same

model). At the plot level, we analyzed ambient soil N (%), P (ppm), and K (ppm) concentrations, measured at the start of the experiment. At the site-level, we analyzed mean annual precipitation (MAP; mm yr^{-1}), mean annual temperature (MAT; °C), average peak season aboveground biomass at the control plots (g m^{-2}), and sand content (% sand), which each had one value per site. Climate data were extracted from the WorldClim dataset using R statistical software based on latitude and longitude of sites. Separate models were fit for site and plot level variables, as these strongly covaried (Appendix 2, Supplementary Figure 2.2), such that variance inflation factors reached concerning values between 4 and 5 (up to 4.8; (O'brien 2007, Zuur et al. 2007) when these were analyzed together. We were also interested in unique effects of ambient soil nutrients (plot-level) as compared to biophysical effects (site-level).

Statistical analyses were carried out using RStudio in R version 4.0.2 (R Core Team 2019). We followed the data exploration procedures outlined in Zuur et al. (2010) and found that many of our response variables and some of our predictor variables had outliers that led to non-homogeneous variances. Data for SOM, MAOM and POM-C and -N concentration, C:N, and ambient soil P concentration were all natural log transformed to mitigate outliers and meet the assumptions of the linear model. Additionally, environmental variables were standardized to a mean of 0 and standard deviation of 0.5, as the variables differed widely in scale, allowing for direct comparison of response estimates (Grueber et al. 2011, Rasmussen 2018). The responses of fMAOM-C and -N were modeled with beta regression (*glmmTMB* package; Brooks et al., 2017) because they are continuous proportion data (Douma and Weedon 2019). SOM, MAOM, and POM C and N concentrations and stoichiometry (C:N) were modeled with the normal distribution. Our analyses follow our hypotheses and the layout of data analyses presented in the above paragraph: (1) To assess overall nutrient effects, we created mixed models (*lme4* package;

Bates et al., 2015) with SOM, MAOM, or POM C:N or fMAOM-C or -N as response variables, nutrient treatment as the predictor variable and block nested within site as the random variable, to account for the blocking structure. We used analysis of variance (ANOVA; *car* package; Fox and Weisberg, 2019) and pairwise comparisons between all nutrient treatments and the control (*emmeans* package; Lenth, 2020) to determine overall effect of treatment and individual nutrient effects, respectively. (2) We determined how nutrient addition effects varied with environmental factors by using the same model structure as for (1) but with the mean percent change in a SOM component under nutrient addition (eq. 1) as the response variable and environmental variables as the predictors. Mean percent changes were calculated as a back-transformed log response ratio for ease of interpretation using the equation:

$$\text{Mean Percent Change} = (\exp(\ln(\bar{X}_{trt})-\ln(\bar{X}_{cntrl}))-1) \times 100 \qquad \text{(eq. 1)}$$

where $\bar{X}_{trt}$ is the mean value under the nutrient treatment (i.e. N, P, $K_{+\mu}$, or $NPK_{+\mu}$) and $\bar{X}_{cntrl}$ is the mean value under the control, with no nutrients added. For this analysis we used the normal distribution, since mean percent change approximates a normal distribution (Hedges et al. 1999). (3) Finally, we determined the relative importance of the treatment and environmental factors by carrying out multi-model averaging with all environmental variables and the treatment following Grueber et al. (2011) using the *MuMIn* package (Barton, 2020). We used the natural averaging method to avoid shrinkage towards zero of predictors that only appear in low-weighted models (Grueber et al. 2011) and a model inclusion criterion of 2AICc (Burnham and Anderson 2002). Data for Cereep could not be included in multi-model averaging because of missing aboveground biomass data. For all hypothesis tests and pairwise comparisons, significance was determined at $p < 0.05$.

3. Results

3.1. Data distribution

Our sites spanned a variety of soil textures and nutrient concentrations, climate factors, and productivities (Table 3.1), leading to a diversity of SOM-C and -N stoichiometries, concentrations, and distributions between SOM components. Carbon stored per unit N (median; range), i.e., C:N stoichiometry, was highest in POM (14.3; 10.3-36.1) and lowest in MAOM (10.6; 7.4-18.3), with SOM falling between (12.0; 7.1-22.4). SOM-C had a median (range) value of 26.6 g OC kg^{-1} soil (3.5-278.0 g OC kg^{-1} soil), with most of that C stored in MAOM (17.0; 1.9-187.3 g OC kg^{-1} soil) as opposed to POM (9.9; 1.6-99.0 g OC kg^{-1} soil). Similarly, SOM-N (2.7; 0.2-18.3 g N kg^{-1} soil) was mostly found in MAOM (1.7; 0.2-12.7 g N kg^{-1} soil) as opposed to POM (0.7; 0.1-6.12 g N kg^{-1} soil). Consequently, proportions of C and N in MAOM (fMAOM-C and -N) had median values of 0.62 (0.18-0.93) and 0.65 (0.23-1.06), respectively.

3.2. Overall responses to nutrient addition

The effect of nutrient treatment on C stored per unit N was subtle and only significant for MAOM (χ^2 (4, N = 163) = 18.0, p = 0.001) and POM (χ^2 (4, N = 163) = 10.4, p = 0.035) but not for total SOM (χ^2 (4, N = 163) = 5.9, p = 0.210; Figure 3.1). Overall SOM C:N was likely unaffected by treatment, because the nutrient treatments caused proportional increases in both SOM-C and -N (Table 3.2; Appendix 2, Supplementary Material B2). For SOM fractions, addition of N (p = 0.002), P (p = 0.028), and $NPK_{+\mu}$ (p = 0.048) decreased MAOM C:N. Similarly, addition of $NPK_{+\mu}$ (p = 0.059) or of N (p = 0.055) decreased POM C:N, but not significantly. Response of POM C:N to nutrient treatment was likely driven by changes in soil N concentration rather than soil C concentration, as POM-N was significantly increased by N and

NPK addition. MAOM-N was also significantly affected by nutrient treatment but was only higher under N addition as compared to K addition, indicating a more subtle response of MAOM C:N (Table 3.2; Appendix 2, Supplementary Material B2). The nutrient treatment significantly affected fMAOM-C and -N (C: χ^2 (4, N = 163) = 10.1, p = 0.039 and N: χ^2 (4, N = 163) = 12.7, p = 0.013, respectively; Figure 3.2). Addition of $NPK_{+\mu}$ decreased the proportion of C in MAOM (p = 0.029), relative to the control, and the proportion of N in MAOM, relative to P addition (p = 0.011; Figure 3.2).

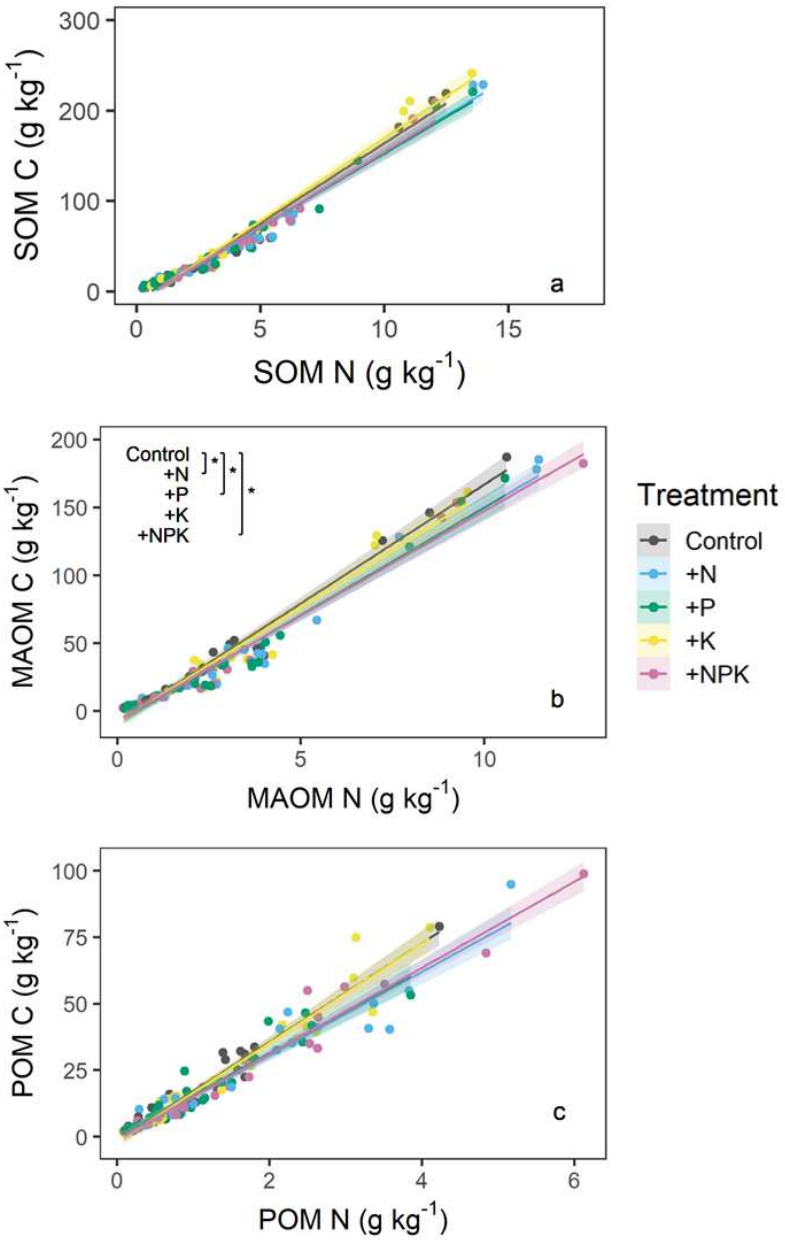

Figure 3.1. Soil (a), mineral-associated (b), and particulate (c) organic matter (SOM, MAOM, and POM) carbon (C) versus nitrogen (N) concentrations under nutrient addition treatments (+N = nitrogen, +P = phosphorus, +K = potassium + micronutrients, +NPK = combined) at 11 globally-distributed grasslands (Table 3.1). There were only significant pairwise comparisons for MAOM at $p < 0.05$, which are denoted with a "*" in the upper left of the MAOM plot. The main effect of nutrient treatment was not significant for SOM C:N (χ^2 (4, N = 163) = 5.9, p = 0.210), but was for MAOM (χ^2 (4, N = 163) = 18.3, p = 0.001) and POM (χ^2 (4, N = 163) = 10.4, p = 0.035) C:N

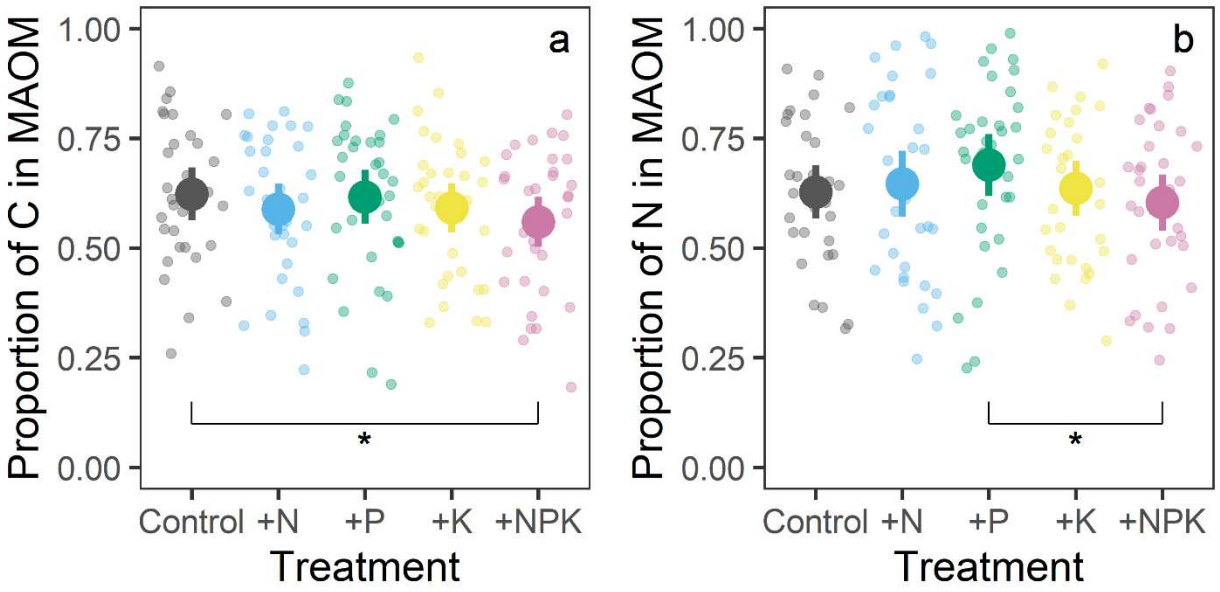

Figure 3.2. Proportion of carbon and nitrogen in MAOM (fMAOM-C and -N) under nutrient addition treatments (+N = nitrogen, +P = phosphorus, +K = potassium + micronutrients, +NPK = combined) at 11 globally-distributed grasslands (Table 3.1). Means with 95% confidence intervals (n=33 for treatments and n=31 for control) are shown as large dots with whiskers and individual observations are behind the large dots. Significantly different pairwise comparisons, at $p < 0.05$, are denoted with a "*"

Table 3.2. Means and standard errors (in parentheses) of soil total, mineral, and particulate organic matter (SOM, MAOM, and POM) carbon (C) and nitrogen (N) concentrations and ratios in response to nutrient treatments at 11 globally-distributed grasslands (Table 3.1). Proportions of C and N in MAOM are denoted as fMAOM-C and fMAOM-N, respectively. An asterisk (*) indicates a significant treatment effect at $p < 0.05$.

Treatment	**Control**	**Nitrogen (N)**	**Phosphorus (P)**	**Potassium + micronutrients ($K_{+\mu}$)**	**Combined ($NPK_{+\mu}$)**
SOM component					
SOM C:N	12.1 (0.5)	12.0 (0.5)	12.6 (0.5)	12.5 (0.5)	12.1 (0.4)
*SOM-C**	45.3 (10.1)	51.6 (10.7)	45.5 (9.1)	46.1 (10.2)	50.5 (10.8)
*SOM-N**	3.3 (0.6)	3.9 (0.6)	3.4 (0.6)	3.3 (0.5)	3.8 (0.7)
*MAOM C:N**	12.1 (0.6)	11.1 (0.4)	11.2 (0.4)	11.7 (0.5)	11.4 (0.5)

MAOM-C	31.5 (7.9)	33.0 (7.9)	30.8 (7.2)	28.1 (6.6)	29.9 (7.6)
*MAOM-N**	2.25 (0.4)	2.6 (0.5)	2.5 (0.4)	2.1 (0.4)	2.4 (0.5)
*POM C:N**	16.7 (0.8)	15.2 (0.9)	15.8 (0.7)	16.0 (0.8)	15.0 (0.6)
POM-C	15.7 (2.8)	20.1 (3.7)	15.7 (2.4)	19.7 (3.7)	21.1 (4.0)
*POM-N**	0.9 (0.1)	1.3 (0.2)	1.0 (0.1)	1.2 (0.2)	1.4 (0.2)
*fMAOM-C**	0.62 (0.03)	0.59 (0.03)	0.62 (0.03)	0.59 (0.03)	0.56 (0.03)
*fMAOM-N**	0.63 (0.3)	0.65 (0.04)	0.69 (0.03)	0.64 (0.03)	0.60 (0.03)

3.3. Responses to nutrient addition as modified by environmental factors

Generally, soil C:N tended to increase with nutrient addition at sites with low ambient soil nutrients, and tended to decrease at sites with high ambient soil nutrients. Specifically, SOM C:N responses to N, P, and $K_{+\mu}$ addition were all negatively related to ambient soil P (Table 3.3; Figure 3.3). In addition, SOM C:N responses to N and $NPK_{+\mu}$ addition were negatively related to ambient soil K concentration (Table 3.3) and SOM C:N responses to P addition were negatively related to ambient soil N concentration (Table 3.3; Figure 3.3). Similarly, MAOM C:N responses to P and $NPK_{+\mu}$ addition were negatively related to ambient soil N (Table 3.3; Figure 3.3). MAOM C:N response to $NPK_{+\mu}$ was also negatively related to ambient soil K concentration (Table 3.3; Figure 3.3). Nitrogen was likely responsible for some SOM C:N responses, as the SOM-N response to $K_{+\mu}$ was positively related to ambient soil P, whereas other nutrient relationships with C and N concentrations were more subtle and not directly connected to SOM and MAOM C:N responses (Appendix 2, Supplementary Material B3). In contrast, the response of POM C:N to nutrient addition was not related to ambient soil nutrient concentrations (Figure 3.3). Both POM-C and -N responses to N and P additions were negatively related to ambient soil

P, likely leading to no change in POM C:N (Appendix 2, Supplementary Material B3). Instead, addition of $NPK_{+\mu}$ tended to decrease POM C:N at sites with low sand content (Table 3.3). Climate also modified this response. In particular, SOM and POM C:N increased more with N addition with increasing MAT (Table 3.3).

Table 3.3. Chi-square estimate and (p-value) from type 3 Wald chi-squared tests for models of the response of total soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) carbon-to-nitrogen ratio (C:N) to nutrient addition, as modified by environmental variables at 11 globally-distributed grasslands (Table 3.1). Columns for N, P, K, and NPK represent responses to nitrogen, phosphorus, potassium + micronutrients and their combined addition. Bolded relationships are significant at $p < 0.05$.

	SOM C:N				**MAOM C:N**				**POM C:N**			
	Df = 1; n = 31				**Df = 1; n = 31**				**Df = 1; n = 31**			
	N	**P**	**K**	**NPK**	**N**	**P**	**K**	**NPK**	**N**	**P**	**K**	**NPK**
Ambient soil N (%)	2.7 (0.097)	**4.1 (0.042)**	0.3 (0.615)	2.0 (0.158)	2.2 (0.138)	**4.1 (0.043)**	0.4 (0.520)	**8.0 (0.005)**	0.2 (0.660)	0.1 (0.722)	0.0 (0.831)	0.2 (0.680)
Ambient soil P (ppm)	**7.7 (0.006)**	**6.4 (0.011)**	**4.6 (0.032)**	1.6 (0.204)	0.4 (0.528)	0.0 (0.877)	0.0 (0.914)	1.6 (0.211)	1.4 (0.231)	0.1 (0.774)	0.2 (0.624)	2.0 (0.153)
Ambient soil K (ppm)	**9.5 (0.002)**	1.8 (0.186)	0.7 (0.394)	**4.4 (0.036)**	0.2 (0.682)	0.2 (0.634)	0.6 (0.444)	**6.2 (0.013)**	0.1 (0.752)	0.2 (0.644)	3.4 (0.066)	0.1 (0.803)
MAT (°C)	**4.3 (0.038)**	3.8 (0.052)	0.2 (0.639)	0.4 (0.522)	0.2 (0.655)	2.0 (0.156)	0.4 (0.527)	1.4 (0.243)	**5.1 (0.024)**	1.0 (0.310)	0.4 (0.505)	1.2 (0.280)
MAP (mm yr^{-1})	1.5 (0.215)	0.3 (0.580)	0.0 (0.901)	0.5 (0.465)	0.7 (0.390)	0.3 (0.613)	0.1 (0.744)	0.0 (0.877)	0.0 (0.937)	0.5 (0.466)	1.1 (0.305)	0.0 (0.922)
Sand content (%)	1.6 (0.206)	2.0 (0.157)	0.1 (0.780)	2.2 (0.137)	0.5 (0.472)	0.3 (0.567)	0.0 (0.982)	0.9 (0.341)	0.2 (0.659)	0.0 (0.994)	0.4 (0.535)	**5.0 (0.025)**
Aboveground biomass (g m^{-2})	0.0 (0.950)	0.1 (0.752)	0.0 (0.883)	0.0 (0.904)	0.2 (0.645)	1.9 (0.164)	0.6 (0.432)	0.7 (0.408)	1.0 (0.329)	2.7 (0.097)	1.3 (0.263)	0.0 (0.922)

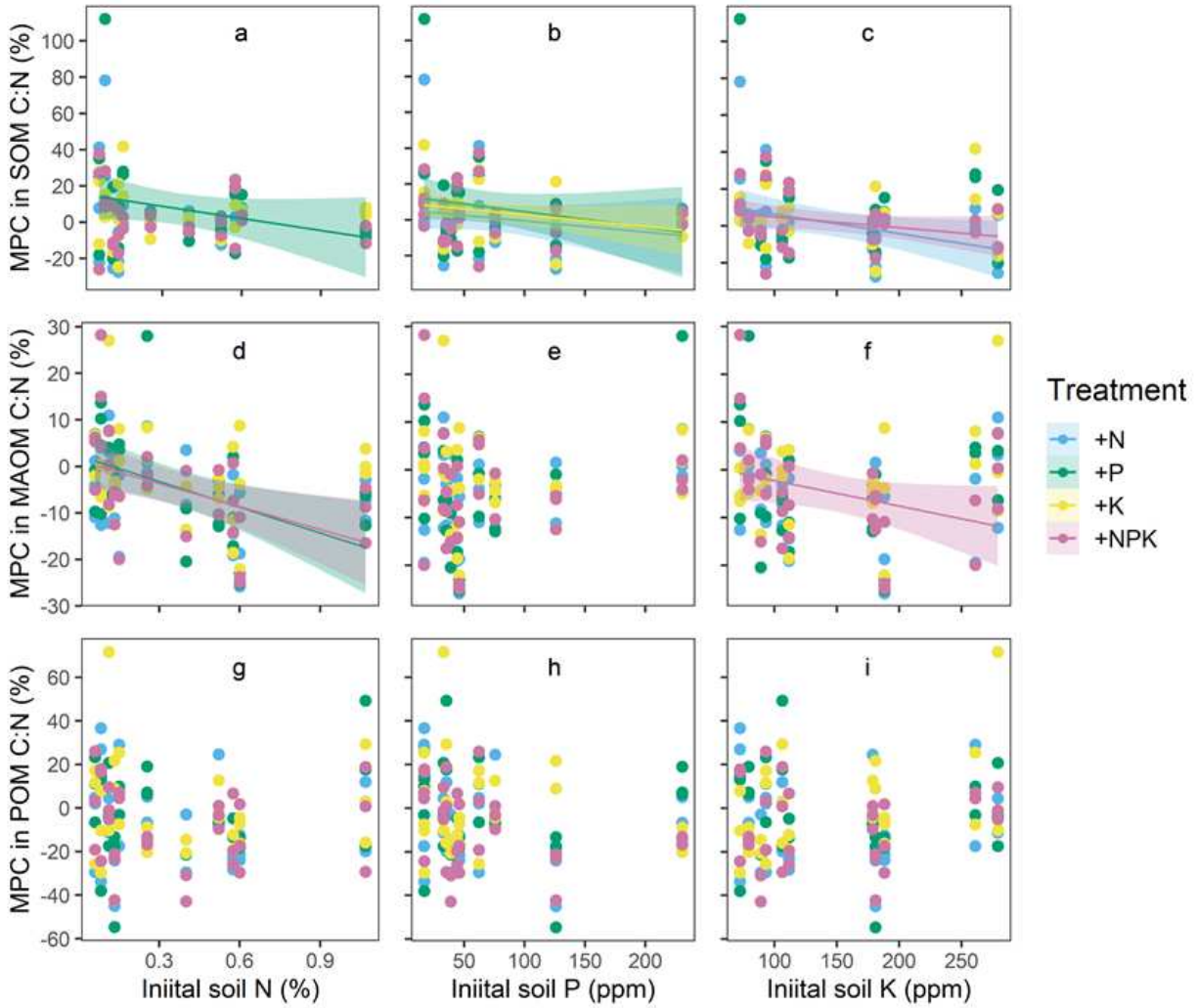

Figure 3.3. Relationships between ambient soil nitrogen (N; a, d, g), phosphorus (P; b, e, h), and potassium (K; c, f, i) concentrations and mean percent change (MPC) in soil, mineral-associated, and particulate organic matter (SOM (a-c), MAOM (d-f), and POM (g-i) carbon to nitrogen ratios (C:N) under +N (blue), +P (green), +K + micronutrinets (yellow) and +NPK + micronutrients (pink) addition at 11 globally-distributed grasslands (Table 3.1). Linear trendlines are reported only for significant relationships from ANOVA. Statistical information for linear trendlines are reported here. For SOM C:N fertilized with each individual nutrient, soil P estimates, p-values, and R^2 were, +N: -11.7, p = 0.010, and r^2 = 0.18; +P: -13.7, p = 0.017, and r^2 = 0.16; +K: -7.4, p = 0.083, and r^2 = 0.14, for SOM C:N fertilized with N and NPK+μ, soil K estimates, p-values, and R^2 were, +N: -0.14, p = 0.005, and r^2 = 0.22, +NPK+μ: -0.08, p = 0.046, and r^2 = 0.02, and for SOM C:N fertilized with P, soil N estimate, p-value, and R^2 were, -26.8, p = 0.052, and r^2 = 0.10. For MAOM C:N fertilized with P and $NPK_{+\mu}$, soil N estimates, p-values, and R^2 were, +P: -19.5, p = 0.085, and r^2 = 0.21; $+NPK_{+\mu}$: -19.5, p = 0.028, and r^2 = 0.26 and for $NPK_{+\mu}$, soil K estimate, p-value, and R^2 were -0.08, p = 0.043, and r^2 = 0.21. Chi-squared estimates and p-values from ANOVA analysis are in Table 3.3.

Addition of all nutrients decreased the proportion of C and N in MAOM relatively consistently across environmental variables. The response of fMAOM-C to nutrient addition was not related to any environmental factor. The response of fMAOM-N to N addition was positively related to MAT and the response to N and $K_{+\mu}$ addition were negatively related to ambient soil P (Appendix 2, Supplementary Table 2.6). These responses were subtle and could not be directly connected to MAOM and POM-C and -N responses (Appendix 2, Supplementary Material B3).

3.4 Relative importance of explanatory variables

To indicate the overall strongest drivers of SOM, MAOM, and POM C:N stoichiometry and C and N distribution (fMAOM), we performed multi-model averaging using all environmental variables and the treatment variables. Soil N was included in the averaged model for all soil components except POM C:N. Soil texture and MAT also emerged as consistently strong (high estimate) predictors across our response variables, whereas other predictor variables were only related to changes in a subset of SOM components or had relatively low estimates (Table 3.4). Multi-model averaging for SOM, MAOM, and POM C and N concentrations followed similar patterns as above (Appendix 2, Supplementary Table 2.5). Treatment was only included in models of the distribution of C and N between MAOM and POM (fMAOM-C and -N).

Table 3.4. Top model (< 2 AICc) estimates and standard errors (in parentheses) from multi-model averaging for bulk soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) carbon (C) and nitrogen (N) stoichiometries (C:N), and distributions (fMAOM) with all environmental variables and the treatment at 11 globally-distributed grassland nutrient addition experiments (Table 3.1). MAT, mean annual temperature; MAP, mean annual precipitation.

Soil component	Number of models	Percent sand	Above-ground biomass	MAT	MAP	Ambient soil N	Ambient soil P	Ambient soil K	Nutrient addition
SOM C:N	3					0.26 (0.08)	-0.21 (0.06)		
MAOM C:N	8	0.24 (0.09)		0.22 (0.09)	0.29 (0.12)	0.34 (0.12)	-0.24 (0.12)		
POM C:N	2						-0.26 (0.10)	-0.28 (0.10)	
fMAOM-C	3	-0.23 (0.09)	0.31 (0.09)	1.14 (0.10)	-0.26 (0.13)	1.04 (0.13)	0.28 (0.10)	0.25 (0.09)	N: -0.19 (0.12) P: -0.05 (0.12) K: -0.03 (0.12) NPK: -0.31 (0.12)
fMAOM-N	3	-0.70 (0.12)	0.14 (0.11)	1.16 (0.13)		0.59 (0.14)	0.39 (0.13)	0.22 (0.10)	N: 0.20 (0.15) P: 0.44 (0.16) K: 0.11 (0.16) NPK: -0.12 (0.15)

4. Discussion

We found that six or more years of experimental nutrient addition, particularly of N and $NPK_{+\mu}$, promoted N accrual more strongly than C accrual in MAOM and POM and reduced average stability of SOM-C, as evidenced by reduced fMAOM-C. However, responses of C:N

stoichiometry to chronic nutrient addition were subtle – total SOM C:N was unaffected by nutrient treatment - and depended on environmental context. Generally, for SOM and MAOM, addition of specific nutrients increased C relative to N at sites with low ambient soil fertility; whereas for POM this occurred at warm sites with sandy soils following N and $NPK_{+\mu}$ addition. In contrast, responses of fMAOM-C were consistent across the wide range of climatic and edaphic conditions represented in this study. These results suggest that increased availability of $NPK_{+\mu}$ could consistently promote C storage in the less stable POM pool but that the response of soil C per unit N is dependent on SOM fraction, nutrient identity, and local environmental conditions.

4.1. Responses to increased nutrient availability

Effects of nutrient addition on soil stoichiometry were subtle, with SOM C:N unaffected by increased nutrient availability. This lack of change was driven by similar increases in SOM-C and -N in response to nutrient addition (Table 3.2) and aligns with previous NutNet work (Crowther et al. 2019, Seabloom et al. 2021a, Keller et al., 2021) and with meta-analyses (Lu et al. 2011a, Lu et al. 2011b, Rocci et al. 2021, Yue et al. 2017, Yue et al. 2016), which all find increased soil C and N with nutrient addition. While there are certainly exceptions to this general finding of increased soil C and N with nutrient addition (e.g., Boot et al. 2016, Mack et al. 2004), these findings might be particularly expected given the relatively long period of treatment (6-10 years) and relatively high nutrient addition rate for this study, as soil C responds more positively at higher N addition durations and amounts (Xu et al. 2020). We might expect individual C and N responses to be stronger on decadal time scales, potentially leading to altered C:N ratios, as has been found for fertilized retired farmland (Seabloom et al., 2021b). However, given the heterogeneity of the total SOM pool, it is important to unpack these findings by analyzing

distinct fractions of SOM. Because POM and MAOM are thought to have distinct pathways of formation and loss, they can indicate both potential mechanisms underlying responses and the stability of SOM following global change.

In contrast to the total SOM pool, and aligned with our hypotheses, we found that greater nutrient (specifically N, P, and $NPK_{+\mu}$) availability reduced MAOM and POM C:N. Since these findings were likely driven by increases in MAOM and POM N, our results suggest nutrient addition promotes MAOM and POM formation or loss pathways that increase N, relative to C. MAOM formation is thought to occur through two main pathways; efficient microbial resource use efficiency and subsequent necromass sorption (i.e., *in vivo*) and exo-enzymatic breakdown of polymeric residues followed by direct DOM sorption (i.e., *ex vivo*; *sensu* Liang et al. 2017). Both of these pathways could reduce MAOM C:N with N addition. Addition of N tended to reduce plant C:N at our sites (Appendix 2, Supplementary Figure 2.3), which aligns with previous NutNet work (Anderson et al. 2018), and is expected to favor MAOM formation via the *in vivo* pathway (Cotrufo et al. 2013). Since microbial necromass is more N-rich than plant inputs (Cleveland and Liptzin 2007), this may decrease MAOM C:N. Direct addition of labile N may also form MAOM through the *ex vivo* pathway. A previous NutNet study of 6 globally-distributed grasslands, including one of our sites, found that N addition increases dissolved N concentrations (Schleuss et al. 2021), and N compounds are thought to sorb strongly to mineral surfaces (Possinger et al. 2020), thus potentially increasing MAOM-N. Carbon stored per unit N in MAOM was also reduced under P addition, which aligns strongly with our expectations, given findings of C desorption with P addition (Spohn and Schleuss 2019). The drivers for MAOM C:N responses to N and P addition can likely be extended to explain reduced MAOM C:N under $NPK_{+\mu}$ addition. Responses of POM C:N were more variable than MAOM C:N but nevertheless

tended to decline with N and $NPK_{+\mu}$ addition. At our sites, addition of N and $NPK_{+\mu}$ increased standing aboveground biomass and $NPK_{+\mu}$ addition also reduced live aboveground plant C:N (Appendix 2, Supplementary Figure 2.3), in line with other NutNet studies (Anderson et al. 2018, Fay et al. 2015). Thus, reduced POM C:N is likely due to greater inputs of lower C:N plant material with N and NPK+μ addition, although responses of live plant biomass may be different than plant litter (Seabloom et al., 2021a). We lack root data for all of our sites but a study at one of our sites (Cowichan) suggested root biomass and C:N responses would mirror aboveground plant responses (Ziter and MacDougall, 2013), potentially adding greater low quality plant input belowground, also potentially reducing POM C:N. Thus, our results suggest increasing nutrient -- especially N -- availability in grasslands will likely store soil C at a higher N cost, which could be a benefit in areas experiencing rapidly increasing N deposition (Ackerman et al. 2019) but may be problematic for management that depends on fertilization.

Beyond studying C relative to N, we also studied content of C and N in MAOM relative to POM, to assess how the average stability of soil C and N may change with greater nutrient availability. Addition of $NPK_{+\mu}$ reduced the relative distribution of C and N in MAOM versus POM (fMAOM-C and -N) as compared to the control and P, respectively. These findings align with previous NutNet work that suggests grassland biomass production is co-limited by N, P, and K, as adding these together increases aboveground plant growth (Fay et al. 2015, Carroll et al. 2021), as we also found in our sites (Appendix 2, Supplementary Figure 2.3). This is expected to favor POM, rather than MAOM, formation. Addition of $NPK_{+\mu}$ may be more effective at promoting N storage in POM than P addition because P addition alone did not increase average aboveground biomass in our sites (Appendix 2, Supplementary Figure 2.3). Overall, our findings of lower fMAOM-C with $NPK_{+\mu}$ addition suggest that providing limiting nutrients to grasslands

will shift distributions of soil C towards storage in POM, potentially making them more susceptible to loss due to warming (Rocci et al. 2021) and management (Grandy and Robertson 2007).

4.2. Responses to nutrient addition along environmental gradients

While C:N stoichiometry responses indicated that, on average, soil N, and C to a lesser extent, increased with greater nutrient availability, we found that nutrient addition in sites with low ambient nutrients may allow for more C stored per unit N added. Some of these relationships (Figure 3.3b, d, f) were aligned with our expectation that adding a nutrient when it has low ambient availability may promote more coupled C and N responses, but only for SOM and MAOM. Thus, these relationships were likely mediated through, for example, increased microbial necromass inputs to MAOM with N addition (3d; Averill and Waring 2018) rather than through plant productivity, as we would expect for POM. However, many of these relationships were not expected and indicated interactions between different nutrients. For example, positive responses of SOM C:N to N addition at low ambient soil P and K suggest that adding a non-limiting nutrient may preserve existing soil C and N through reduced microbial acquisition of those elements (Schleuss et al. 2019). In contrast, responses of POM C:N to the addition of N and $NPK_{+\mu}$ did not vary with soil fertility but rather with MAT and soil texture, respectively, which is likely related to the formation pathway of POM. Warmer temperatures are generally associated with higher plant tissue C:N (Anderson et al. 2018, Reich and Oleksyn 2004). Since aboveground biomass increased with N addition at our sites (Appendix 2, Supplementary Figure 2.3), N addition in warm grasslands may promote more structural (C-rich, nutrient poor) plant inputs to the POM pool (Cotrufo et al. 2015). Our finding of increased POM C:N with $NPK_{+\mu}$ addition in sandy soils is strongly in line with findings for total soil C in a

previous NutNet study (Crowther et al. 2019) and for fertilized retired farmland (Seabloom et al. 2021b). Notably, we corroborate this finding for the POM pool only, which may be due to relatively smaller MAOM pool size in sandy soils (Appendix 2, Supplementary Figure 2.4). Because our fractionation method defines POM C as the C in the sand-sized fraction, this is not a surprising finding, but, nevertheless, suggests that increased soil C with fertilization of sandy soils may be occurring in the less stable POM pool. Overall, findings of environmental influence on responses of SOM, MAOM, and POM C:N align with relatively high estimates for ambient soil N, MAT, and sand content for top models of SOM components C and N (Table 3.4). In addition, they emphasize the importance of site edaphic and climatic conditions, as found in other NutNet studies (Keller et al. 2021), in determining the strength and direction of soil responses to nutrient addition (Appendix 2, Supplementary Table 2.7 & Figure 2.5). These results confirm and extend previous NutNet results to show that findings of increased soil C in sandy vs. low nutrient sites may be realized in POM and MAOM, respectively, with implications for the stability of soil C.

In contrast to C:N stoichiometry responses to environmental gradients, $NPK_{+\mu}$ addition consistently reduced C content in MAOM, relative to POM, across a wide range of climatic and edaphic conditions. This may be because plant growth is often co-limited by multiple nutrients (Fay et al. 2015), so addition of $NPK_{+\mu}$ is able to reduce plant growth limitation under a wide range of baseline conditions, thus increasing C content preferentially in POM. Responses of fMAOM-N were slightly less consistent but were subtle and could not be directly related to POM or MAOM-N responses (Appendix 2, Supplementary Table 2.3). It is possible that increased fMAOM-N with N addition at warmer sites could be due to greater microbial anabolism and decomposition in these conditions (Averill and Waring 2018, Li et al. 2019, Lu et

al., 2013), increasing MAOM-N and decreasing POM-N, respectively. However, a study at 21 globally-distributed NutNet sites found increased decomposition rate with N addition in cool, not warm, sites (Ochoa-Hueso et al. 2020), suggesting reduced POM-N from heightened decomposition is not likely to drive the fMAOM-N response. The generally uniform response of the distribution of C between MAOM and POM to nutrient addition may represent a consistent soil response that could be considered in management decisions and soil modeling.

4.3. Conclusions

Because nutrient availability is expected to increase with global environmental change, it is important we evaluate not just the total soil C responses to nutrient addition, but also the coupling of C and N and the distribution of C and N between soil components, to improve our understanding of future grassland biogeochemistry. While C and N were coupled in the total SOM pool, evaluating MAOM and POM allowed us to find more subtle decreases in C:N after N, P, and $NPK_{+\mu}$ addition, suggesting that increased nutrient supplies may cause C and N to decouple in MAOM and POM. Furthermore, MAOM and POM responded differently to nutrient addition across environmental gradients. This revealed that nutrient addition-driven increases in soil C in low fertility soils may be driven by increases in MAOM C:N whereas for sandy soils increases in soil C may be driven by increases in POM C:N. By evaluating distribution of C and N between MAOM and POM, we were able to show that addition of macro- and micronutrients in combination ($NPK_{+\mu}$) drives C accumulation preferentially in POM, suggesting reduced soil C stability. Our results highlight that more mechanistic and practical insight can be gained by investigating coupled C and N and SOM heterogeneity in addition to bulk soil C.

5. References

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CHAPTER 4: SOIL ORGANIC MATTER STOCKS AND DISTRIBUTION AS MODIFIED BY THIRTY YEARS OF INCREASED PRECIPITATION AT A TALLGRASS PRAIRIE[3]

1. Introduction

Globally, future climate is expected to include increased amounts of precipitation and greater seasonal amplification of precipitation, with wetter wet seasons and drier dry seasons (Lee et al. 2021). In the central mesic region of the U.S. Great Plains, annual precipitation is expected to increase in both the cool and warm seasons, primarily through larger individual precipitation events (Meehl et al. 2006; Schoof et al. 2010). Plant productivity has proven very responsive to changes in moisture availability in grasslands of the Great Plains (Knapp et al. 2001; Sala et al. 1988). Despite this, belowground responses in mesic grasslands have been limited and hard to anticipate, potentially because they are understudied compared to aboveground responses (Vilonen et al. 2022; Wilcox et al. 2016; Yue et al., 2017). Soil organic matter (SOM) is a central component of belowground processes, as it stores nutrients and carbon (C) to be used by plants and microbes, and SOM has been shown to be unresponsive to increased precipitation (Rocci et al. 2021). Given the diverse controls of SOM formation and loss and the decadal timescale of SOM turnover, it is not yet clear if limited belowground responses to changes in precipitation in mesic grasslands are evidence of an unresponsive system or are the net balance from changes in multiple SOM component pools and input-output fluxes. Improved understanding of SOM responses to increased precipitation will be important for predicting future land-biogeochemical feedbacks to climate change in mesic grasslands.

[3] To be developed into a peer-review publication with John Blair, Alan Knapp, and M. Francesca Cotrufo

The response of SOM to increased water availability is the result of combined responses of plants and microbes. It is generally found that plants respond more strongly to climate manipulation than soil microbes (Yue et al. 2017), suggesting plant responses may be the dominant control of SOM responses to increased precipitation. Plants generally increase their aboveground and belowground growth in response to increased precipitation, although this response is dampened in wetter systems (Knapp et al. 2017; Wang et al. 2021; Wang et al. 2020). Plants may also change their quality, either directly or through community change. There is varied support for direct changes in plant quality under increased precipitation, with increased plant N, P, and lignin content in two semi-arid grassland studies (Ren et al. 2015; Wang et al. 2015) but contrasting responses (increase and no response) of plant N in meta-analyses (Dumont et al. 2015; Wu et al. 2022). Meta-analyses also show minimal change in species diversity or richness, except for dryland grasslands after long-term and large increases in precipitation (Komatsu et al. 2019; Korell et al. 2021; Yue et al. 2020). However, at individual sites, increased precipitation increased dominance of grass, shallow-rooted species, and N-fixers, potentially increasing plant input quality (Eskelinen and Harrison 2015; Yang et al. 2011; Zhang et al. 2019a). Greater plant input, potentially of higher quality, may promote SOM formation under increased precipitation, since removing plant input strongly reduces C content in prairie soils (Lajtha et al., 2014).

Although plants generally respond more strongly than soil microbes to altered precipitation experiments, microbes have evolved a number of processes to manage deficit and excess water (Jansson and Hofmockel 2020). Generally, soil microbial biomass increases with increased precipitation although, like for plants, this response is stronger for extreme precipitation manipulations (> 60% of annual precipitation) in arid sites (Xu et al. 2020; Zhou et

al. 2018). Greater microbial biomass likely translates into greater heterotrophic respiration, as a meta-analysis found that heterotrophic respiration was more responsive to increased precipitation than autotrophic respiration (Liu et al. 2016), although this is not a fully consistent finding (Moinet et al. 2016; Zhang et al. 2022b). Increased microbial biomass generally occurs across different functional groups, with little to no change in the functional soil microbial community composition under increased precipitation in both arid and alpine ecosystems (Chen et al. 2015; Cregger et al. 2012; Huang et al. 2015a; Xu et al. 2020; Yang et al. 2017; Zhang et al. 2018a). However, increases in arbuscular mycorrhizal fungi and changes in certain operational taxonomic units or enzyme activities have been found with increased precipitation, suggesting some community change may occur that is not captured by assessing coarse functional microbial groupings (Hu et al. 2020; Ma et al. 2020; Yang et al. 2017; Zhou et al. 2018). Given plant and microbial responses can have variable effects on the total SOM pool, predicting the response of SOM to increased water availability has proven difficult.

Soil organic matter is made of a diversity of compounds. As explained in Chapter 1, by separating SOM into constituent components, we can investigate more homogenous pools that may have more specific plant and microbial controls. Different SOM components, or fractions, have different formation pathways and protection mechanisms, and have been shown to respond differently to global change (Rocci et al. 2021). For example, the free particulate organic matter (fPOM), which is not protected by aggregates, is most similar to plant inputs in its quality and lacks protection from decomposition, reducing its persistence (Heckman et al. 2022; Six et al. 2001b). Larger soil respiration, likely resulting from heterotrophic decomposition, compared to plant inputs under increased precipitation could lead to reduced fPOM (Wang et al. 2021; Liu et al., 2016). In contrast, the occluded POM (oPOM), which is protected in aggregates, and is

largely of plant origin, may increase with increased precipitation (Six et al. 2001b). This would result from increased plant input coupled to increased aggregation, also shown to occur under higher precipitation (Bai et al. 2020; Caplan et al. 2019; Wang et al. 2021). The mineral-associated organic matter (MAOM) is thought to form via two main pathways. These are the *ex vivo* pathway, where dissolved organic matter (DOM), produced either directly (i.e. exudation and leaching) or following exoenzyme processing of structural inputs and POM, sorbs to mineral surfaces and the *in vivo* pathway, where DOM substrates are taken up by microbes whose products and necromass sorb to mineral surfaces (Cotrufo et al. 2013; Liang et al. 2017). It has been suggested that the *ex vivo* pathway may be more important in the mineral-rich, microbe-poor bulk soil and that the *in vivo* pathway may dominate in the high-input, microbe-rich rhizosphere (Sokol et al. 2019). Greater DOM from higher root production and turnover, and greater microbial biomass with increased precipitation may spur MAOM formation via both *ex vivo* and *in vivo* pathways (Bai et al. 2010; Wang et al. 2021; Zhou et al. 2018). However, little is known about the influence of increased precipitation on microbial necromass and MAOM desorption, important controls on the formation and loss of MAOM, respectively. This potential for increased MAOM and oPOM but decreased fPOM under increased precipitation has been supported by other studies but the mechanisms driving these responses have not been explored (He et al. 2012; Rocci et al. 2021). Further, unique responses of SOM fractions may explain findings of both no change and increased SOM C under increased precipitation, where specific fraction responses could be driving the overall SOM response (Wang et al. 2021; Yue et al. 2017).

Plants and microbes are not the only determinants of SOM dynamics - soil properties also play a crucial role. Soil properties often change with soil depth, as do plant inputs and microbial

density. Variation in the response of SOM at depth compared to surface soil has been documented in response to warming, elevated CO_2 and N fertilization (Rocci et al. 2021; Soong et al. 2021). However, the available literature indicates that soil C and N responses to increased precipitation do not vary with soil depth (Wilcox et al. 2016; Zhang et al. 2018a), despite, He et al. (2012) found unique responses of the sand-sized fraction of SOM (similar to POM) to increased precipitation, depending on depth. This suggests that investigating SOM fraction responses may be able to provide different information than investigating the total SOM pool. In particular, we might expect stronger responses of MAOM to increased precipitation at depth, due to greater DOM under increased precipitation that could be stabilized on mineral surfaces at depth, where there is large mineralogical capacity (Haddix et al. 2020; Wang et al. 2021; Sokol et al., 2019; Georgiou et al. 2022). By investigating the response of SOM fractions to increased precipitation, we may be able to better connect soil C and N responses to their biophysical drivers.

The Konza Prairie Irrigation Transect Experiment (ITEX) has increased precipitation to a tallgrass prairie by 29% on average for 30 years across a landscape transect (Appendix 3, Supplemental Figure 3.1). Results from this experiment have revealed increases in aboveground plant growth and microbial activity and changes in plant community due to increased precipitation but have found no changes in total soil C and N pools (Knapp et al. 1998; Broderick et al. 2022, accepted; Wilcox et al. 2016). However, because these total C and N pools integrate responses of SOM fractions, the character of SOM may have changed at this site, without changes in the absolute amount of bulk soil C and N pools. Because fPOM, oPOM, and MAOM have different functional roles in the soil, changes in the relative distribution of C and N between

these pools could modify long-term C and N stability and ecosystem functioning (Cotrufo & Lavallee, 2022).

To that end, we used the ITEX experiment to determine the influence of increased precipitation on SOM dynamics and the associated mechanisms. We had the following three hypotheses related to influence of increased precipitation on SOM components and the moderating influence of soil properties: (1) We expected increased precipitation to increase plant inputs and quality. This would translate to greater microbial processing of plant inputs, expected to increase the MAOM pool. In addition, MAOM would increase through greater DOM which could be directly sorbed on minerals, and oPOM would increase through higher aggregation (Caplan et al. 2019). In contrast, fPOM would decrease due to greater microbial decomposition (Broderick et al. 2022) than plant input. (2) We expected soils under increased precipitation to have greater accumulation of MAOM in the subsoil as compared to control soils, due to increased DOM leaching to depth, where there are also more mineral surfaces available for sorption. Since landscape position influences soil properties, with feedbacks to SOM dynamics (Berhe and Kleber 2013; Brubaker et al. 1993; Staub & Rosenzweig, 1992), (3) we also expected landscape position to interact with increased precipitation, and to see higher responses in the lowland due to increased water retention in the deeper soil profile.

2. Methods

2.1. Study Site and Experimental Design

This study was conducted at the ITEX experiment at Konza Prairie Biological Station (Kansas, USA, 39°09′N, 96°55′W). Konza Prairie has a mean annual temperature of 12.8°C and mean annual precipitation of 863 mm (30-year averages). Precipitation at this site exhibits high intra- and inter-annual variability and ranges from 569-1674 mm per year (Appendix 3,

Supplementary Figure 3.1). The ITEX experiment is described in detail elsewhere (Knapp et al. 2001) but, briefly, the ITEX site is an annually burned native tallgrass prairie that has experienced a growing season irrigation treatment that intends to maintain soil water content at 0.25 cm^3 cm^{-3} or higher to 30 cm depth (Knapp et al., 2001). This causes the magnitude of water addition to vary year-to-year but has resulted in a 29% increase in annual precipitation on average (Appendix 3, Supplementary Figure 3.1). We focus on this average precipitation increase because we are largely investigating slow-turnover pools, that will be minimally affected by climate at the time of sampling (Delgado-Baquerizo et al. 2017a; Delgado-Baquerizo et al. 2017b). The irrigation transect spans a topographic gradient, with the upland soils classified as fine, mixed mesic Udic Haplustolls (0-10cm: 15% sand, 58% silt, 27% clay) and the lowland soils classified as fine, mixed Pachic Argiustolls (0-10cm: 15% sand, 51% silt, 34% clay; Broderick et al. 2022). The site has two irrigation transects, initiated in 1991 and 1993, respectively (Appendix 3, Supplementary Figure 3.2). For each transect (2 each in the upland and lowland), each irrigation treatment is replicated 3 times, providing a total of 24 plots (2 landscape positions x 2 irrigation treatments x 2 transects x 3 replicates).

2.2. Sampling

In September 2021, we performed soil coring at each plot. We took 4 cores per plot using a 5.25 cm diameter push corer to 30cm, where soil depth allowed, since some upland plots had shallow soil profiles. In the field, we separated each core into three depth increments: 0-5 cm, 5-15 cm, and 15-30 cm, and bulked the 4 increments according to depth. This provided 72 individual observations (n = 24 plots x 3 depths). For several cores in the upland we were unable to reach 30cm and, for a few samples, 15cm, so this was accounted for in our bulk density

calculations. Soils were then transported to Colorado State University in coolers for subsequent analyses.

2.3. Soil and plant analyses

All soil samples were first sieved though 8 mm mesh and a subsample was subsequently sieved through 2 mm mesh. We collected roots and coarse material (aboveground plant material and rocks) during sieving and used the weight of these and the soil to calculate bulk density and to determine root biomass distribution (Mosier et al., 2021). For root biomass, we only report coarse roots (from 8 mm sieving) since they were the dominant root mass in the soil and roots from 2 mm sieving were unaffected by treatment or landscape position. We used air-dried 2 mm sieved soil to perform a combined density and size fractionation following Haddix et al. (2020), on the 0-5 cm and 5-15 cm depth soils (n = 48). Because there were limited effects of the treatment on SOM fraction C and N for these soils (Figure 2), we did not fractionate the 15-30cm depth soils. Briefly, 10.5 g of soil was shaken with deionized water and then centrifuged and filtered through a 20 µm nylon filter to retrieve the DOM pool. The soil was then centrifuged with sodium polytungstate at a density of 1.85 g cm^{-3} to separate the light and heavy fractions of SOM. The light, floating, fraction was aspirated as free POM (fPOM). The remaining soil (the heavy fraction) was then dispersed using 0.5% sodium hexametaphosphate and 12 glass beads. After shaking for 18 hours, the slurry was poured over a 53 µm sieve, with the fraction passing through designated as MAOM and the fraction >53 µm designated as occluded POM and heavy, coarse OM (oPOM+hcOM). Each fraction was subsequently weighed to determine recovery, which, on average, was 98.3% and varied from 94.6-102.7%. Concentrations of C and N in SOM, fPOM, oPOM+hcOM, and MAOM were measured on a VELP 802 elemental analyzer

(VELP Scientific, Inc., Long Island, New York). Concentrations of C and N in DOM were measured on a Shimadzu TOC-L (Shimadzu Scientific Instruments, Inc., Columbia, Maryland).

In addition to soil analyses, we also performed two procedures to assess root quality. We chose to focus on roots, rather than aboveground material, because this site is annually burned and so it is assumed that roots are the more quantitatively important form of plant inputs in this system. All root analyses were performed on coarse roots (collected during 8 mm sieving) and root material from 5-15 and 15-30 cm depths were combined to ensure sufficient material for the analyses. We determined acid unhydrolyzable residue (AUR) and cellulose contents in the roots using the ANKOM Acid Detergent Fiber and Acid Detergent Lignin methods (ANKOM Technology, 2022). Briefly, 0.45-0.55 g of ground root material were measured into filter bags. Filter bags were placed in an ANKOM A200 (ANKOM Technology, Macedon, NY) and agitated for 1 hour in acid detergent solution containing 93.2% water, 4.8% sulfuric acid, and 2% cetyltrimethylammonium bromide (Midland Scientific, Inc., Omaha, NE). Bags were then rinsed with deionized water and dried to 105°C and weighed to determine acid detergent fiber (W1). Bags were then submerged in H_2SO_4 for 3 hours and agitated every 30 minutes to remove cellulose. Bags were rinsed with deionized water until the pH was neutral and then dried to 105°C and weighed to determine AUR + ash (W2). Bags were then ashed in a muffle furnace at 525°C for three hours to remove AUR and weighed to determine residual ash (W3). Cellulose and AUR were determined as the difference between W1 and W2 and W2 and W3, respectively. Two filter bags (an upland and lowland irrigated replicate) opened during this procedure and, because we did not have enough root material to repeat the procedure, these were removed from analysis (below). In addition, we analyzed the amount of hot water extractable (HWE) material in the roots, approximating readily available plant compounds, using the method by Tappi

(1981), as modified by Soong et al. (2015). We digested 0.35 g of root material cut into 1 cm pieces with deionized water at 100°C for 3 hours. We then filtered the mixture through a 20 µm nylon mesh to obtain HWE (< 20 µm) and the hot water residue (> 20 µm). The HWE was then frozen until being run on a Shimadzu TOC-L (Shimadzu Scientific Instruments, Inc., Columbia, Maryland) to obtain HWE-C and HWE-N.

2.4. Data Analyses

Statistical analyses were caried out in RStudio v4.0.2 (R Core Team 2019). Response variables for this study were C and N in SOM, DOM, oPOM+hcOM, MAOM, and root HWE, as well as root biomass, AUR, and cellulose. We followed the data exploration procedures outlined in Zuur et al. (2010) and found that root biomass, bulk density, SOM C and N stock and C:N, oPOM+hcOM C and N stock, MAOM C stock and C:N, and fPOM, oPOM+hcOM, DOM, and HWE C and N concentrations had influential outliers or unequal variance and were log transformed to mitigate these. We investigated whether increased precipitation modified the response variables by building random effect models with treatment, landscape position, soil depth, and their interactions as predictors and land position nested within transect as the random variable, effectively creating four blocks. Soil depth was removed as a main effect in models for C and N stocks and root biomass because soil depth segments were uneven (e.g., 5 cm, 10 cm, and 15 cm) but interactions with treatment and landscape position were retained. Bulk density was not significantly influenced by treatment, so we investigated the effect of treatment on both soil C and N stock and concentrations. Significance was determined at $p < 0.05$.

3. Results

3.1. Soil organic matter carbon and nitrogen

Total soil C and N stocks for 0-30 cm were similar between the increased precipitation treatment and control plots, with an average of 9019 *versus* 9144 g C m^{-2} and 774 *versus* 775 g N m^{-2}, respectively. The majority of C and N was found in MAOM and the distribution of C and N between fractions was also largely unaffected by the irrigation treatment (Figure 4.1; Appendix 3, Supplementary Table 3.1). While the interaction of landscape position and soil depth was a significant predictor of SOM C and N, fPOM C, and oPOM N stocks, there were no significant differences between those values in the upland and lowland at any depth (Figure 4.1). In contrast, the C and N stocks in MAOM varied with landscape position, depending on soil depth, such that upland MAOM C and N stocks were higher than in the lowland in the 5-15 cm depth (Appendix 3, Supplementary Table 3.1). The interaction of treatment and landscape position was significant for N stock in MAOM, which was marginally higher in the control than increased precipitation plots in the upland (Appendix 3, Supplementary Table 3.1).

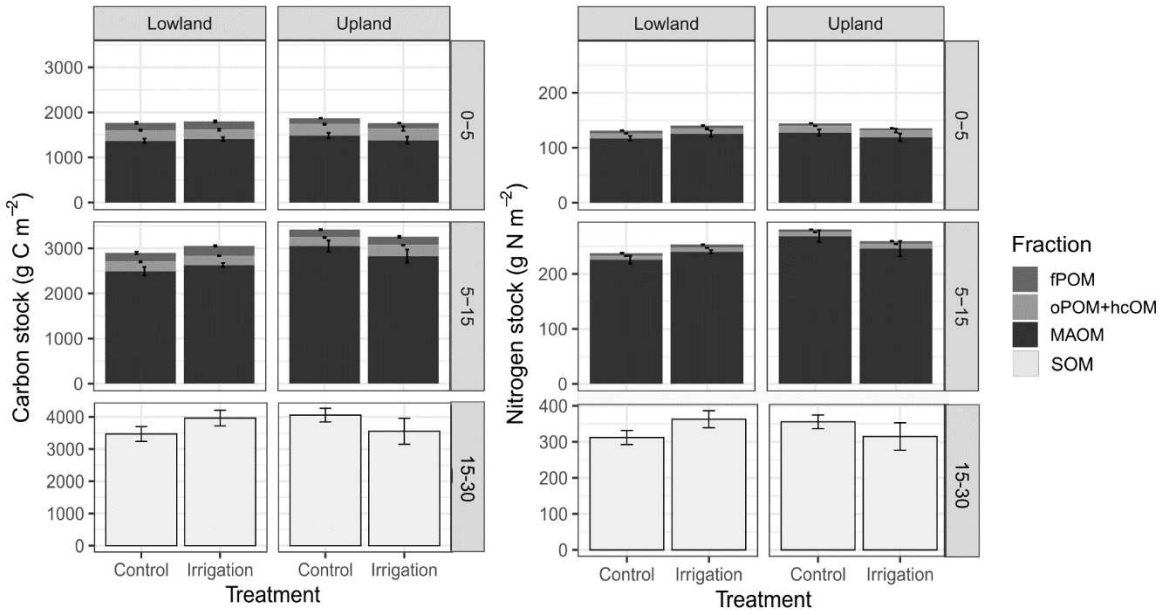

Figure 4.1. Carbon and nitrogen stocks in soil organic matter fractions (top two panels) and total soil organic matter (bottom panel) for the three soil depth increments (0-5, 5-15, or 15-30 cm) and landscape positions (lowland or upland) of our study of increased precipitation (irrigation).

Carbon and N concentrations were more responsive to increased precipitation, despite similar bulk density values between increased precipitation and control plots (1.00 *versus* 1.03 g cm^{-3}; Appendix 3, Supplementary Table 3.2). The concentration of N in fPOM was 8% higher in increased precipitation compared to control plots ($p = 0.045$; Appendix 3, Supplementary Figure 4.3). Additionally, MAOM C and N were 7 and 9% higher and oPOM+hcOM C:N was 7% lower in the increased precipitation as compared to the control plots, but only in the lowland (all $p < 0.05$; Figure 4.2; Appendix 3, Supplementary Figure 3.3). In contrast, DOM C was 18% higher under control than increased precipitation conditions in the 5-15 cm depth ($p = 0.007$). This drove 13% higher DOM C:N in the control compared to increased precipitation treatment ($p = 0.022$; Figure 4.2). The interaction of landscape position and soil depth was also significant for SOM C and C:N and MAOM C and N (Appendix 3, Supplementary Table 3.3, Figure 4.2; Supplementary Figure 3.3). SOM C and MAOM C and N were higher in the upland than lowland in 5-15 cm depth (all $p < 0.05$) and SOM C:N was higher in lowland than upland plots in the 0-5 cm depth ($p = 0.031$; Figure 4.2; Appendix 3, Supplementary Figure 3.3). Soil depth was a significant predictor of all SOM C and N concentrations and stoichiometries with higher values for all response variables in 0-5 cm, except for fPOM, oPOM+hcOM, and DOM C:N, which were higher in 5-15 cm depth (Figure 4.2; Appendix 3, Supplementary Table 3.3, Supplementary Figure 3.3).

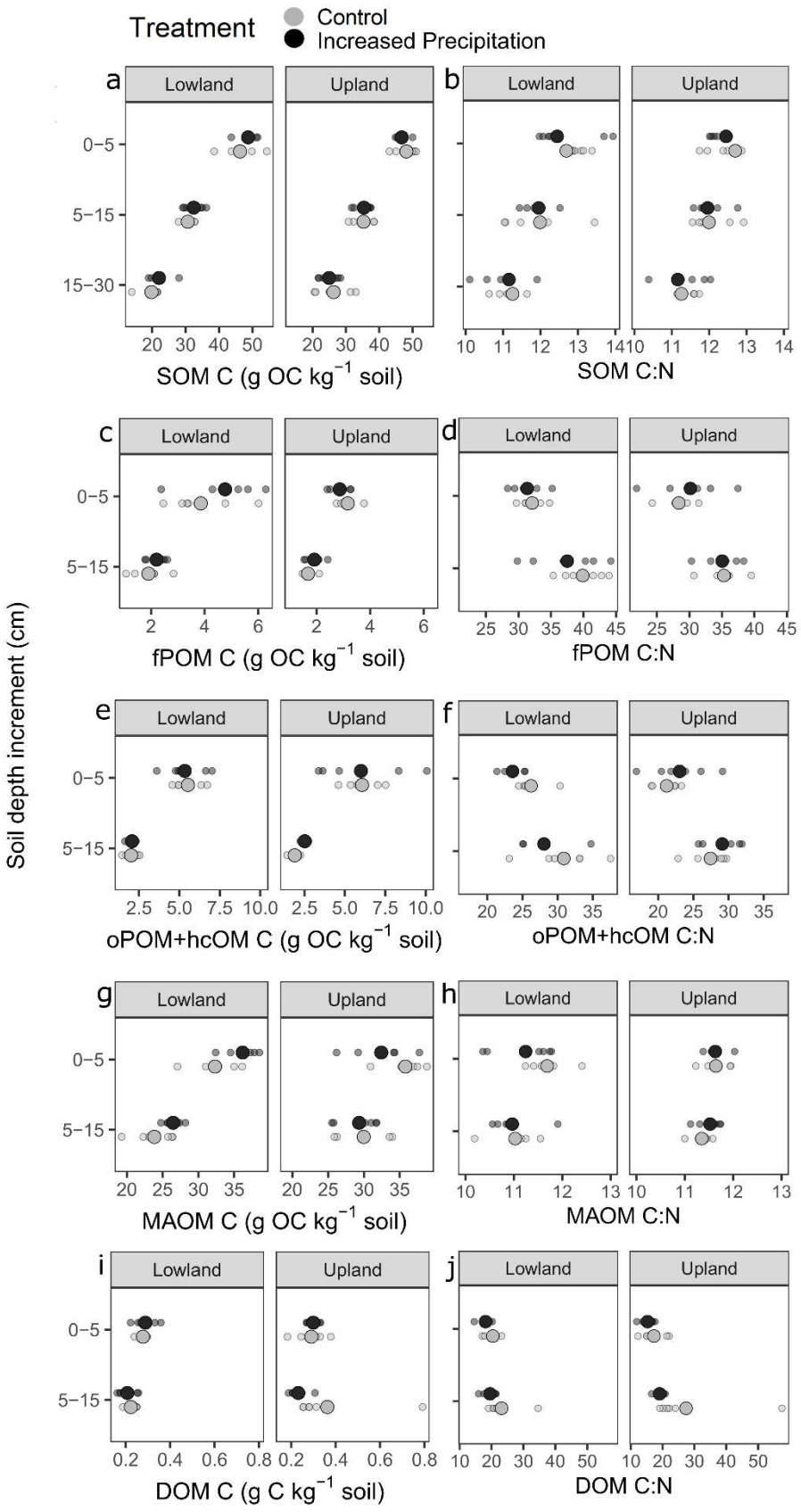
Treatment
Control
Increased Precipitation
a
Lowland
Upland
b
Lowland
Upland
0–5
5–15
15–30
20 30 40 50
SOM C (g OC kg^{-1} soil)
10 11 12 13 14
SOM C:N
c
d
2 4 6
fPOM C (g OC kg^{-1} soil)
25 30 35 40 45
fPOM C:N
e
f
2.5 5.0 7.5 10.0
oPOM+hcOM C (g OC kg^{-1} soil)
20 25 30 35
oPOM+hcOM C:N
g
h
MAOM C (g OC kg^{-1} soil)
MAOM C:N
i
j
0.2 0.4 0.6 0.8
DOM C (g C kg^{-1} soil)
10 20 30 40 50
DOM C:N
Soil depth increment (cm)

Figure 4.2. Soil organic matter (SOM) carbon (C) and carbon-to-nitrogen (C:N; a+b), free particulate OM (fPOM) C and C:N (c+d), occluded POM and heavy, coarse OM (oPOM+hcOM) C and C:N (e+f), mineral-associated OM (MAOM) C and C:N (g+h), and dissolved OM (DOM) C and C:N (i+j) in response to increased precipitation (black) *versus* a control (gray) across soil depths and landscape positions. Large circles are mean values and small circles below are individual observations.

3.2. Root biomass and quality

Root biomass increased by 26% with increased precipitation ($p = 0.001$; Figure 4.3). Root biomass was also significantly higher in the lowland than upland but only in the 0-5 cm depth ($p = 0.039$). No other pairwise comparisons were significant for root biomass (Figure 4.3; Appendix 3, Supplementary Table 3.4). Root AUR increased by 11% with increased precipitation in the lowland ($p = 0.010$) but was otherwise unaffected by predictor variables (Appendix 3, Supplementary Table 3.4). Root cellulose was influenced by soil depth ($p = 0.011$) and its interaction with landscape position ($p = 0.024$), such that there was 12% more cellulose in roots in the 5-30 cm compared to the 0-5 cm depth in the upland. Root HWE %C, %N, and C:N were unaffected by any predictor variables (Appendix 3, Supplementary Table 3.4).

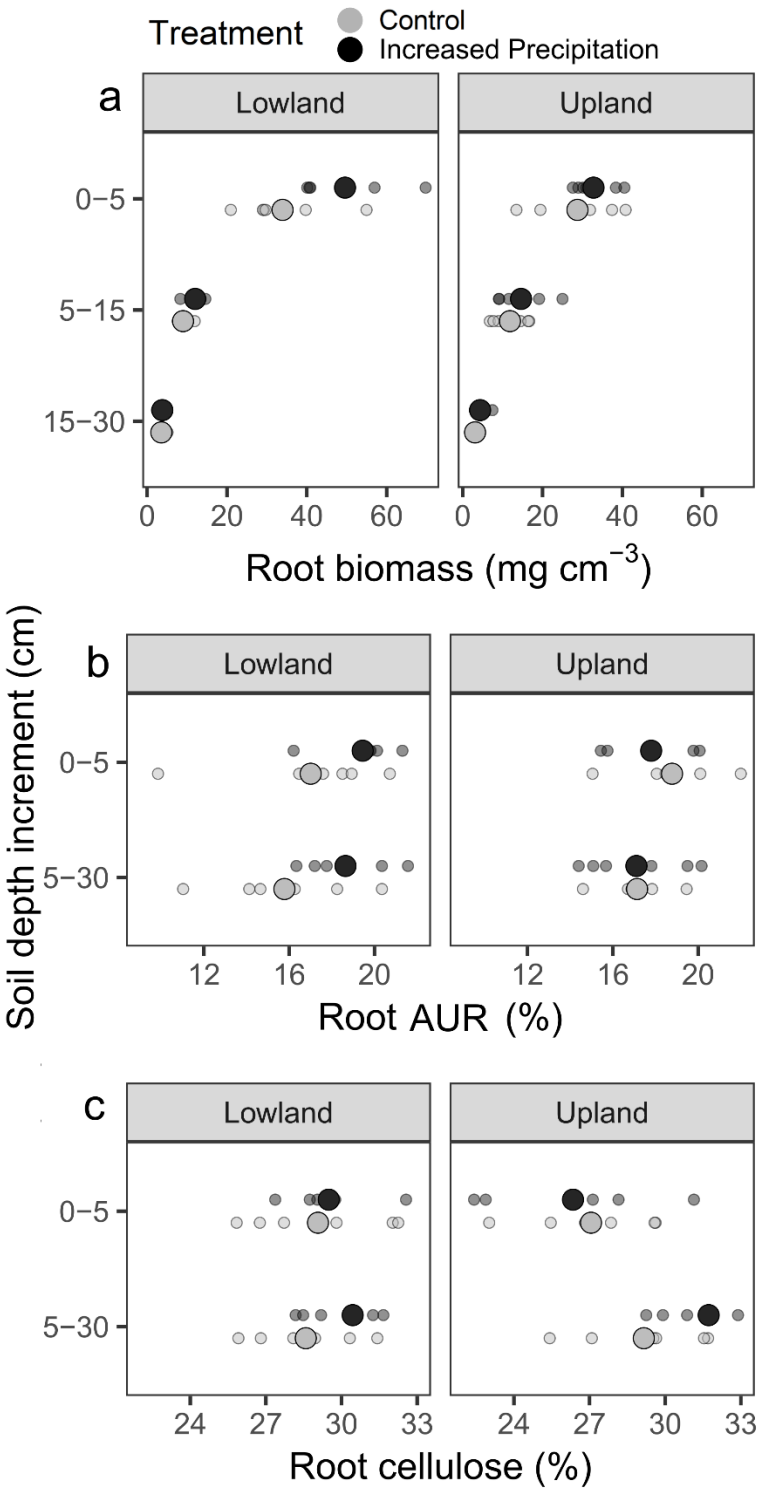

Figure 4.3. Root biomass (a), acid unhydrolyzable residue (AUR; b), and cellulose (c) in response to increased precipitation (black) *versus* a control (gray) across soil depths and landscape positions.

4. Discussion

Despite 30 years of 29% higher average water availability, that caused increased plant inputs (Knapp et al. 2001; Wilcox et al. 2016), the amount of C and N stocks and their distribution across SOM fractions was largely unchanged at this increased precipitation experiment in a mesic grassland. Although unresponsiveness of soil C and N has been reported for this site (Wilcox et al. 2016), this finding and the lack of distribution changes are noteworthy considering the high interest in C sequestration in grasslands (Bai and Cotrufo, 2022), and the general understanding that managing grasslands to increase plant production would result in increased soil C. While we found minimal influence on C and N stocks, increased precipitation modified C and N in SOM fractions in ways that align with our understanding of SOM formation and loss.

4.1. Limited responses of SOM C and N stocks

Limited responses of SOM C and N stocks found in this study are likely driven by increased C and N cycling, with minimal change in stocks of C and N. This notion is supported by our findings and previous work, which suggested greater aboveground (Wilcox et al. 2016) and belowground plant inputs with increased precipitation (Figure 4.3) but also higher respiration, potentially leading to balanced C inputs and outputs (Broderick et al. 2022; Knapp 1998). Previous work also supports the potential for higher N losses with greater water availability, with higher root $\delta^{15}N$, soil ammonium concentrations, and N mineralization and nitrification under increased precipitation at our site, depending on landscape position (Wilcox et al. 2016; Broderick et al., accepted). Thus, it is likely that increased precipitation increased C and N inputs and outputs similarly at our site, resulting in minimal change in soil C and N stocks and their distribution across SOM fractions.

Notably, while there were not significant differences in C and N stocks between increased precipitation and control plots, we did find differences in C and N concentrations. Specifically, increased MAOM C and N concentrations under increased precipitation in lowland (see section 4.2) did not translate to stock changes because bulk density responded oppositely (Supplementary Table 4.2). Reduced bulk density in lowland, increased precipitation plots is likely due to greater root biomass in both increased precipitation and lowland 0-5 cm soils (Figure 4.3). This suggests that MAOM C and N per unit mineral material likely increased, potentially allowing for more stable SOM storage under increased precipitation. However, despite small changes in the MAOM pool, our results suggest that total soil C storage in mesic grasslands under increased precipitation is unlikely to provide a negative feedback to climate change.

4.2. SOM fraction responses to increased precipitation

While we found minimal effects of increased precipitation on soil C and N stocks, we did find small responses of standing roots and SOM fraction C and N concentrations. In the lowland, there is likely faster C and N cycling under increased precipitation that is associated with greater microbial activity and likely inputs. Both N cycling and microbial biomass N increased in the lowland under increased precipitation, and respiration was elevated across the landscape transect (Broderick et al. 2022, accepted). These responses indicate greater microbial activity under increased precipitation, a response found by other studies in semi-arid grasslands (Zhao et al. 2016; Yang et al. 2017; Zhang et. 2013) but less common in mesic grasslands like our site (Ochoa-Hueso et al. 2020a; Slaughter et al. 2015). Because MAOM is formed by microbially mediated pathways, greater microbial activity aligns with higher MAOM C and N. Specifically, MAOM formation could have increased through both *in vivo* and *ex vivo* pathways. We found

greater root biomass under increased precipitation, which is associated with higher root exudation (Figure 4.3; (Eisenhauer et al. 2017). This potential for greater root inputs in the microbially-dense rhizosphere could promote efficient microbial utilization of plant inputs (Sokol et al. 2019; Villarino et al. 2021). This is aligned with increased microbial biomass N under increased precipitation at our site (Broderick et al., accepted), suggesting microbial utilization of greater root inputs may promote MAOM formation via the *in vivo* pathway. We also found lower DOM C and DOM C:N with increased precipitation, across the landscape transect (Figure 4.2). When we removed a particularly high DOM C outlier, the DOM C response became nonsignificant but the DOM C:N response was robust. The *in vivo* pathway could also operate here, with efficient microbial utilization of high-quality soluble OM that is more available with increased precipitation (Cotrufo et al. 2013). Relatively N-rich inputs of DOM under increased precipitation may have also formed MAOM via the *ex vivo* pathway, given greater stabilization of N-rich molecules (Possinger et al. 2020). We expect MAOM responses to be dominated by changes in inputs, since mineral protection reduces MAOM availability for decomposition. Overall, slight increases in C and N concentrations in MAOM in the lowland support our expectation that increased precipitation will promote formation of stable OM through greater microbial activity.

The oPOM+hcOM pool could also be considered a stable pool of OM, given similar ^{14}C ages of MAOM and oPOM (Heckman et al. 2022). The controls of hcOM are less well-known but since it generally makes up a small portion of total soil C, our interpretations focus on oPOM (Leuthold et al. 2022). We found decreased oPOM+hcOM C:N under increased precipitation in the lowlands, suggesting the quality, but not quantity, of material in aggregates was modified by increased precipitation. As noted above, the lowlands exhibited increased N cycling and

microbial biomass N under increased precipitation (Broderick et al., accepted), suggesting greater microbial activity. This may indicate that a greater relative proportion of microbial products may have been entrapped in aggregates in lowland under increased precipitation. The tallgrass prairie contains some of the highest concentrations of amino sugars (a proxy for microbial necromass) of all global grasslands (Bai and Cotrufo 2022). Necromass-C has been shown to increase up to approximately 1000 mm of annual precipitation, which is slightly lower than the annual precipitation we would expect at our site with the irrigation treatment (Bai and Cotrufo 2022). Thus, increased microbial necromass occlusion in the lowlands is a feasible pathway for decreased oPOM+hcOM C:N under increased precipitation. Changes in plant quality may be less likely to explain reduced oPOM+hcOM C:N in lowland, increased precipitation plots, given our findings of increased root AUR, expected to largely consist of N-poor lignin. However, AUR from decomposing plant material has been suggested to also contain microbial structures which would be more N-rich (McKee et al. 2016), potentially driving lower oPOM+hcOM C:N if root litter was the main constituent of this pool. Regardless, the variation in AUR found here is small relative to studies of litter quality impacts on SOM formation, and so may not be influential in our study (Lavallee et al. 2018; Vityakon et al. 2000). Altogether, greater microbial activity in the lowland likely promoted changes in stable SOM fractions that were not exhibited in the upland, suggesting the character of SOM may be modified under increased precipitation when microbial activity is also modified. We will measure amino sugars in these soils to investigate this potential mechanism.

We expected to find reduced fPOM under increased precipitation at our site, due to higher microbial decomposition under increased precipitation (Broderick et al. 2022; Knapp 1998). Rather, N in fPOM increased across the landscape transect (Figure 4.2). This may be

because evidence for higher microbial activity was more strongly exhibited in the lowland (Brodderick et al., 2022; accepted), indicating decomposition of the unprotected fPOM pool may not have been as high as expected. Rather, greater plant input under increased precipitation may explain this fPOM response. Forb, legume, and grass biomass all increased at our site under increased precipitation (Wilcox et al. 2016), which may promote more structural (forb) and N-rich (legume and grass) plant input that could contribute to the fPOM pool (Cotrufo et al., 2015; Six et al. 2001b; Vázquez-de-Aldana et al. 2000). Meta-analytical findings of greater plant N under increased precipitation suggest this may be a widespread phenomenon (Wu et al. 2022). Responses of fPOM may also be explained by changes in microbial input; particulate OM has been found to contain microbial structural components, particularly those of fungi (Six et al. 2001b). Greater fungal amino sugars and biomass associated with increased annual precipitation may promote greater quantities of fungal structures in POM, potentially making POM more N-rich, since amino sugars are enriched in N relative to plant material (Amelung 2003; Bai et al. 2020; Bai and Cotrufo 2022). However, microbial drivers of fPOM responses may have less support at our site, since increases in microbial biomass N were restricted to the lowland, whereas the fPOM N response was not. These results suggest that the response of SOM C and N distribution under increased precipitation was dependent on changes in microbial activity, particularly related to N cycling, which we found solely in lowland soils.

The stronger lowland responses in this study are similar to studies of legacy effects of precipitation at this site (Broderick et al., accepted; Broderick et al. 2022). The lowland part of this site is a depositional setting, where material eroded from the upland deposits in the lowland. Depositional settings may have more stable organic matter than erosive landscapes, given inputs of OM that may have been freed from aggregates during erosion and freshly deposited reactive

minerals (Berhe and Kleber 2013). Newly deposited OM and minerals may interact and then get buried by successive erosion, further protecting them from decomposition (Berhe and Kleber 2013). The potentially higher availability of OM and reactive materials at the lowland site may facilitate the stronger responses of minerally-protected OM pools to increased precipitation in this study. However, we find that MAOM C stock is higher in the upland than the lowland in the 5-15cm depth, suggesting greater reactive minerals and OM in the lowland may be less important for our results (Figure 4.1). Alternatively, the depositional setting of the lowland created deeper soils, which have been shown to have higher soil water content under increased precipitation that may promote N cycling and stable SOM responses in the lowland (Broderick et al. 2022; accepted). Differences in depth between lowland soils and upland soils may be why we did not find interactions of increased precipitation treatment and soil depth at our site, as we had expected. The shallow upland soils prevented us from consistently sampling down to 30 cm. More studies on precipitation alteration in deeper soils will help clarify if variable responses occur over depth, as has been found for other global changes (Rocci et al. 2021; Soong et al. 2021).

4.3. Looking forward

The results of this study and others from this site paint a picture of faster C and N cycling under increased precipitation in mesic grasslands. In the lowland at our site, this likely occurred efficiently enough to form MAOM C and N, due to greater N cycling. This suggests that in grassland sites limited by both water and N, stable SOM formation may only occur when both N and C cycling are increased (Broderick et al. accepted). Co-limitation of SOM formation by water and N may be particularly common in mesic grasslands, as N addition has the strongest influence on decomposition in wetter grasslands (Ochoa-Hueso et al. 2020b). In the uplands,

where soil moisture may not have been sufficiently retained to influence N cycling, C and N storage in stable OM was unaffected. Thus, when considering the potential of grassland soils for C storage to mitigate climate change (Bai and Cotrufo, 2022), it will be important to consider C and N cycling in tandem. Furthermore, our study suggests that soil C may be preferentially stored in MAOM under increased precipitation when N cycling is also increased, since C was only responsive in MAOM. This could promote a more resilient soil C pool to other global changes, like warming (Rocci et al. 2021). However, current results of this study suggest that changes in the stable OM pool under increased precipitation will likely not be sufficient to provide a significant negative feedback to climate change.

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CHAPTER 5: PROXIMITY TO ROADS DOES NOT MODIFY INORGANIC NITROGEN DEPOSITION IN A TOPOGRAPHICALLY COMPLEX, HIGH TRAFFIC, SUBALPINE FOREST[4]

1. Introduction

Excess reactive nitrogen (N) is a byproduct of many anthropogenic activities leading to cascading ecological effects in terrestrial ecosystems such as soil acidification and changes in biodiversity (Galloway et al. 2003). This excess N is sourced to the atmosphere as nitrogen oxides (NO_x) and ammonia (NH_3) from the transportation sector, energy production, and agriculture. Reactive N species are transformed in the atmosphere into biologically available forms of nitrate (NO_3^-) and ammonium (NH_4^+) and may then enter natural ecosystems in wet or dry atmospheric deposition. While we have a good understanding of regional trends of atmospheric N deposition in the USA through programs like the National Atmospheric Deposition Program National Trends Network (NTN; wet deposition) and the Clean Air Status and Trends Network (CASTNET; dry deposition), we have less understanding of how local and point sources, like vehicles, feedlots, and smokestacks (Bettez et al. 2013; Elliott et al. 2009), may contribute to fine scale N deposition.

Vehicle exhaust is emitted close to the ground and deposited locally and may be an overlooked important local N deposition source in high traffic areas (Cape et al. 2004; Wetherbee et al. 2019). Vehicle emissions of N constitute the largest source of NO_2 emissions in the USA (EPA, 2020). Higher N deposition has been found close to roads with a variety of traffic densities in the northeast USA, Scotland, England, and in Grand Canyon National Park (Bettez et al. 2013; Cape et al. 2004; Gadsdon and Power 2009; Kenkel et al. 2016; Redling et al.

[4] To be developed into a peer-reviewed publication with M. Francesca Cotrufo and Jill Baron

2013). Both NO_x and NH_3 are emitted from vehicles, leading to deposition of NO_3^- and NH_4^+ along roadsides (Bishop and Stedman 2015; Fenn et al. 2018b; Liu et al. 2017). Oxidized N is a byproduct of internal combustion engines, but reduced N is becoming increasingly important as catalytic converters convert NO_x to NH_3 (Fenn et al. 2018b). This is true for both gasoline and diesel vehicles, which have three-way and selective catalytic converters, respectively (Bishop and Stedman 2015; McDonald et al. 2012). While catalytic converters have been effective in reducing NO_x emissions and thus overall vehicle N emissions, they do not consistently reduce NH_3 emissions (Bishop and Stedman 2015; Carslaw et al. 2019). Because different N species have nonidentical effects on ecosystem properties, evaluating the relative input of NO_3^- *versus* NH_4^+ is important for determining the influence of N deposition on ecosystems. In several studies addition of NH_4^+ generally had a more positive effect on carbon storage in roots and soil and a more negative effect on dissolved organic carbon than addition of NO_3^-, likely due to the acidifying effect of NH_4^+ addition (Evans et al. 2008; Lu et al. 2011; Yue et al. 2016). Vehicles are likely important sources for both oxidized and reduced N deposition to ecosystems adjacent to high traffic roads.

The influence of vehicle emissions on roadside ecosystems is likely to be modified by vegetation cover. Nearly one third of the USA (including Alaska and Hawaii) is forested (Vogt and Smith, 2017) and N deposited through tree or shrub canopies (e.g., throughfall deposition) can be modified by interactions with canopies. Wet N deposition occurs when nitrogenous gases dissolve in atmospheric liquid and fall as NO_3^-, NH_4^+, or organic N in precipitation whereas dry deposition occurs when atmospheric particles or gases deposit via gravity or aerodynamic transport followed by reaction with or adsorption to a surface (Jacob 1999; Zhang et al. 2021). Tree foliage is a surface aerosols and gases can sorb to, so throughfall includes both wet and dry

deposition, together termed bulk deposition (Bettez et al. 2013; Fenn and Bytnerowicz 1993). In contrast, bulk deposition collection in non-forested areas, hereafter termed open deposition, is generally comparable to wet deposition alone, due to the absence of an active absorbing surface (Fenn et al. 2018a). Gaseous N species in vehicle exhaust may be particularly susceptible to dry deposition, particularly in dry climates, that would be better captured by throughfall than open deposition measurements, because vehicle emissions deposit locally (Cape et al. 2004). Indeed, a study of N deposition adjacent to roadways in Massachusetts, USA found higher roadside inorganic N deposition in throughfall but not open measurements, which they attribute to greater capture of gaseous N in throughfall collectors (Bettez et al. 2013). In support of this, a study in southern England found stronger declines in NO_2 and NH_3 deposition moving away from roads for under-canopy compared to within-canopy measurements (Gadsdon and Power 2009). More broadly, the amount and composition of throughfall N deposition could be modified by foliar uptake, primarily via diffusion of NH_3 and NO_x through leaf stomata, and transformation of N (Harrison et al. 2000). Direct leaf uptake of atmospheric N has been shown to contribute anywhere from 0-50% of plant N demand (Harrison et al. 2000; Sparks 2009). There is generally higher uptake of NH_4^+ than NO_3^- across tree species, particularly for deciduous trees and senescing leaves, potentially increasing the deposition of NO_3^- *versus* NH_4^+ to the forest floor (Adriaenssens et al. 2011). Microbial transformations of N in the phyllosphere (the canopy habitat for microbes) could also modify the species of N deposited. In forests in Spain, the United Kingdom, and Norway, isotopic evidence and the presence of nitrifiers and the amoA gene (which encodes the enzyme that catalyzes nitrification) suggest nitrification occurs in tree canopies, increasing the proportion of NO_3^- captured in throughfall collectors (Guerrieri et al. 2020; Guerrieri et al. 2015; Papen et al. 2002). Importantly, throughfall deposition has been

shown to have different effects on ecosystems than open N deposition. Li et al. (2021) found that addition of N in the canopy increased fine root biomass, but direct N addition to the forest floor decreased fine root biomass. By investigating throughfall N deposition in addition to open N deposition, we may be able to better capture deposition from vehicle N emissions and infer how local N emissions could differently influence forested and non-forested ecosystems.

Ecosystems that evolved with low N availability are vulnerable to even small changes in N input (Baron et al. 2000). In Colorado, USA, much of the literature points to lower elevation oxidized and reduced N emissions from agricultural and urban N sources on the Great Plains (Benedict et al. 2013b; Benedict et al. 2018; Clow et al. 2015; Nanus et al. 2018; Thompson et al. 2015) resulting in increased soil, plant, lake, and stream N in high elevation ecosystems of the Rocky Mountains to the west (Baron et al. 2000; Clark et al. 2021; Mast et al. 2014; Rueth and Baron 2002). Local, rather than regional emission sources, such as highway and road vehicular emissions, have not been quantified, prompting the study we report here.

Rocky Mountain National Park (RMNP) in Colorado, USA, is experiencing a large increase in local vehicle emissions. Visitation at RMNP increased by 44% since 2012, with 4.67 million visitors in 2019 (Patterson, 2019). Most visitors tour the park by car, with approximately 670,000 vehicles entering through the most popular entrances (Beaver Meadows and Fall River) in June through September in 2021 (IRMA, 2022) and an average of ~230 cars on the popular Bear Lake road area per hour during peak entry time in 2021 (Creany and Monz, 2022). Rocky mountain NP is dominated by complex, mountainous topography, which has been shown to cause high variation in N deposition (Hoffman et al. 2019).

We asked whether local N sources from vehicles influence open and throughfall N deposition in high traffic, mountainous terrain. Specifically, we investigated (1) whether there is

a measurable increase in atmospheric N deposition to vegetation directly adjacent to the roadway that may be attributed to vehicles; (2) whether N deposition patterns vary in throughfall versus open sites adjacent to a highly trafficked road in RMNP; (3) how roadside N deposition compares to regional N deposition estimates from NTN and CASTNET. We expected (1) higher N deposition directly adjacent to the roadway (Bettez et al. 2013; Cape et al. 2004; Redling et al. 2013) dominated by NH_4^+ due to catalytic converters increasing relative NH_3 emission (Bishop and Stedman 2015; Fenn et al. 2018a). We also expected (2) higher throughfall than open N deposition, particularly at the roadside, due to greater dry deposition of NO_3^- and not NH_4^+ due to potential preferential NH_4^+ uptake by trees and canopy nitrification. Due to vehicle emissions, we expected (3) higher roadside N deposition than N deposition measured at regional NTN and CASTNET sites.

2. Methods

2.1 Study Site and Experimental Design

Bulk deposition collectors were installed near the Bear Lake parking lot in Rocky Mountain National Park (RMNP) USA (40°18'42"N, 105°38'42"W), which has an average annual precipitation and temperature of 88 cm and 3.1°C (USDA, 2022). Vegetation in subalpine forest stands was dominated by subalpine fir (*Abies lasiocarpa*) and Engelmann spruce (*Picea engelmannii*) that were at elevations ranging from 2800-2900 m. Nitrogen emission sources to RMNP include energy production east and west of the continental divide, and agricultural and urban sources from east of the continental divide (Benedict et al. 2013b; Benedict et al. 2018; Clow et al. 2015; Nanus et al. 2018; Thompson et al. 2015). Emissions from the east, which is the largest regional source area, move to the mountains via upslope wind patterns primarily in spring and summer (Benedict et al. 2013b).

We created two transects of sites that moved away from the road (Figure 5.1); a throughfall transect where collectors were placed under the canopies of subalpine fir trees; and an open transect, where sites were placed in forest openings (design modeled after Redling et al. 2013). The throughfall transect consisted of six sites at approximately 5, 10, 20, 50, 150, and 750 m from the road. The open transect consisted of five sites at approximately 5, 10, 20, 60, and 225 m from the road. The open transect was limited to 225 m because of the lack of forest openings in the Bear Lake area. Previous research found 90% of NH_3 and NO_2 deposition to occur within 15 m of the road and that the effects of roadways are only detectable to within 100 m (Bignal et al. 2007; Cape et al. 2004), so we treated the furthest sites in each transect as controls that should be unaffected by N emitted by vehicles, following the method of Redling et al. (2013). Due to the proximity of the sites, we assumed they had the same background N deposition from regional sources described above. At each site, we installed passive N collectors that collected both wet and dry deposition (e.g., bulk deposition; Fenn et al. 2018a). Collectors were placed on low slope terrain but occupied topographically complex terrain and were mostly adjacent to steeper slopes (Appendix 4, Supplementary Figure 4.1). None of the collectors were installed at the same elevation as the road to minimize their visibility. Each bulk or throughfall site had three collectors approximately 5 m apart from one another and parallel to the road, to account for spatial variability. This provided a total of 18 individual observations at throughfall sites and 15 individual observations at open sites. Both throughfall and open collectors were operated from June 28-October 7, 2021 (101-day exposure period). The dates of sample collection corresponded with the period of peak visitation at RMNP (NPS 2022). Throughfall was also collected from May 25-June 28, 2021 and full summer data (May 25-October 7, 2021; 132-day exposure period) for the throughfall transect can be found in the Appendix 3.

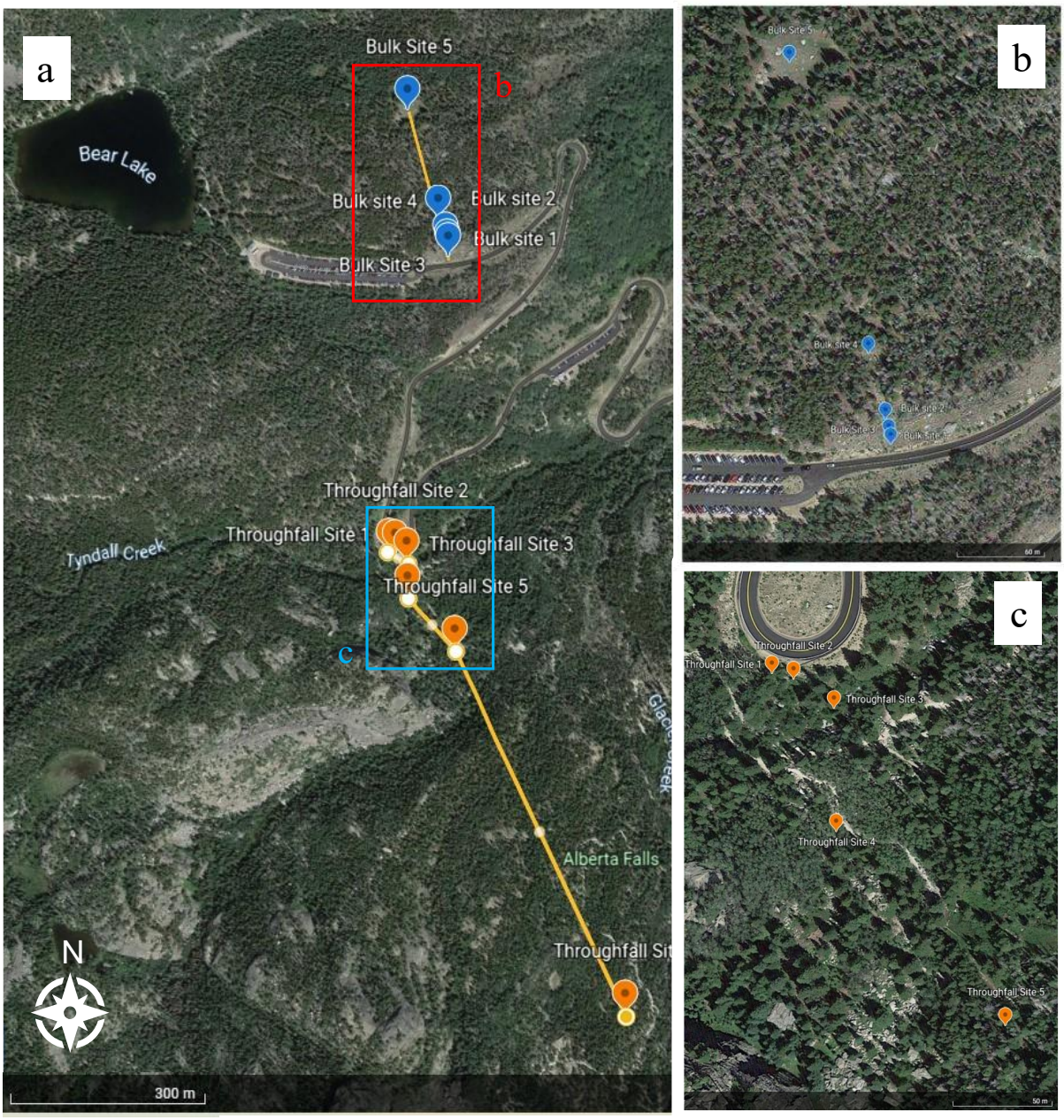

Figure 5.1. (a) Map of Bear Lake area in Rocky Mountain National Park showing open (blue) and throughfall (orange) transects. (b) Closeup of the red box in (a) showing all five open sites moving north away from the Bear Lake parking lot. (c) Closeup of the blue box in (a) showing the five throughfall sites closest to the road. Images are from Google Earth.

Collectors consisted of an ion exchange resin (IER) column, which is a PVC tube that contains a nuclear grade mixed bed IER (Amberlite IRN-150, Fisher Scientific, LLC.) with both cation and anion exchange resins that capture NH_4^+ and NO_3^-, respectively. The IER column was

fit with a fiber filter plug to prevent debris from entering the tube and a funnel to provide a consistent collection area. The IER apparatus was attached to a pole (Appendix 4, Supplementary Figure 4.2a). At each site, one capped IER column was attached to the pole as a blank and used to correct for any resin contamination. At open sites, bird rings were added to the collectors to discourage birds from landing on and defecating in the collectors (Appendix 4, Supplementary Figure 4.2b). At throughfall sites, collectors were placed under branches approximately halfway between the canopy edge and tree trunk.

Diameter at breast height (DBH) of trees with throughfall samplers was recorded as a proxy for tree size. Approximately monthly during the collection period, we looked for disturbance to the collectors, such as skewed bird rings or bent poles, such that the collectors were not perpendicular to the forest floor. After noting disturbances, which were uncommon, these were corrected. We looked for, but found no evidence of, bird droppings in the open collectors. At the end of the collection period (October 2021), the collectors and IER columns were brought to the Colorado State University EcoCore laboratory for analysis.

2.2 Laboratory Analyses and Calculations

We extracted the IER columns following the methodology in Fenn et al. (2016). Columns were first rinsed with deionized water, then extracted with 2M potassium chloride (KCl) solution to desorb the NO_3^- and NH_4^+ ions. We used two KCl extractions per column because two extractions have been found to recover 98-100% of the ions on the IER (Fenn and Poth 2004). A ~60 mL subsample was frozen until analysis. Ammonium and NO_3^- concentrations were analyzed colorimetrically (Alpkem Flow Solution IV Automated wet chemistry system; O.I. Analytical, College Station, TX). To measure NO_3^- colorimetrically we raised the pH of the

extractant by adding 0.2 mL of NaOH to 2 mL of the extraction, as suggested by Fenn et al. (2016).

To determine deposition rates of NO_3^- and NH_4^+, we first summed the N concentrations (g/L) from both KCl extractions for each column and multiplied them by the extracted sample volume (L) to determine the total amount of NO_3^- or NH_4^+-N collected by the IER column. The total NO_3^-- or NH_4^+-N was scaled to mg N ha^{-1} day^{-1} using the following equation:

$$\mathrm{NO_3^-or\ NH_4^+\ deposition} = Total\ \mathrm{NO_3^-}\ or\ \mathrm{NH_4^+} - N * 1000 * \frac{1}{\text{collection area}} * 10000 * \frac{1}{\text{collection time}} \quad \text{(Eq. 1)}$$

where collection area is the area of the funnel opening (0.0346 m^2) and collection time is the total length of time the IER columns were in the field (101 days). Nitrate and NH_4^+ deposition were summed to calculate total inorganic N (TIN) deposition. We also determined the ratio of NH_4^+ to NO_3^- deposition.

We compared our data to nearby NTN and CASTNET sites (Appendix 4, Supplementary Figure 4.3). Loch Vale (CO98) and Beaver Meadows (CO19) NTN sites are located approximately 3000 m and 7800 m from the Bear Lake parking lot, near where our transects start. We added a single additional throughfall IER collector at the Loch Vale NTN site for comparison; the resin was extracted and analyzed as for the other IER collectors. The Loch Vale NTN site is at an elevation of 3159 m and Beaver Meadows is at an elevation of 2477 m. The CASTNET site in RMNP (ROM206) is south of the Beaver Meadows NTN site, ~8700 m from the Bear Lake parking lot, and is at an elevation of 2742 m (Appendix 4, Supplementary Figure 4.3). NTN and CASTNET samples are collected each Tuesday, so our IER data had 14 weeks of overlap. NTN and CASTNET data were retrieved from

https://nadp.slh.wisc.edu/networks/national-trends-network/ and https://java.epa.gov/castnet/clearsession.do, respectively. There was not N concentration data from the NTN dataset for 2 and 3 of the overlapping weeks for Loch Vale and Beaver Meadows, respectively, so the number of weeks in equation 2 were scaled back from 14 weeks to 12 and 11 weeks, respectively. For CASTNET, no weeks were missing. To calculate the wet deposition at Loch Vale and Beaver Meadows, we calculated precipitation-weighted mean concentrations of NO_3^- and NH_4^+ as (Wetherbee et a., 2019; NADP, 1987):

$$Precipitation\ weighted\ mean\ (Pwm) = \frac{\sum_{i=1}^{n}(C\ x\ Ppt)}{\sum_{i=1}^{n} Ppt} \quad \text{(Eq. 2)}$$

Where, n is the number of weeks samples were collected, C is the concentration of NO_3^- or NH_4^+ in mg N L^{-1}, and Ppt is weekly precipitation, in centimeters, measured at co-located rain gauges. Deposition load (mg N ha^{-1} day^{-1}) was then calculated as:

$$Depositon\ load = Pwm \text{ x } (\text{Ppt x } time^{-1}) * 0.1 * 1000000 \quad \text{(Eq. 3)}$$

Where, (Ppt x $time^{-1}$) is the total amount of precipitation divided by the time included in the Pwm. We compared precipitation data measured at the rain gauges associated with Loch Vale and Beaver Meadows NTN sites with precipitation data from a Snowpack Telemetry (SNOTEL) site at the Bear Lake parking lot (SNOTEL site 322), which is likely representative of the precipitation at our transects, as it is approximately 330 and 450 m from the open and throughfall transects, respectively (USDA, 2022; Appendix 4, Supplementary Figure 4.3).

CASTNET data were downloaded as kg NO_3^- or NH_4^+ ha^{-1} $week^{-1}$ for each of the 14 overlapping weeks and were converted to mg N ha^{-1} day^{-1}. Details on calculation of CASTNET dry deposition loads can be found in Schwede and Lear (2014), but briefly, these are calculated using concentration of NO_3^- or NH_4^+ collected on filters and modeling of dry deposition rates

using the Community Multiscale Air Quality model. CASTNET does not collect NH_3 deposition, which has been shown to be the largest contribution of dry N in RMNP (Benedict et al. 2013a).

2.3 Statistical analyses

Statistical analyses were conducted in RStudio in R version 4.0.2 (R Core Team 2019). The response variables of interest were TIN, NO_3^- and NH_4^+ deposition rates and the ratio of NH_4^+ to NO_3^- deposition rates. We assessed the following for each response variable: (1) the effect of distance from the road separately at throughfall and open sites, (2) the difference in throughfall *versus* open sites, and (3) the relationship between throughfall deposition and tree size. We followed the data exploration procedures outlined in Zuur et al. (2010) and found that there were outliers in every response variable when grouped by open or throughfall but not when grouped by site. Transforming the variables did not remove outliers so we chose to determine the influence of these outliers by evaluating models testing throughfall versus open deposition with and without outliers in the dataset; these results are presented below.

To assess the effect of distance from the road, we created separate linear models for open and throughfall transects for each response variable with distance from the road as the predictor. We then used Analysis of Variance (ANOVA) and pairwise comparisons in the *emmeans* package (Lenth, 2020) to determine effect of site overall and differences between sites, respectively.

To evaluate open *versus* throughfall deposition, we created linear models with the same response variables but with site as a random variable to account for non-independence between observations at a site and site type (throughfall *versus* open) as the predictor variable. We used

ANOVA and pairwise comparisons for the full dataset and with the outliers removed to determine their influence on the statistical outcomes.

To assess the relationship between N deposition and tree size, we performed correlations between response variables and tree DBH. For all tests, significance was determined at $p < 0.05$. Graphs were created using the *ggplot2* (Wickham, 2016) and *ggbreak* (Xu et al., 2021) packages.

3. Results

Total inorganic N deposition at our throughfall sites varied from 3,815.3 – 23,842.4 mg N ha^{-1} day^{-1} with the majority deposited as NO_3^- (mean NH_4^+:NO_3^- = 0.33). At open sites, TIN deposition was less variable, 3,643.6 – 8,100.8 mg N ha^{-1} day^{-1}, and also dominated by NO_3^- (mean NH_4^+:NO_3^- = 0.68).

There was no relationship with distance from the road and any form of open N deposition (Table 5.1; Figure 5.2a-d). However, there were significant differences between sites for throughfall NH_4^+ deposition (Table 5.1). The highest NH_4^+ deposition was at the site 50 m from road, which was 81% higher than the site 750 m from the road ($p = 0.041$). Generally, the sites 50 and 150 m from the road were higher than sites 10, 20, and 750 m from the road, although these relationships were not statistically significant (Figure 5.3). There was no relationship with distance from the road and any other form of throughfall N deposition.

Table 5.1. Outcome of ANOVAs for the response of nitrogen deposition to site location at open (open canopy) and throughfall (under trees) sites. Response variables that varied significantly ($p < 0.05$) with distance from the road are bolded. Ammonium = NH_4^+; Nitrate = NO_3^-; Total inorganic nitrogen (ammonium + nitrate) = TIN; ammonium-to-nitrate ratio = NH_4^+: NO_3^-.

Tree Cover	Response Variable	F-value	p-value
Open	NH_4^+	0.276	0.887
	NO_3^-	2.477	0.112
	TIN	1.083	0.415
	NH_4^+: NO_3^-	0.149	0.959
Throughfall	**NH_4^+**	**4.500**	**0.015**

NO_3^-	0.612	0.693
TIN	0.703	0.632
NH_4^+: NO_3^-	1.645	0.222

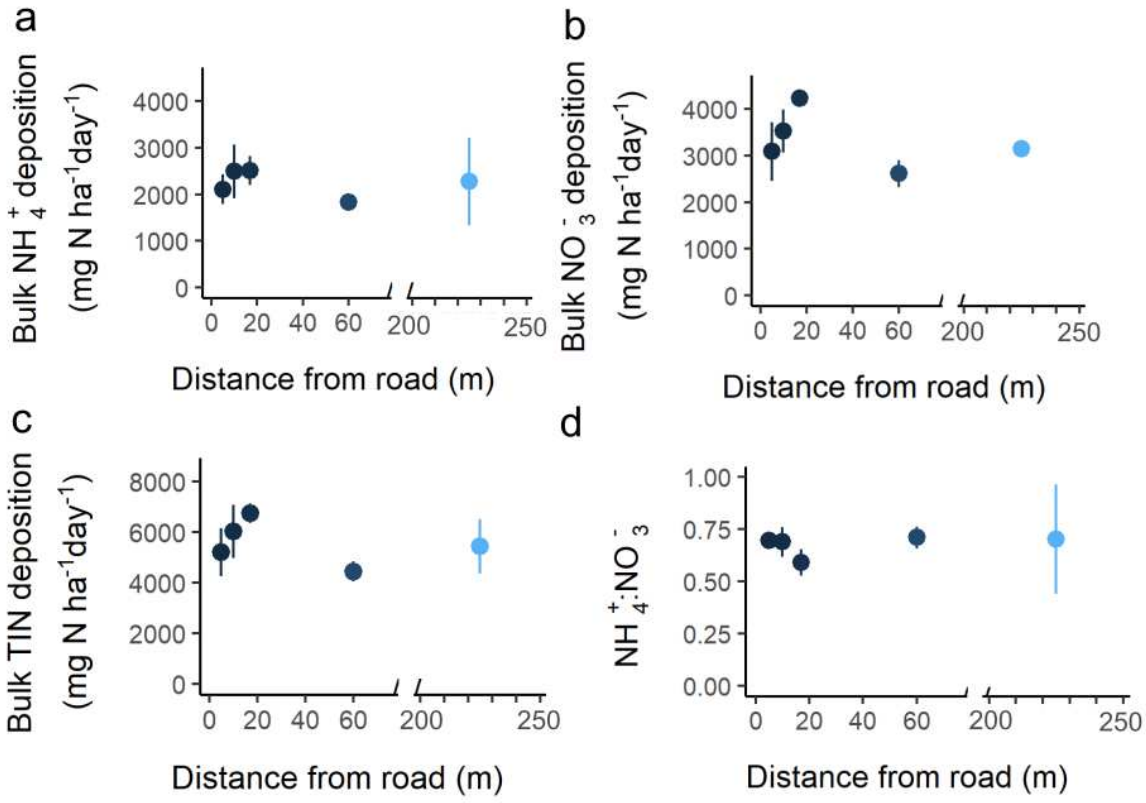

Figure 5.2. Lollipop plots of a) ammonium (NH_4^+), b) nitrate (NO_3^-), c) total inorganic nitrogen (TIN), and d) the ratio of NH_4^+ to NO_3^- in open deposition in July-September at five sites moving away from Bear Lake Road in Rocky Mountain National Park. Darker blue dots are closer to the road.

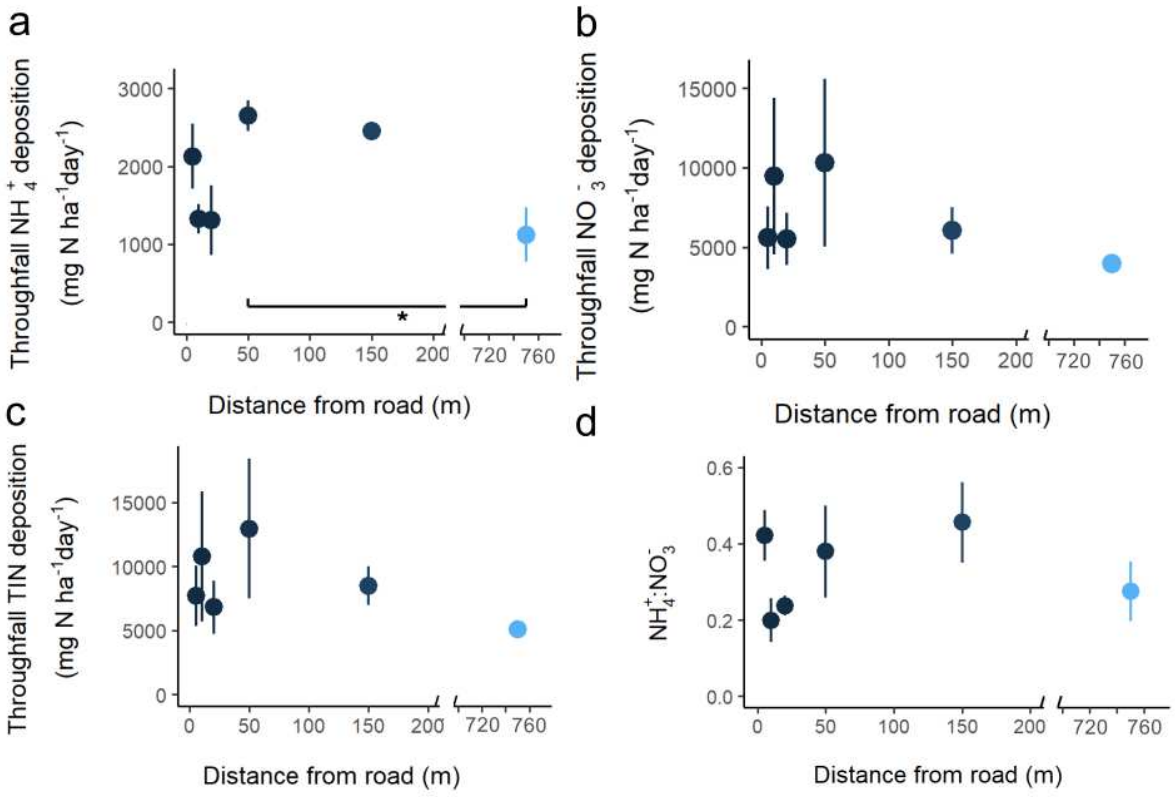

Figure 5.3. Lollipop plot of a) ammonium (NH_4^+), b) nitrate (NO_3^-), c) total inorganic nitrogen (TIN), and d) the ratio of NH_4^+ to NO_3^- throughfall deposition in July-September at six sites moving away from Bear Lake road in Rocky Mountain National Park. Lines with "*" connects sites with significantly different deposition rates at $p < 0.05$. Darker blue dots are closer to the road. Note the difference in scale between NH_4^+ and other concentrations.

When open deposition was compared with throughfall deposition we found that open deposition had a higher NH_4^+ to NO_3^- ratio (mean = 0.68) as compared to throughfall deposition (mean = 0.33; $F = 33.571$; $p < 0.001$; Figure 5.4d). This was driven by 69% lower open NO_3^- deposition compared to throughfall NO_3^- deposition ($F = 6.610$; $p = 0.010$; Figure 5.4b). Lower NO_3^- deposition also drove 43% lower TIN open deposition compared to throughfall deposition ($F = 4.291$; $p = 0.038$; Figure 5.4c). Removing outliers from the NH_4^+ to NO_3^- ratio and NO_3^- and TIN deposition and had minimal influence on statistical outcomes (NH_4^+:NO_3^-: $F = 40.292$; $p < 0.001$; NO_3^-: $F = 10.584$; $p = 0.001$; TIN: $F = 3.184$; $p = 0.074$). The deposition of NH_4^+ was similar between open and throughfall deposition (2243 versus 1832 mg N ha^{-1} day^{-1}), such that

site type was not significant (F = 1.664; p = 0.197), regardless of removal of outliers (F = 0.610; p = 0.453).

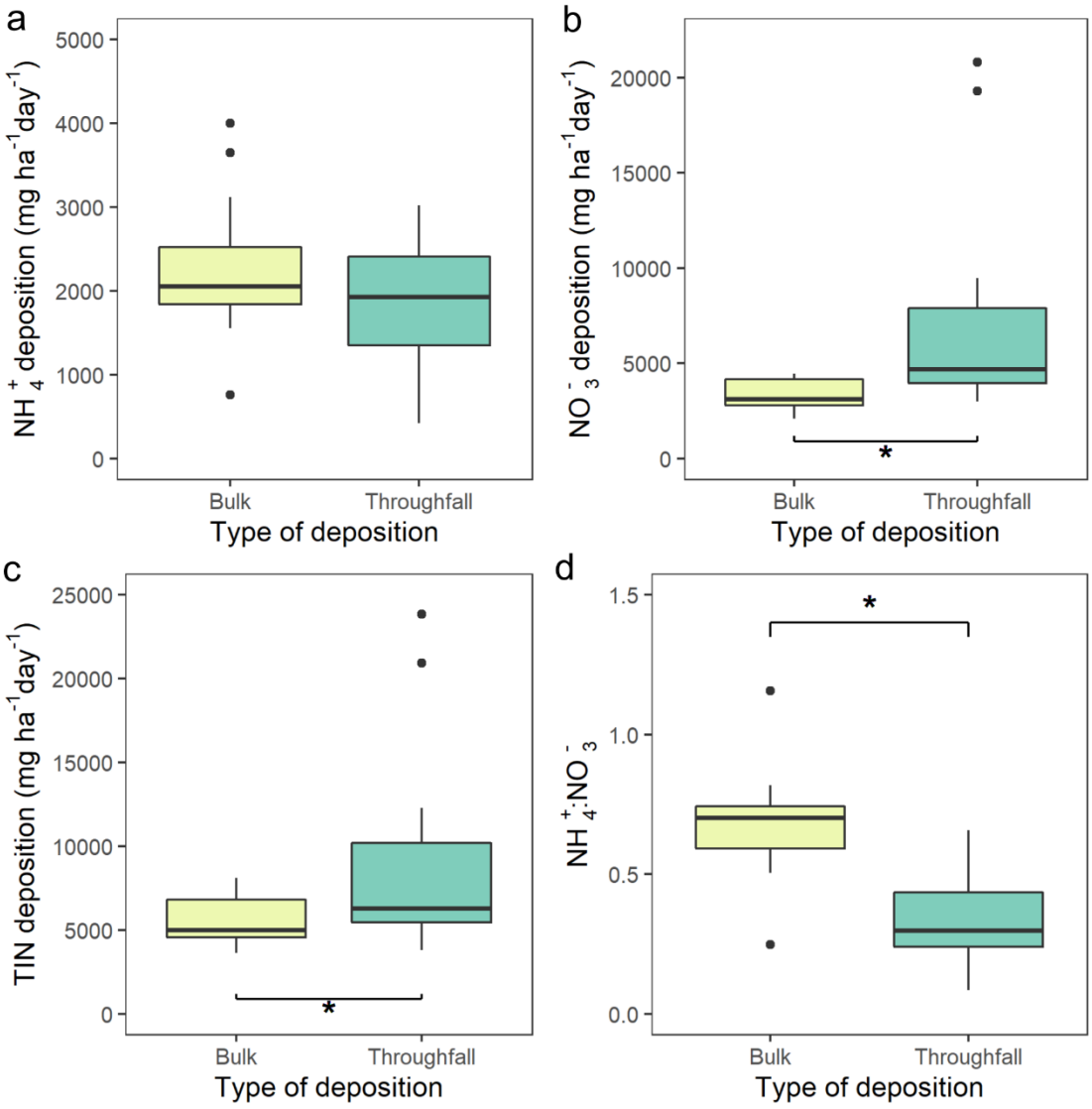

Figure 5.4. Boxplots of a) ammonium (NH_4^+), b) nitrate (NO_3^-), c) total inorganic nitrogen (TIN), and d) the ratio of NH_4^+ to NO_3^- deposition in July-September at open vs throughfall sites along Bear Lake Road in Rocky Mountain National Park. Brackets with "*" connect sites with significantly different deposition rates at $p < 0.05$.

We compared throughfall deposition to a proxy for tree size, DBH. Throughfall NH_4^+ deposition was positively related to DBH ($r^2 = 0.217$; p = 0.051; Figure 5.5), although not

statistically significant. No other form of throughfall N deposition was clearly correlated to DBH (NO_3^-: $r^2 = 0.114$; $p = 0.171$; TIN: $r^2 = 0.142$; $p = 0.122$; NH_4^+:NO_3^-: $r^2 = 0.007$; $p = 0.741$; Figure 5.5). Average DBH was highest for the site 50m from the road (Table 5.2).

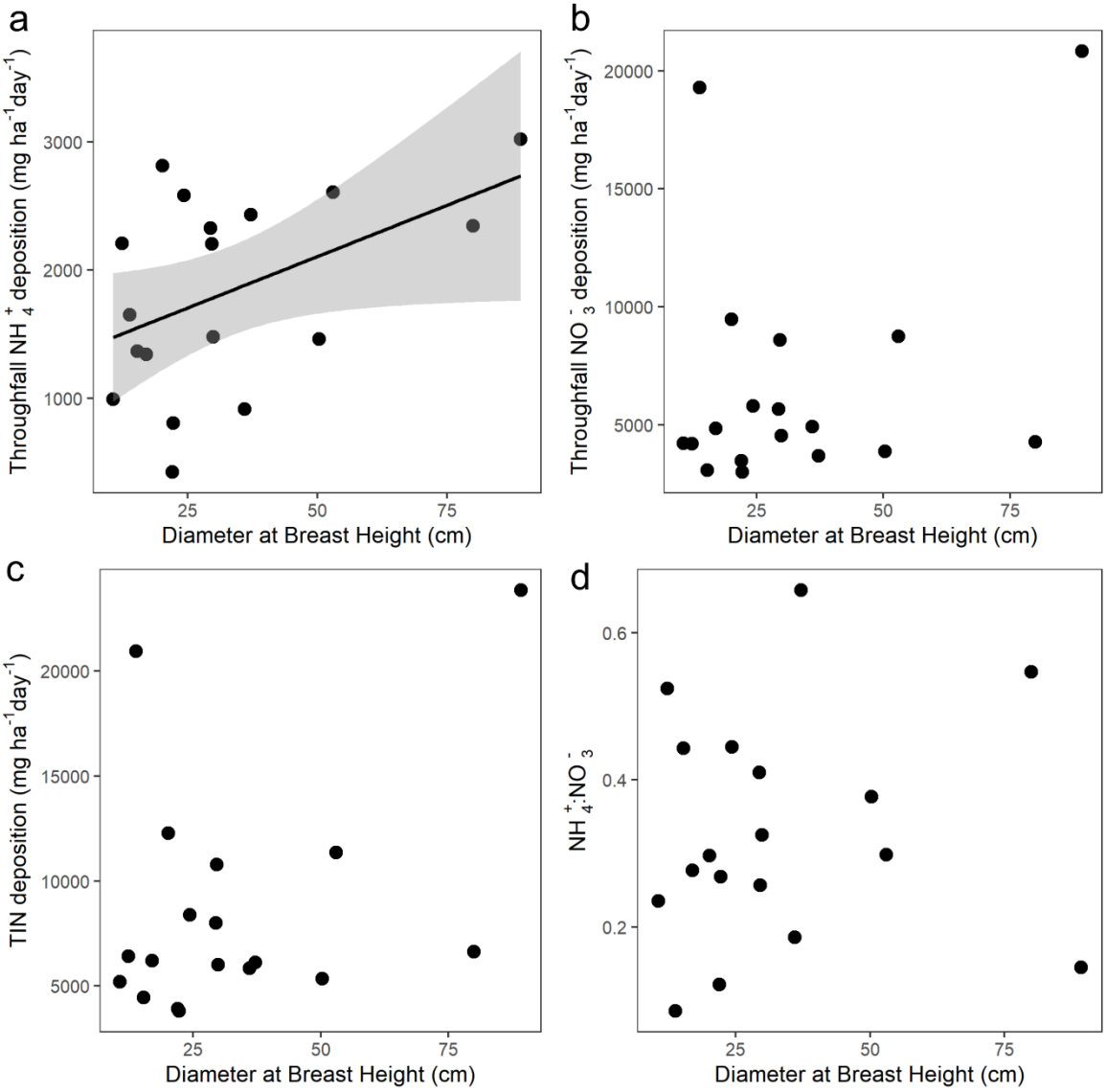

Figure 5.5. Scatter plots of a) ammonium (NH_4^+), b) nitrate (NO_3^-), c) total inorganic nitrogen (TIN), and d) the ratio of NH_4^+ to NO_3^- throughfall deposition in July-September vs diameter at breast height (DBH) of trees at sites along Bear Lake Road in Rocky Mountain National Park. Plots with a correlation at $p < 0.1$ have a linear regression line.

Table 5.2. Mean (standard error) of diameter at breast height for three trees at each throughfall site used in study of nitrogen deposition in the Bear Lake area of Rocky Mountain National Park.

Distance from road (m)	Mean diameter at breast height (cm)
5	15.9 (2.27)
10	13.8 (1.85)
20	29.3 (3.99)
50	64.5 (20.3)
150	39.9 (6.94)
750	34.1 (8.43)

We compared our results to nearby CASTNET and NTN sites (Table 5.3). Open and throughfall NH_4^+, NO_3^-, and TIN measurements from the IER were generally lower than NTN deposition values, with the exception of throughfall NO_3^- which was higher than both the Loch Vale and Beaver Meadows estimates of wet NO_3^- deposition (Table 5.3). The single throughfall Loch Vale IER collector had higher TIN values than the wet N deposition reported from CO98. All IER measurements, including from the site adjacent to the Loch Vale NTN site, showed NO_3^- as the dominant N species, in contrast with both the CASTNET and NTN sites (Table 5.3). Precipitation values proximal to our transects from the Bear Lake SNOTEL site were 43% lower than that at the Loch Vale NTN site and were similar (4% higher) to that at the Beaver Meadows NTN site (Table 5.3).

Table 5.3. Comparison of means of Open, Throughfall, and Loch Vale IER sites and nearby dry (CASTNET) and wet (NTN) deposition sites. The Loch Vale IER values are from a single blank-corrected throughfall collector. Precipitation data are from NTN data for Loch Vale and Beaver Meadows and are from the Bear Lake SNOTEL site for Open and Throughfall estimates. Nitrogen in ammonium = NH_4^+-N; Nitrogen in nitrate = NO_3^--N; Total inorganic N = NH_4^+-N + NO_3^--N.

Site	NH_4^+-N (mg N day^{-1} ha^{-1})	NO_3^--N (mg N day^{-1} ha^{-1})	Total Inorganic N (mg N day^{-1} ha^{-1})	NH_4^+: NO_3^-	Mean precipitation (cm day^{-1})
Loch Vale – NTN (CO98)	7557.73	4175.09	11732.82	1.81	0.231
Beaver Meadows – NTN (CO19)	6720.30	3603.32	10323.62	1.87	0.144
ROM206 - CASTNET	316.09	77.63	393.72	4.07	N/A
Open	2242.88	3322.21	5565.09	0.68	0.150
Throughfall	1831.60	6809.94	8641.54	0.33	0.150
Loch Vale IER	4427.5	9127.9	13555.4	0.49	0.231

4. Discussion

4.1 Lack of proximity to road effects

Higher N deposition in roadside ecosystems has been found in both urban and rural areas (Bettez et al. 2013; Cape et al. 2004; Gadsdon and Power 2009; Redling et al. 2013) including in Grand Canyon National Park (Kenkel et al., 2016), in stark contrast to our results. We describe four potential factors that might influence the fate of N emissions from vehicles. Possible causes for our results include insufficient vehicle emissions of N to cause an effect, changes in N deposition with elevation, rapid sorption of N to soils and herbaceous vegetation adjacent to roads, or the interaction of N deposition with forest vegetation.

Nitrogen oxides emissions have been declining in the USA since the 1990s with reductions in national annual emissions of NO_x from on-road mobile sources of about 50% from 2008 to 2017 (6,941 *versus* 3,495 thousand tons). In contrast, national annual mobile emissions of NH_3 have reduced less dramatically from 138 to 101 thousand tons in that same time period (EPA, 2021). While vehicle N pollution is clearly still an issue for the USA as a whole, it is

possible that emissions in non-urban areas are too low to be detected using the transect method. Kenkel et al. (2016) found no relationship between NOx concentrations and distance from the road in a relatively low traffic area in Grand Canyon National Park, which had slightly higher visitation overall than RMNP in the year of our study (4.5 vs 4.4 million people; NPS, 2022). Roadside NH_3 and NO_2 deposition increases linearly with number of vehicles on the road, so there may not have been sufficient vehicle inputs at our site (Cape et al. 2004). If we assume one-quarter of the 670,000 vehicles entering RMNP during our experimental period travelled to the Bear Lake parking lot and that our transects are affected by approximately one-quarter mile of road, and use vehicle emission estimates of 33 mg NH_3 per mile and 160 mg NO_x per mile (Davison et al. 2020; Farren et al. 2020), potential N inputs from vehicles at our transects were ~14 g NH_3 day^{-1} and ~66 g NO_x day^{-1}. Given the daily input nationally is 2.5 x 10^8 g NH_3 day^{-1} and 8.7 x 10^9 g NO_x day^{-1} (EPA, 2021) and the USA has an area of 9.8 x 10^6 km^2, our values seem to be considerably lower than an average square kilometer in the USA. These are very coarse estimates, but they support the possibility of insufficient N inputs from vehicles at our site to be measurable with the transect method. Alternatively, this lack of a relationship between distance from the road and N deposition could result from other changes in environmental factors along our transects.

Because RMNP consists of highly complex terrain, other factors changed along our transects that may be more influential on patterns of N deposition than proximity to the road. Site elevation generally increased with distance from the road. Clow et al. (2015) found increased NO_3^- deposition with increased elevation using open IER collectors in RMNP, which they attributed to higher precipitation at higher elevations. This pattern has been seen in other mountainous areas (Hoffman et al. 2019; Weathers et al. 2000). However, sites in other studies

spanned much larger elevation gradients, 100s to 1000s of meters, compared with our sites, which spanned 40-100 m (Clow et al. 2015; Hoffman et al. 2019; Weathers et al. 2000). So, higher precipitation across the elevation gradient at our site probably did not influence N deposition. It is possible that the elevation between the road and the site closest to the road caused immediate deposition on the small hillsides (Hasselrot and Grennfelt 1987). Indeed, the throughfall site 10 m from the road was slightly below the road (~5 m) and had higher average NO_3^- deposition than the site 5 m from the road, which was slightly above the road (~2 m; Figure 5.3b). In northeast USA forests, mountainous terrain contained "hotspots" of deposition associated with landscape heterogeneity, such as proximity to forest edges and topographic exposure, where wind exposure and higher precipitation drive increased deposition (Hasselrot and Grennfelt 1987; Weathers et al. 2000; Weathers et al. 2006). These landscape features were stronger and more consistent predictors than proximity to roads (Weathers et al. 2006). At our site, wind direction is highly variable, so it is likely that the wind blew vehicle N emissions towards the small hillsides between the closest collectors and the road at least some of the time (USDA, 2022). At the "tailpipe scale" the small hillsides between the road and the closest N deposition collectors could have caused edge effects that prevented vehicle N emissions from reaching our collectors.

Another potential, and related, reason vehicle emissions may not have reached our collectors is due to interactions with the land surface. More exposed soils and vegetation on small hillsides at roadside edges may have adsorbed or absorbed NH_3, respectively, before the emissions could rise to the elevation of our collectors. Ammonia sorption is enhanced for soils with organic layers that experience high temperatures and both plant and soil NH_3 uptake are enhanced at higher atmospheric NH_3 concentrations (Hanawalt 1969; Huber et al. 2002;

Langford and Fehsenfeld 1992). Our coniferous forest sites would be expected to experience these conditions in the high-visitation summer period at RMNP. Since NH_3 can be the dominant species of N in vehicle emissions (Fenn et al. 2018b), NH_3 sorption on small hillsides could explain the lack of vehicle influence in our study.

Our data also suggest that tree cover and tree size may both influence N deposition (Figures 5.4 and 5.5), which could mitigate effects of proximity to the road. We expected that throughfall deposition may better capture gaseous emissions from vehicles, as found by transect studies in Massachusetts, USA and southern England (Bettez et al. 2013; Gadsdon and Power 2009). However, we saw no evidence of higher N deposition at sites closer to the road in either throughfall or open deposition (Figures 5.2 and 5.3). Differences between throughfall and open deposition instead represent the influence of canopy interactions on regional N deposition, regardless of proximity to the road. These findings do not inform our understanding of local N deposition but may provide insight on the role of vegetation interactions in N deposition more broadly.

There was higher NO_3^- deposition at throughfall sites than open sites, which caused higher TIN and lower NH_4^+:NO_3^- deposition at throughfall sites (Figure 5.4). This pattern of higher N deposition in throughfall compared to open sites has been found for many places and vegetation types, including the Greater Yellowstone Ecosystem, south China, California, and the Sonoran desert (Cook et al. 2018; Fang et al. 2011; Fenn and Poth 2004; Hoffman et al. 2019; Tulloss and Cadenasso 2015), although it is not fully consistent and depends on N species (García-Gomez et al. 2016; Jovan et al. 2021). At our site, higher deposition of throughfall NO_3^-, specifically, would likely occur due to: (1) higher canopy uptake of NH_4^+ (Adriaenssens et al. 2011), (2) higher dry deposition of NO_3^-, since dry deposition is expected to be more represented

in throughfall deposition compared to open deposition (Fenn et al. 2018a), or (3) nitrification in tree canopies transforming NH_4^+ to NO_3^- coupled with greater dry deposition of reduced N species (Guerrieri et al. 2020; Guerrieri et al. 2015). Higher canopy uptake of NH_4^+ compared to NO_3^- might be unlikely for our coniferous forest stands, given findings similar and low uptake of NH_4^+ and NO_3^- in a coniferous tree (Scots pine; *Pinus sylvestris* L.) compared to deciduous trees (Adriaenssens et al. 2011). Both previous studies and our comparisons with NTN and CASTNET sites suggest dry deposition of reduced N species is dominant over oxidized species in RMNP (Benedict et al. 2013a; Table 5.3). This lends support to the idea that nitrification of reduced species in higher dry deposition may explain the higher NO_3^- and TIN deposition at throughfall sites. While we do not have data on canopy nitrification for our site, nitrifiers have been found in needles of other coniferous (spruce and pine) trees and also in beech and Mediterranean holm oak stands, where net flux (throughfall minus open) of NO_3^-, but not NH_4^-, has been shown to occur (García-Gomez et al. 2016; Guerrieri et al. 2020; Guerrieri et al. 2015; Papen et al. 2002). Canopy nitrification may be a widespread, albeit understudied, phenomenon. Notably, the magnitude of change in NH_4^+:NO_3^- deposition in throughfall versus open sites is comparable to changes in both deposition and soil inorganic N ratios that influenced soil pH and organic carbon and plant N isotopes (Huang et al. 2015b; Rocci et al. 2019). Thus, changes in relative deposition of NH_4^+ and NO_3^- could have marked environmental effects and are worth investigating further.

Tree size was related to N deposition – larger trees were associated with higher throughfall NH_4^+ deposition. This may suggest that larger canopies hold greater dry deposition of reduced N. Our finding of significantly higher throughfall NH_4^+ deposition at the site 50 m from the road compared to the site 750 m from the road, which also had approximately double the average DBH of the far site (64.5 versus 34.1 cm), supports this suggestion (Figure 5.3; Table

5.2). Previous studies also support greater throughfall deposition of ions associated with or within dry deposition, including NH_4^+ and NO_3^-, under taller trees (De Schrijver et al. 2008; Erisman and Draaijers 2003). Greater throughfall NH_4^+ deposition under larger trees is somewhat at odds with our suggestion of canopy nitrification driving higher throughfall NO_3^- deposition; why this potential higher dry deposition of NH_4^+ would not be nitrified is unclear. Notably, our full summer dataset for throughfall deposition indicates significant positive relationships between DBH and throughfall NO_3^- and TIN deposition and not with NH_4^+, potentially aligning more with canopy nitrification (Appendix 4, Supplementary Material A). Regardless, this association of larger trees with higher N deposition may indicate that the trees in close proximity to the road, which were generally smaller (Table 5.2), may have not captured sufficient gaseous vehicle emissions to be detected in our transects. Environmental variables may be more important for shaping patterns of N deposition at our sites than proximity to the road, which could apply more broadly to other areas with complex, mountainous terrain (Hoffman et al. 2019).

4.2 Comparison to Regional Data

In the USA, regional N deposition is well represented by NTN and CASTNET measurements, so we can use IER collectors to investigate local influences on N deposition, like vehicles, and compare to regional estimates. While samples collected from IER collectors are not directly comparable to samples from any one national monitoring network that measures dry or wet deposition, we expected to find higher deposition at the roadside that exceeded regional N deposition estimates. Instead, we found no evidence of vehicle emissions contributing to our transects and that our estimates of average TIN deposition were lower than those reported for the NTN and CASTNET sites in RMNP (CO98, CO19, ROM206). Importantly, we do not include

dry deposition estimates of nitric acid (HNO_3) and NH_3 in our regional estimates, which would be measured in our IER collectors as NO_3^- and NH_4^+, respectively. Nitric acid deposition from the CASTNET site during our collection period, calculated as for NO_3^--N, was 1503.4 mg N ha^{-1} day^{-1} and NH_3 deposition has been reported as quantitatively similar to wet NO_3^- deposition (3603.3-4175.1 mg N ha^{-1} day^{-1}), making the regional estimates even higher than our IER estimates. Differences in precipitation at the IER sites and CO98, CO19, and ROM206 are unlikely to explain these results, since precipitation at our transects was comparable to that at the Beaver Meadows NTN site (CO19; Table 5.3). Hoffman et al. (2019) studied N deposition in the Greater Yellowstone Ecosystem (GYE) using IER collectors and, in contrast to our results, found their open N deposition estimates to be higher than those reported from NTN and CASTNET sites in the GYE. They attribute this to high spatial variation in their mountainous study terrain (Hoffman et al. 2019). Indeed, high spatial variation may explain the discrepancy between our IER estimates of TIN deposition and those from the RMNP NTN and CASTNET sites (CO98, CO19, ROM206). Our throughfall TIN estimates (which likely represent dry and wet deposition) ranged between 3,815.3 – 23,842.4 mg N ha^{-1} day^{-1}, which encompassed the estimates from sites CO98, CO19, and ROM206 (Table 5.3). Furthermore, our single IER collector at Loch Vale measured higher TIN deposition than the Loch Vale NTN site (Table 5.3). Our IER measurements were decent but variable approximations of regional N deposition but, due to lack of road proximity effects, did not allow us to compare N deposition from roadside ecosystems to regional deposition.

It is notable that the distribution of N deposition into NH_4^+ and NO_3^- was very different between the IER measurements and estimates at CO98, CO19, and ROM206. The IER measurements are majorly NO_3^-, whereas RMNP NTN and CASTNET estimates are majorly

NH_4^+. The greater relative proportion of oxidized N at our sites may be due to (1) different sources of N, (2) methodological differences, or (3) environmental complexity. As this was designed as a study to investigate the influence of vehicles on N deposition in RMNP, it is possible that our sites, which were closer to roads than CO98, CO19, and ROM206, were indeed influenced by vehicle emissions. However, this might be unlikely given highly local vehicle emission deposition (within 100 m from the road), potentially greater emissions of NH_3 than NO_x from vehicles with catalytic converters, and the lack of road proximity effect in our findings (Bignal et al. 2007; Fenn et al. 2018b; Figures 5.2 and 5.3). Alternatively, NH_4^+ deposition may have been underestimated in our IER collectors due to reduced adsorption efficiency or recovery of NH_4^+ compared to NO_3^- or increased NH_4^+ volatilization in IER collectors due to pooling water or high pH (García-Gomez et al. 2016). However, co-located open IER collectors and NTN sites along an elevation gradient from Boulder, Colorado to the Loch Vale NTN site both had NH_4^+ as the dominant N species (Heindel et al. 2022), potentially suggesting these methods are comparable in our study area. As suggested for the lack of road proximity effects, exposed soils directly adjacent to the roadway may have promoted NH_3 sorption to soils at our IER sites, potentially driving the lower NH_4^+: NO_3^- for our sites compared to CO98, CO19, and ROM206. Our sites have acidic organic layers overlying the mineral soil, and presumably high atmospheric NH_3 concentration, due to vehicle emissions, all of which are associated with greater NH_3 sorption to soils (Hanawalt 1969; Huber et al. 2002; Stratton et al. 2019). Altogether our data indicate a magnitude of deposition that is likely to negatively impact ecosystems in RMNP (Baron et al. 2000), and therefore confirms past work and extends it to a finer, yet more variable, spatial scale.

5. Conclusion

Our study did not find an N signature from tailpipe emissions from vehicles approaching a highly popular and crowded parling lot in RMNP in either open or throughfall IER measurements. This may be attributed to the lack of sufficient N emissions from vehicles or to confounding effects from topographically complex terrain surrounding our collectors. While differences between open and throughfall N deposition were not related to road proximity, they indicate that tree cover may modify regional N deposition, with greater and more NO_3^- rich N deposition under tree canopies. Our results suggest that higher roadside N deposition, found in many studies, cannot be assumed for topographically complex ecosystems.

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CHAPTER 6: CONCLUSION

The goals of this dissertation were to evaluate the responses of soil and plant C and N cycling to global environmental change. Specifically, this was accomplished by investigating soil organic matter (SOM) as an ecosystem property, as well as ecosystem N input, in the form of N deposition, in response to global changes and human activity. Chapters 2-4 all supported the benefits of considering the responses of SOM fractions, and not solely the total SOM pool, to global change. Investigating SOM fractions was both methodologically and functionally useful. In Chapters 3 and 4, SOM fractions were more responsive, statistically, than the total SOM pool. This may be because separating SOM into smaller and more homogenous pools allowed us to find slight changes in SOM imposed by global change. Perhaps more importantly, separating SOM into components provided more functional insight. In Chapter 2, I found that particulate organic matter (POM) was increased and decreased by elevated atmospheric carbon dioxide (eCO_2) and warming, respectively. Increased POM under eCO_2 both reconciles previous research which found that the response of soil C to eCO_2 depended on N availability and provides a path to C storage in soils under eCO_2, which could provide a negative feedback to climate change. Decreased POM under warming aligns with understanding that POM-rich soils, like in the Arctic and sub-Arctic are particularly susceptible to atmospheric warming. In Chapter 3, investigating fractions revealed that while nutrient fertilization did not change the total soil C and N pools, it did reduce the stability of soil C by increasing relative C storage in POM. Given the findings from Chapter 2, this suggests that the soil C pool under nutrient fertilization may be more susceptible to future warming. In Chapter 4, changes in fractions were associated with mechanistic changes in C and N cycling under increased precipitation, even though the total soil C and N stocks were unaffected by greater water availability. This provided greater insight into changes in the character of SOM under increased precipitation, even if the magnitudes of total

soil C and N were unaffected. Altogether, Chapters 2-4 supported the use of SOM fractions to further our understanding of ecosystem responses to global change.

Chapter 5 focused in on a specific human activity, vehicle use, and its influence on small scale N deposition. Global changes are not felt equally across the globe and certain ecosystems (and people, although that is not the focus of this dissertation) may be more susceptible to negative influence from human-induced global change. The findings in Chapter 5 suggest vehicle use is not contributing a significant input of N to roadside ecosystems in Rocky Mountain National Park. This was in contrast to many studies that found greater roadside deposition of N and highlights the importance of evaluating a variety of ecosystems and local conditions to understand the influence of global changes.

Overall, the studies in this dissertation used global synthesis, a global research network, and local case studies to investigate the responses of terrestrial ecosystem C and N cycling to global changes. Chapters 2 and 3 provided insight on the globally averaged responses of POM and MAOM to global change, as well as how these may vary with environmental variation. Chapters 4 and 5 indicated the importance of local context for understanding POM and MAOM responses to increased precipitation and for determining fine scale N deposition. It is my hope that the studies in this dissertation help improve our understanding of terrestrial ecosystem feedbacks and responses to human-induced changes in the Earth system, to better predict future climate and protect ecosystems.

APPENDIX 1: SUPPLEMENTARY MATERIAL FOR CHAPTER 2

Supplementary Table 1.1. The search terms used for a given element in our systematic meta-analysis of global change effects on soil organic carbon pools. "*" indicates that any ending of the word was searched for (e.g., fraction, fractions, fractionation). Soil organic matter search terms were combined with each global change factor. The same search terms were used for both databases but with slightly different syntax to account for differences in database structure. All searches included a phrase to only allow for studies in English.

Parameter	Search Terms
Soil Organic Matter	Mineral-associated organic matter, MAOM, MOM, particulate organic matter, POM, silt and clay, light fraction, heavy fraction, physical separation, size separation, density separation, physical fractionation, density fractionation, soil organic matter fraction*, soil fraction*, SOM fraction*, soil NEAR fraction*, soil organic matter NEAR stability, SOM NEAR stability
Nitrogen Fertilization	nitrogen fertilization, nitrogen addition, nitrogen deposition, nitrogen application
Warming	climate change, warming, elevated temperature
Elevated CO_2	free-air carbon dioxide enrichment, free-air CO_2 enrichment, elevated CO_2, elevated atmospheric CO_2, eCO_2
Precipitation	changing precipitation, increased precipitation, decreased precipitation, increased rainfall, decreased rainfall, changing rainfall, increased snowfall, decreased snowfall, changing snowfall

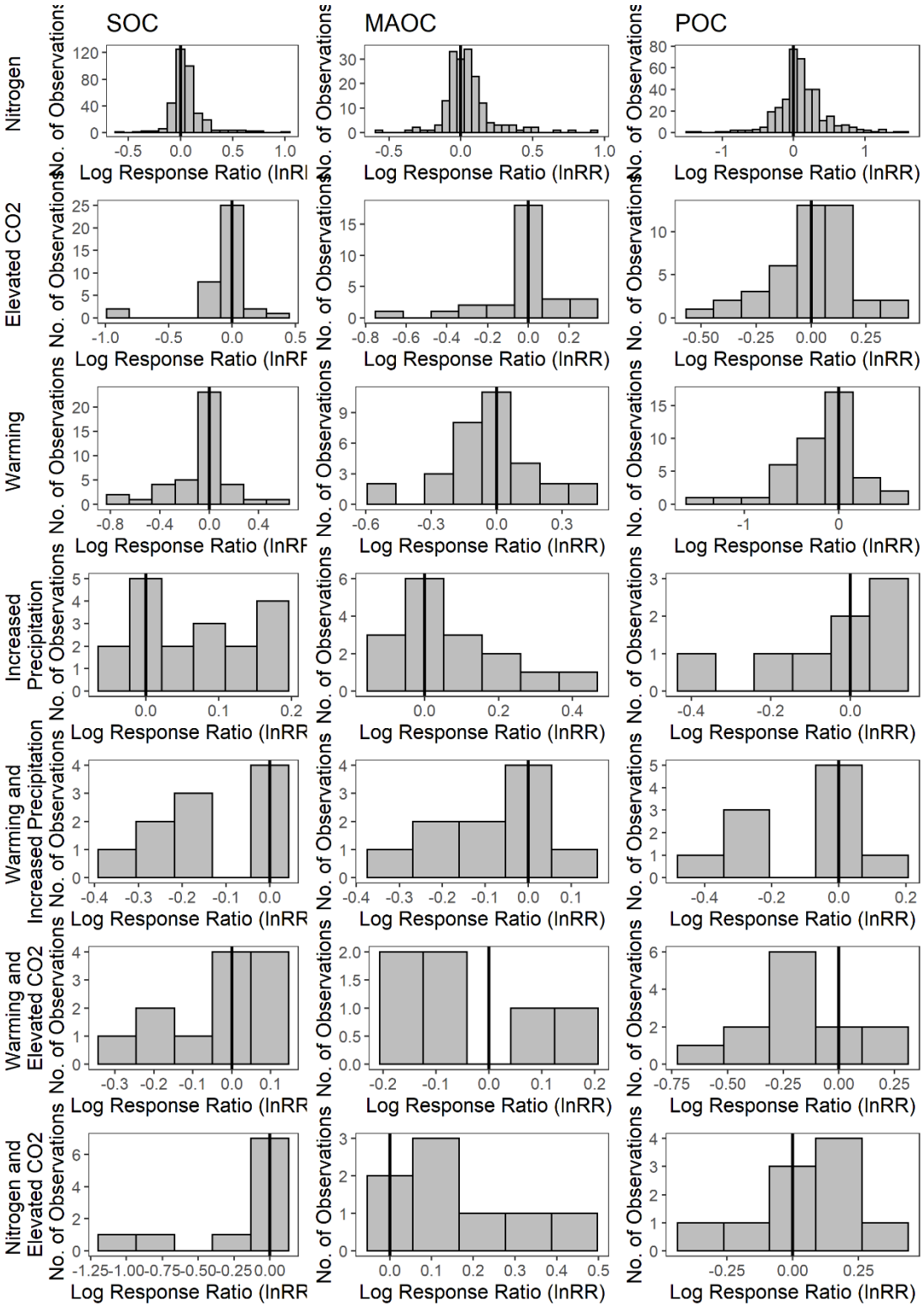
Supplementary Table 1.2. List of references used in a systematic meta-analysis on the response of soil organic carbon pools to global environmental change. Global change factors are nitrogen (N) fertilization, elevated carbon dioxide (CO_2), warming, increased precipitation, and combinations of these. Response parameters are total soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC). Number of observations represents the number of log response ratios, per fraction, calculated from each study. All data collected are available at (add link to database if accepted).

Global Change Factor	Response Parameters	Number of Observations	Reference
N fertilization	SOC, MAOC, POC	9	Fang et al. (2014)
N fertilization	SOC, POC	1	Malhi and Lemke (2007)
N fertilization	SOC, MAOC, POC	2	Volk et al. (2018)
N fertilization	SOC, MAOC	10	Wang et al. (2019b)
N fertilization	SOC, MAOC, POC	5	Song et al. (2014)
N fertilization	SOC, MAOC, POC	2	Yang et al. (2018)
N fertilization	SOC, MAOC, POC	8	Cheng et al. (2018)
N fertilization	SOC, MAOC, POC	5	Rodriguez et al. (2014)
N fertilization	SOC, MAOC, POC	2	Parker et al. (2002)
N fertilization	SOC, POC	36	Yanni et al. (2016)
N fertilization	SOC, MAOC, POC	2	Shahbaz et al. (2017)

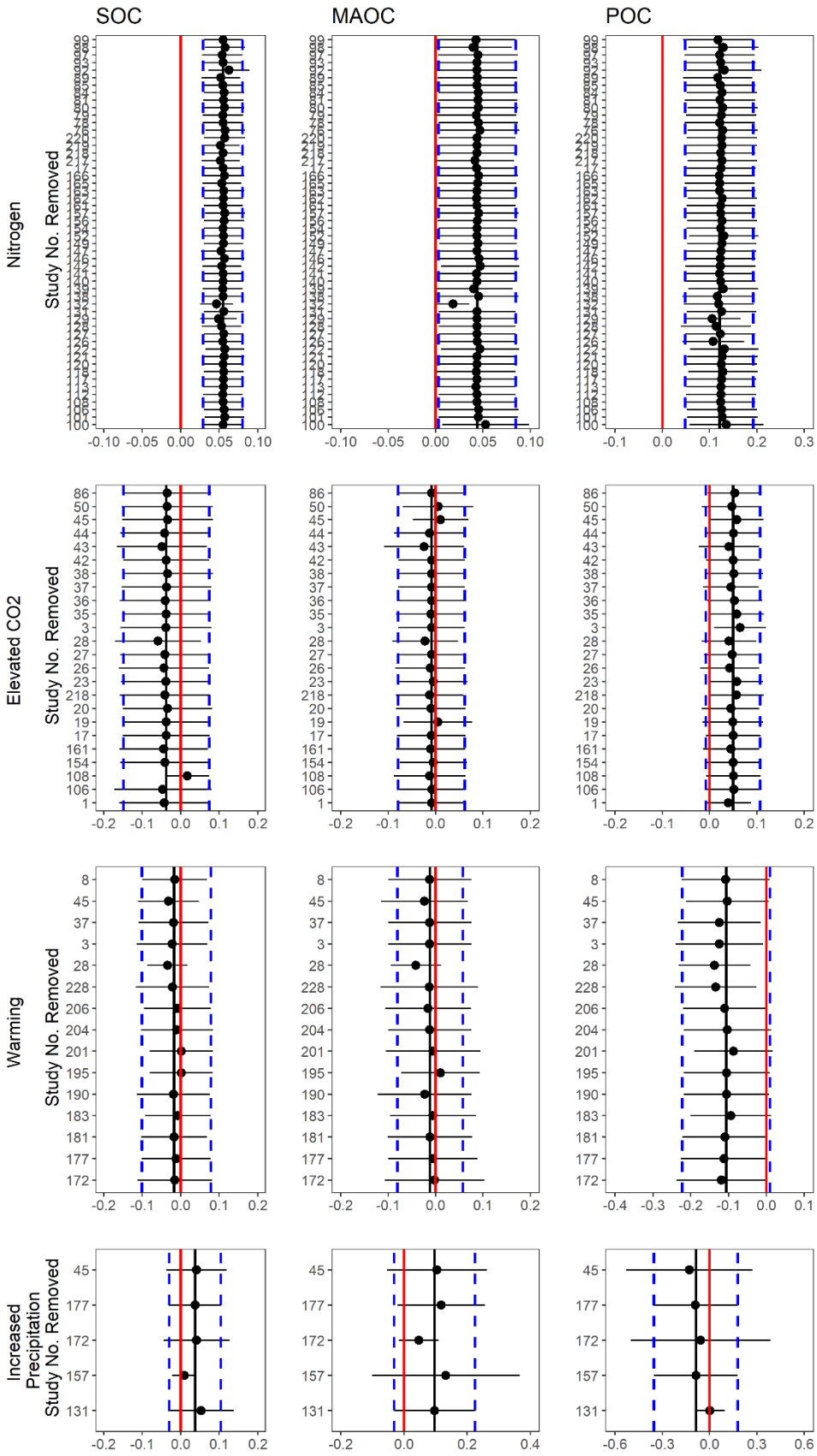
N fertilization	SOC, MAOC, POC	2	Griepentrog et al. (2015)
N fertilization	SOC, MAOC, POC	4	Hagedorn et al. (2003)
N fertilization	SOC, MAOC, POC	2	Van Groenigen et al. (2002)
N fertilization	SOC, MAOC, POC	3	Xie et al. (2005)
N fertilization	SOC, POC	5	Liu et al. (2013a)
N fertilization	SOC, POC	3	Jantalia and Halvorson (2011)
N fertilization	SOC, MAOC, POC	40	Zhong et al. (2015)
N fertilization	SOC, MAOC, POC	1	Haile-Mariam et al. (2000)
N fertilization	SOC, POC	3	Li et al. (2019)
N fertilization	SOC, MAOC, POC	2	Lagomarsino et al. (2009)
N fertilization	POC	1	Cui et al. (2014)
N fertilization	SOC, MAOC, POC	3	Ma et al. (2009)
N fertilization	SOC, MAOC, POC	3	Chen et al. (2018a)
N fertilization	SOC, POC	60	Coulter et al. (2009)
N fertilization	SOC, POC	2	Moinet et al. (2016)
N fertilization	SOC, POC	6	Tian et al. (2013)
N fertilization	SOC, MAOC, POC	12	Ferreira et al. (2018)
N fertilization	SOC, MAOC, POC	6	Dou and Hons (2006)
N fertilization	SOC, MAOC, POC	8	Mujuru et al. (2016)
N fertilization	SOC, MAOC, POC	4	Cusack et al. (2011)
N fertilization	SOC, MAOC, POC	1	Liu et al. (2013b)
N fertilization	SOC, POC	2	Purakayastha et al. (2008)
N fertilization	MAOC, POC	6	Oliveira et al. (2018)
N fertilization	SOC, MAOC, POC	16	Finn et al. (2016)
N fertilization	SOC, MAOC, POC	3	Bremer et al. (1994)
N fertilization	SOC, MAOC, POC	2	Bremer et al. (2008)
N fertilization	SOC, MAOC, POC	4	Frasier et al. (2016)
N fertilization	SOC, MAOC, POC	1	Liang et al. (2014)
N fertilization	SOC, POC	8	Liebig et al. (2002)
N fertilization	SOC, MAOC, POC	5	Maillard et al. (2015)
N fertilization	SOC, POC	6	Malhi et al. (2011)
N fertilization	SOC, POC	6	Malhi et al. (2005)
N fertilization	SOC, MAOC, POC	1	Swanston et al. (2004)
N fertilization	SOC, MAOC, POC	1	Zak et al. (2017)
N fertilization	SOC, MAOC, POC	4	Bradford et al. (2008a)
N fertilization	SOC, MAOC, POC	4	Bradford et al. (2008b)
N fertilization	SOC, POC	56	Stewart et al. (2016)
N fertilization	SOC, POC	36	Stewart et al. (2017)
N fertilization	SOC, POC	12	Stewart et al. (2018)
Elevated CO_2	SOC, MAOC, POC	1	Griepentrog et al. (2015)
Elevated CO_2	MAOC, POC	1	Niklaus et al. (2001b)

Elevated CO_2	SOC, MAOC, POC	1	Niklaus et al. (2001a)
Elevated CO_2	SOC, MAOC, POC	2	Thaysen et al. (2017)
Elevated CO_2	SOC, MAOC, POC	3	Xu et al. (2019)
Elevated CO_2	SOC, MAOC, POC	4	Singh et al. (2019)
Elevated CO_2	SOC, MAOC, POC	1	Jastrow et al. (2000)
Elevated CO_2	SOC, MAOC, POC	2	Hagedorn et al. (2003)
Elevated CO_2	SOC, MAOC, POC	1	Van Groenigen et al. (2002)
Elevated CO_2	SOC, MAOC, POC	2	Xie et al. (2005)
Elevated CO_2	SOC, MAOC, POC	2	Six et al. (2001a)
Elevated CO_2	SOC, MAOC, POC	1	Ma et al. (2009)
Elevated CO_2	MAOC, POC	3	Hofmockel et al. (2011)
Elevated CO_2	SOC, MAOC, POC	1	Insam et al. (1999)
Elevated CO_2	SOC, MAOC, POC	2	Liu et al. (2018)
Elevated CO_2	SOC, MAOC, POC	1	Hoosbeek and Scarascia-Mugnozza (2009)
Elevated CO_2	SOC, MAOC, POC	1	Lichter et al. (2005)
Elevated CO_2	SOC, MAOC, POC	1	Haile-Mariam et al. (2000)
Elevated CO_2	SOC, POC	2	Pendall et al. (2011)
Elevated CO_2	SOC, POC	1	Allard et al. (2005)
Elevated CO_2	SOC, POC	4	Black et al. (2017)
Elevated CO_2	SOC, POC	1	Chen et al. (2012a)
Elevated CO_2	SOC, POC	4	Peralta and Wander (2008)
Warming	SOC, MAOC, POC	2	Link et al. (2003)
Warming	SOC, MAOC, POC	1	Song et al. (2012)
Warming	SOC, MAOC, POC	3	He et al. (2012)
Warming	SOC, MAOC, POC	2	Guan et al. (2018)
Warming	SOC, POC	2	Pendall et al. (2011)
Warming	SOC, MAOC, POC	1	Cheng et al. (2011)
Warming	SOC, MAOC, POC	5	Zhang et al. (2019b)
Warming	SOC, POC	4	Black et al. (2017)
Warming	POC	1	Garten et al. (2009)
Warming	SOC, MAOC, POC	8	Poeplau et al. (2017)
Warming	SOC, MAOC, POC	2	Schnecker et al. (2016)
Warming	SOC, MAOC, POC	4	De Feudis et al. (2019)
Warming	SOC, POC	3	Qi et al. (2016)
Warming	SOC, MAOC, POC	2	Liu et al. (2018)
Warming	SOC, MAOC, POC	2	Thaysen et al. (2017)
Increased precipitation	SOC, MAOC	10	Wang et al. (2019a)
Increased precipitation	SOC, MAOC, POC	1	Song et al. (2012)
Increased precipitation	SOC, MAOC, POC	3	He et al. (2012)

Increased precipitation	SOC, POC	2	Moinet et al. (2016)
Increased precipitation	SOC, MAOC, POC	2	Thaysen et al. (2017)
N fertilization + Elevated CO_2	SOC, POC	1	Chen et al. (2012b)
N fertilization + Elevated CO_2	SOC, POC	1	Henry et al. (2005)
N fertilization + Elevated CO_2	SOC, MAOC, POC	1	Griepentrog et al. (2015)
N fertilization + Elevated CO_2	SOC, MAOC, POC	2	Ma et al. (2009)
N fertilization + Elevated CO_2	SOC, MAOC, POC	2	Hagedorn et al. (2003)
N fertilization + Elevated CO_2	SOC, MAOC, POC	1	Van Groenigen et al. (2002)
N fertilization + Elevated CO_2	SOC, MAOC, POC	1	Xie et al. (2005)
N fertilization + Elevated CO_2	SOC, MAOC, POC	1	Haile-Mariam et al. (2000)
Warming + Elevated CO_2	SOC, POC	4	Black et al. (2017)
Warming + Elevated CO_2	POC	1	Garten et al. (2009)
Warming + Elevated CO_2	SOC, MAOC, POC	2	Liu et al. (2018)
Warming + Elevated CO_2	SOC, POC	2	Pendall et al. (2011)
Warming + Elevated CO_2	SOC, MAOC, POC	4	Thaysen et al. (2017)
Warming + Increased precipitation	SOC, MAOC, POC	2	Thaysen et al. (2017)
Warming + Increased precipitation	SOC, MAOC, POC	3	He et al. (2012)
Warming + Increased precipitation	SOC, MAOC, POC	1	Song et al. (2012)
Warming + Increased precipitation	SOC, MAOC, POC	4	Puissant et al. (2017)



Supplementary Figure 1.1. Histograms of log response ratios of observations for each global change factor (row) and soil carbon pool (column). Black lines are at zero.


SOC
MAOC
POC
Nitrogen
Elevated CO2
Warming
Increased Precipitation
Study No. Removed

Supplementary Figure 1.2. Sensitivity analysis for each individual global change factor (row) and soil carbon pool (column). The plots show the mean effect size and confidence interval when the given study on the y-axis is removed. The red lines are at zero, the black lines are the mean of all observations, and the blue dotted lines are the 95% confidence intervals using all observations. If the confidence interval of a recalculated mean changes the significance of the mean (i.e. crosses zero when the overall mean did not cross zero), then the overall mean is sensitive to inclusion of that study.

Supplementary Table 1.3. Results of all moderator tests carried out for a meta-analysis of the responses of soil organic carbon (SOC), mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) to global changes.

Global Change Factor	Moderator	Levels	n	Fraction	Effect Size	Lower Confidence Interval	Upper Confidence Interval
Nitrogen Fertilization	Soil depth sampled	Surface	181	SOC	9.145	5.906	12.482
		Subsoil	70	SOC	0.642	-2.982	4.401
		Deep soil	69	SOC	2.308	-4.368	9.449
		Profile	106	SOC	7.691	7.261	8.123
	Experiment Length	<5 yrs	105	SOC	1.349	-2.274	5.106
		5-10 yrs	171	SOC	8.073	4.384	11.891
		>10 yrs	159	SOC	6.759	3.255	10.382
	Land Cover Type	Cropland	337	SOC	5.537	2.350	8.824
		Grassland	38	SOC	4.884	-1.330	11.488
		Forest	52	SOC	7.686	1.879	13.824
	Experiment Type	Field	405	SOC	5.573	2.821	8.400
		Lab	24	SOC	5.639	-4.377	16.705
	N Fertilizer Type	Organic	150	SOC	5.963	2.465	9.579
		Inorganic	183	SOC	6.143	2.485	9.931
		Combo	92	SOC	2.184	-1.725	6.249
	Initial soil pH	Continuous	267	SOC	-0.004	-0.023	0.015
	Change in soil pH	Continuous	37	SOC	-0.149	-0.352	0.055
	MAT	Continuous	337	SOC	0.000	-0.004	0.004
	MAP	Continuous	335	SOC	0.000	0.000	0.000
	Latitude	Continuous	333	SOC	-0.001	-0.001	0.001
	% sand	Continuous	232	SOC	0.001	-0.001	0.001
	% silt	Continuous	232	SOC	0.000	-0.001	0.001
	% clay	Continuous	232	SOC	-0.001	-0.003	0.001
	Aboveground biomass	Continuous	90	SOC	0.055	0.014	0.096
	Belowground biomass	Continuous	33	SOC	0.118	0.033	0.203
	Soil depth sampled	Surface	181	MAOC	4.310	0.995	9.374
		Subsoil	71	MAOC	3.283	-4.524	11.739
		Deep soil	69	MAOC	4.823	-2.586	12.784

Experiment Length	Profile	110	MAOC	2.460	0.692	4.269
	<5 yrs	110	MAOC	2.491	-3.062	8.361
	5-10 yrs	171	MAOC	9.177	1.847	17.035
	>10 yrs	159	MAOC	2.071	-7.049	12.075
Land Use Type	Agriculture	338	MAOC	4.289	-1.390	10.076
	Grassland	53	MAOC	4.603	-5.257	15.373
	Forest	41	MAOC	4.603	-2.664	12.412
Experiment Type	Field	410	MAOC	5.443	0.703	10.407
	Lab	24	MAOC	0.501	-7.596	9.308
N Fertilizer Type	Organic	188	MAOC	3.459	-1.587	8.763
	Inorganic	150	MAOC	4.917	-0.199	10.186
	Combo	92	MAOC	6.716	-1.686	15.835
Fractionation Method	Size	96	MAOC	2.092	-3.198	7.670
	Density	72	MAOC	6.962	0.110	14.271
	Size + Density	11	MAOC	0.451	-8.616	10.418
Initial soil pH	Continuous	145	MAOC	-0.010	-0.020	0.001
Change in soil pH	Continuous	23	MAOC	0.016	-0.165	0.197
MAT	Continuous	166	MAOC	-0.001	-0.006	0.005
MAP	Continuous	149	MAOC	0.000	0.000	0.000
Latitude	Continuous	162	MAOC	0.000	-0.002	0.001
% sand	Continuous	111	MAOC	0.000	-0.001	0.001
% silt	Continuous	111	MAOC	0.000	-0.001	0.001
% clay	Continuous	111	MAOC	-0.001	-0.002	0.001
Aboveground Biomass	Continuous	90	MAOC	0.021	-0.003	0.046
Belowground biomass	Continuous	12	MAOC	0.067	-0.298	0.432
Soil depth sampled	Surface	181	POC	15.754	6.471	25.835
	Subsoil	71	POC	-6.293	-4.763	6.003
	Deep soil	69	POC	-23.799	-33.655	-12.480
Experiment Length	Profile	110	POC	7.713	6.035	9.417
	<5 yrs	110	POC	6.577	-4.228	18.590
	5-10 yrs	171	POC	6.780	-5.020	20.057
	>10 yrs	159	POC	25.671	13.247	39.459
Land Use Type	Agriculture	338	POC	11.963	2.737	22.14
	Grassland	53	POC	27.125	4.707	54.188
	Forest	41	POC	9.090	-5.635	25.986
Experiment Type	Field	410	POC	14.111	5.654	23.244
	Lab	24	POC	4.394	-13.411	25.86
N Fertilizer Type	Organic	188	POC	12.975	2.429	24.608
	Inorganic	150	POC	10.960	0.401	22.63
	Combo	92	POC	13.202	-0.299	28.66

	Fractionation Method	Size	285	POC	8.091	-1.351	18.436
		Density	129	POC	19.208	5.717	34.420
		Size + Density	11	POC	35.785	0.743	83.015
	Initial soil pH	Continuous	301	POC	-0.012	-0.072	0.047
	Change in soil pH	Continuous	37	POC	-0.252	-0.634	0.129
	MAT	Continuous	362	POC	-0.007	-0.017	0.003
	MAP	Continuous	344	POC	0.000	0.000	0.000
	Latitude	Continuous	358	POC	0.001	-0.001	0.004
	% sand	Continuous	244	POC	0.000	-0.003	0.003
	% silt	Continuous	244	POC	0.002	-0.001	0.006
	% clay	Continuous	244	POC	-0.005	-0.010	0.001
	Aboveground Biomass	Continuous	90	POC	0.123	-0.050	0.295
	Belowground biomass	Continuous	34	POC	0.007	-0.159	0.173
Elevated CO_2	Land Cover Type	Cropland	12	SOC	-4.967	-25.475	21.184
		Grassland	16	SOC	2.037	-14.962	22.436
		Forest	17	SOC	-8.621	-24.002	9.873
	Aboveground Biomass	Continuous	11	SOC	0.004	-0.004	0.011
	Belowground Biomass	Continuous	9	SOC	0.025	-0.017	0.067
	MAT	Continuous	38	SOC	0.008	-0.015	0.031
	MAP	Continuous	37	SOC	0.000	-0.001	0.001
	Latitude	Continuous	33	SOC	0.000	-0.001	0.002
	% sand	Continuous	18	SOC	-0.001	-0.003	0.002
	% silt	Continuous	17	SOC	0.001	-0.002	0.004
	% clay	Continuous	17	SOC	0.005	-0.005	0.015
	Nitrogen fixers	Yes	17	SOC	-0.044	-0.162	0.075
		No	44	SOC	-0.033	-0.114	0.047
	Nitrogen Fertilizer	Continuous	21	SOC	0.001	0.000	0.002
	Soil Nitrogen	Continuous	21	SOC	0.001	-0.034	0.035
	Experiment Length	< 5 yrs	22	SOC	-6.707	-20.579	9.586
		> 5 yrs	22	SOC	-1.032	-16.699	17.582
	Soil Depth Sampled	Surface	17	SOC	-7.946	-20.918	7.153
		Deeper	6	SOC	-20.939	-36.005	-2.325
		Profile	23	SOC	1.002	-11.507	15.280
	Land Cover Type	Cropland	12	MAOC	-4.781	-20.388	13.871
		Grassland	16	MAOC	-3.546	-15.211	9.779
		Forest	17	MAOC	2.092	-6.751	11.773
	Fractionation Type	Size	34	MAOC	3.252	-3.729	10.738
		Density	11	MAOC	-13.238	-22.586	-2.858
		Size/density	3	MAOC	1.106	-19.507	26.998

Aboveground Biomass	Continuous	8	MAOC	0.005	-0.001	0.012
Belowground Biomass	Continuous	6	MAOC	0.032	-0.019	0.083
MAT	Continuous	29	MAOC	0.004	-0.007	0.015
MAP	Continuous	28	MAOC	0.000	0.000	0.001
Latitude	Continuous	38	MAOC	0.001	-0.003	0.004
% sand	Continuous	18	MAOC	-0.003	-0.007	0.001
% silt	Continuous	17	MAOC	0.004	-0.003	0.011
% clay	Continuous	17	MAOC	0.006	-0.003	0.014
Nitrogen fixers	Yes	17	MAOC	-0.025	-0.181	0.132
	No	44	MAOC	-0.009	-0.085	0.068
Nitrogen Fertilizer	Continuous	14	MAOC	0.000	0.000	0.001
Soil Nitrogen	Continuous	20	MAOC	-0.003	-0.082	0.088
Experiment Length	< 5 yrs	22	MAOC	6.781	-3.773	18.493
	> 5 yrs	22	MAOC	-7.427	-15.909	1.911
Soil Depth Sampled	Surface	17	MAOC	-0.928	-11.464	10.862
	Deeper	6	MAOC	-3.429	-35.634	44.890
	Profile	23	MAOC	-0.652	-10.088	9.774
Land Cover Type	Cropland	12	POC	-2.750	-10.293	5.428
	Grassland	16	POC	9.683	0.058	20.234
	Forest	17	POC	5.721	-1.069	12.976
Fractionation Type	Size	34	POC	9.308	2.122	16.999
	Density	11	POC	-2.274	-14.444	11.628
	Size/density	3	POC	12.524	-10.685	41.765
Aboveground Biomass	Continuous	12	POC	0.003	-0.005	0.011
Belowground Biomass	Continuous	10	POC	0.018	-0.035	0.072
MAT	Continuous	41	POC	-0.001	-0.012	0.011
MAP	Continuous	40	POC	0.000	0.000	0.000
Latitude	Continuous	24	POC	-0.001	-0.003	0.000
% sand	Continuous	18	POC	-0.001	-0.004	0.002
% silt	Continuous	17	POC	-0.001	-0.005	0.004
% clay	Continuous	17	POC	0.002	-0.004	0.008
Nitrogen fixers	Yes	17	POC	0.046	-0.042	0.134
	No	44	POC	0.078	0.011	0.145
Nitrogen Fertilizer	Continuous	21	POC	0.000	-0.001	0.001
Soil Nitrogen	Continuous	25	POC	0.007	-0.044	0.058
Experiment Length	< 5 yrs	22	POC	3.477	-4.668	12.317
	> 5 yrs	22	POC	6.994	-2.528	17.445
Soil Depth Sampled	Surface	17	POC	2.246	-8.125	13.788
	Deeper	6	POC	-16.168	-37.266	12.025

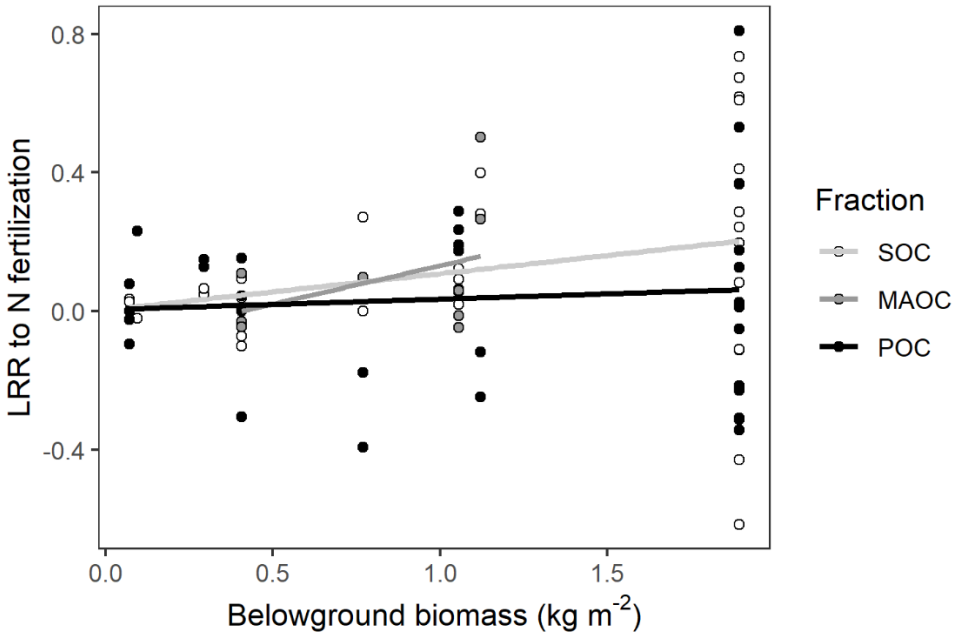
		Profile	23	POC	7.05	-0.741	15.451
Warming	Latitude	Continuous	34	SOC	-0.005	-0.014	0.004
	Soil Depth Sampled	Surface	12	SOC	-10.506	-19.989	0.050
		Subsoil	6	SOC	-5.484	-15.532	5.770
		Profile	19	SOC	3.956	-6.593	15.685
	Land Cover Type	Cropland	4	SOC	-1.745	-21.683	23.269
		Grassland	27	SOC	-2.703	-13.012	8.839
		Forest	8	SOC	-2.166	-18.470	17.386
	Aboveground Biomass	Continuous	9	SOC	-0.151	-0.580	0.278
	MAT	Continuous	41	SOC	0.000	-0.015	0.014
	MAP	Continuous	41	SOC	0.012	-0.001	0.025
	% sand	Continuous	22	SOC	0.003	-0.001	0.007
	% silt	Continuous	22	SOC	-0.004	-0.009	0.002
	% clay	Continuous	22	SOC	-0.012	-0.033	0.010
	Experiment Length	< 5 yrs	19	SOC	0.773	-10.854	13.906
		> 5 yrs	19	SOC	-2.010	-15.945	14.236
	Latitude	Continuous	28	MAOC	-0.003	-0.011	0.005
	Soil Depth Sampled	Surface	12	MAOC	-10.094	-19.852	0.854
		Subsoil	6	MAOC	-9.154	-20.084	3.283
		Profile	19	MAOC	-5.295	-16.389	7.262
	Land Cover Type	Cropland	0	MAOC	NA	NA	NA
		Grassland	27	MAOC	-3.825	-10.138	2.932
		Forest	8	MAOC	16.044	-0.040	34.716
	Aboveground Biomass	Continuous	7	MAOC	0.620	-0.162	1.402
	MAT	Continuous	32	MAOC	-0.006	-0.019	0.008
	MAP	Continuous	32	MAOC	0.018	-0.003	0.039
	Fractionation Type	Size	15	MAOC	-0.598	-11.219	11.293
		Density	14	MAOC	4.185	-7.133	17.000
	% sand	Continuous	19	MAOC	0.002	-0.001	0.004
	% silt	Continuous	19	MAOC	-0.002	-0.004	0.001
	% clay	Continuous	19	MAOC	-0.004	-0.020	0.012
	Experiment Length	< 5 yrs	19	MAOC	1.633	-11.494	16.696
		> 5 yrs	19	MAOC	-1.558	-16.222	15.662
	Latitude	Continuous	35	POC	-0.013	-0.021	-0.004
	Soil Depth Sampled	Surface	12	POC	-9.108	-23.746	8.350
		Subsoil	6	POC	-5.993	-21.518	12.603
		Profile	19	POC	-9.226	-19.836	2.778
	Land Cover Type	Cropland	4	POC	-9.968	-32.060	19.315
		Grassland	27	POC	-12.164	-23.241	0.511
		Forest	8	POC	-11.130	-29.602	12.176
	Aboveground Biomass	Continuous	9	POC	-0.143	-0.776	0.490

	MAT	Continuous	42	POC	0.011	-0.007	0.029
	MAP	Continuous	42	POC	0.016	0.000	0.031
	Fractionation Type	Size	27	POC	-8.972	-19.908	3.458
		Density	14	POC	-13.142	-28.064	4.875
	% sand	Continuous	22	POC	-0.001	-0.008	0.005
	% silt	Continuous	22	POC	0.001	-0.007	0.009
	% clay	Continuous	22	POC	0.014	-0.016	0.044
	Experiment Length	< 5 yrs	19	POC	-5.219	-17.832	9.319
		> 5 yrs	19	POC	-15.278	-30.218	2.860
Increased Precipitation	Experiment Length	Continuous	18	SOC	0.001	-0.001	0.003
	% sand	Continuous	13	SOC	-0.004	-0.011	0.004
	% silt	Continuous	13	SOC	-0.009	-0.007	0.025
	% clay	Continuous	13	SOC	-0.012	-0.229	0.205
	MAT	Continuous	18	SOC	-0.005	-0.015	0.006
	Experiment Length	Continuous	16	MAOC	0.002	-0.005	0.009
	Fractionation Method	Size	13	MAOC	14.305	-2.459	33.937
		Density	3	MAOC	-0.717	-23.609	29.033
	% sand	Continuous	13	MAOC	-0.001	-0.011	0.010
	% silt	Continuous	13	MAOC	0.002	-0.021	0.025
	% clay	Continuous	13	MAOC	0.030	-0.280	0.340
	MAT	Continuous	16	MAOC	-0.010	-0.077	0.056
	Experiment Length	Continuous	8	POC	0.004	-0.001	0.010
	Fractionation Method	Size	5	POC	-11.077	-33.068	18.140
		Density	3	POC	4.582	-29.447	55.022
	% sand	Continuous	3	POC	-0.003	-0.135	0.128
	% silt	Continuous	3	POC	0.007	-0.286	0.300
	% clay	Continuous	3	POC	0.006	-0.232	0.244
	MAT	Continuous	8	POC	-0.019	-0.043	0.004

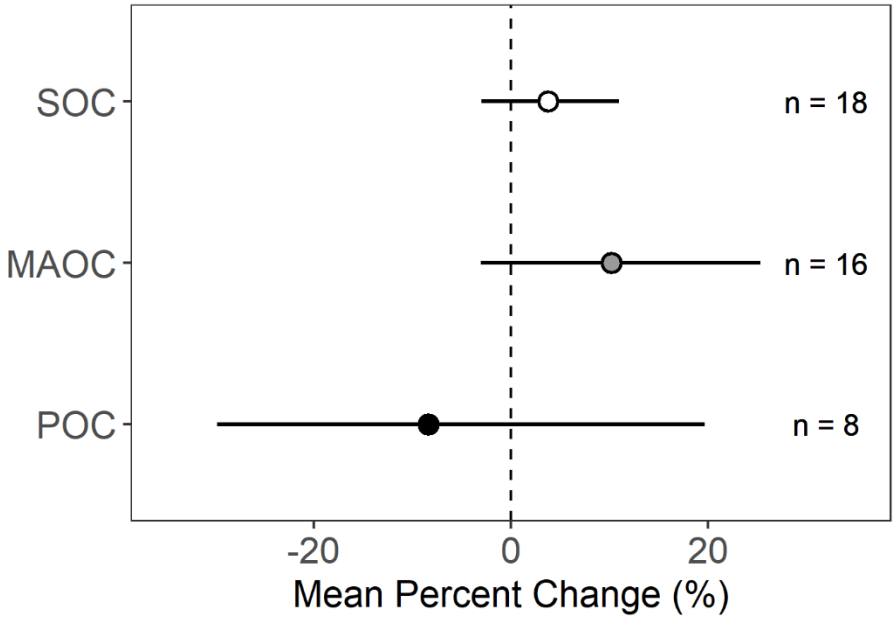
Supplementary Table 1.4. P-values for all moderator tests carried out for a meta-analysis of the responses of soil organic carbon (SOC), mineral-associated organic carbon (MAOC) and particulate organic carbon (POC) to global changes. P-values greater than 0.05 were considered non-significant and are denoted with "ns". Multiple moderator tests were carried out when more than one moderator was significant for a given global change factor - soil fraction pairing. When one or no moderators were significant, multiple moderator tests did not apply, and are denoted with "NA". For the multiple moderator model for POC response to warming, air warming and soil warming were tested separately for the full and reduced (removing warming >4°C) datasets, which had the same outcome.

Global Change Factor	Moderator	Number of observations	Soil Organic Carbon	Particulate Organic Carbon	Mineral-associated Organic Carbon
Nitrogen Fertilization	Soil Depth Sampled	431	**< 0.001**	**< 0.001**	ns
	Experiment Length	440	**0.003**	**0.028**	ns
	Land Cover Type	432	ns	ns	ns
	Experiment Type	434	ns	ns	ns
	N Fertilizer Type	430	ns	ns	ns
	pH	268	ns	ns	ns
	MAT	339	ns	ns	ns
	MAP	336	ns	ns	ns
	Latitude	335	ns	ns	ns
	% sand	232	ns	ns	ns
	% silt	232	ns	ns	ns
	% clay	232	ns	ns	ns
	Aboveground biomass	90	ns	ns	ns
	Belowground biomass	33	**0.008**	ns	ns
	Fractionation Method	179	NA	ns	ns
	Soil Depth Sampled + Experiment Length	SOC: 33 POC: 347	**Belowground biomass significant**	**All significant**	NA
Elevated CO_2	Land Cover Type	60	ns	ns	ns
	Aboveground Biomass	11	ns	ns	ns
	Belowground Biomass	9	ns	ns	ns
	MAT	52	ns	ns	ns
	MAP	51	ns	ns	ns
	Latitude	47	ns	ns	ns
	% sand	26	ns	ns	ns
	% silt	25	ns	ns	ns
	% clay	25	ns	ns	ns
	Fractionation Method	48	NA	ns	ns
	Nitrogen Fixers	61	ns	ns	ns
	Nitrogen Fertilizer	29	ns	ns	ns
	Soil Nitrogen	33	ns	ns	ns
	Experiment Length	59	ns	ns	**0.048**

	Soil Depth Sampled	60	**0.018**	ns	ns
Warming	Latitude	28	ns	**0.005**	ns
	Degree of air warming	26	ns	**0.048**	ns
	Degree of soil warming	10	**0.011**	**0.013**	**0.038**
	Degree of air warming (>4°C removed)	22	ns	ns	ns
	Degree of soil warming (>4°C removed)	8	**0.038**	**0.015**	ns
	Experiment length	38	ns	ns	ns
	Soil Depth Sampled	39	**<0.001**	ns	ns
	Fractionation Method	41	NA	ns	ns
	Land Cover Type	41	ns	ns	**0.026**
	Aboveground Biomass	9	ns	ns	ns
	MAT	41	ns	ns	ns
	MAP	41	ns	ns	ns
	% sand	22	ns	ns	ns
	% silt	22	ns	ns	ns
	% clay	22	ns	ns	ns
	Latitude + degree of warming	Soil: 10(full), 8 (reduced) Air: 20 (full), 9 (reduced)	None significant	**Degree of soil warming significant**	**Degree of soil warming significant**
Increased Precipitation	Experiment Length	18	ns	ns	ns
	Fractionation Method	16	ns	ns	ns
	% sand	13	ns	ns	ns
	% silt	13	ns	ns	ns
	% clay	13	ns	ns	ns
	MAT	18	ns	ns	ns



Supplementary Figure 1.3. Log response ratio (LRR) of carbon concentrations (g C kg soil^{-1}) in total soil organic matter (SOC, open symbol), mineral-associated organic matter (MAOC, grey symbol), and particulate organic matter (POC, black symbol) in response to N fertilization regressed against belowground biomass.



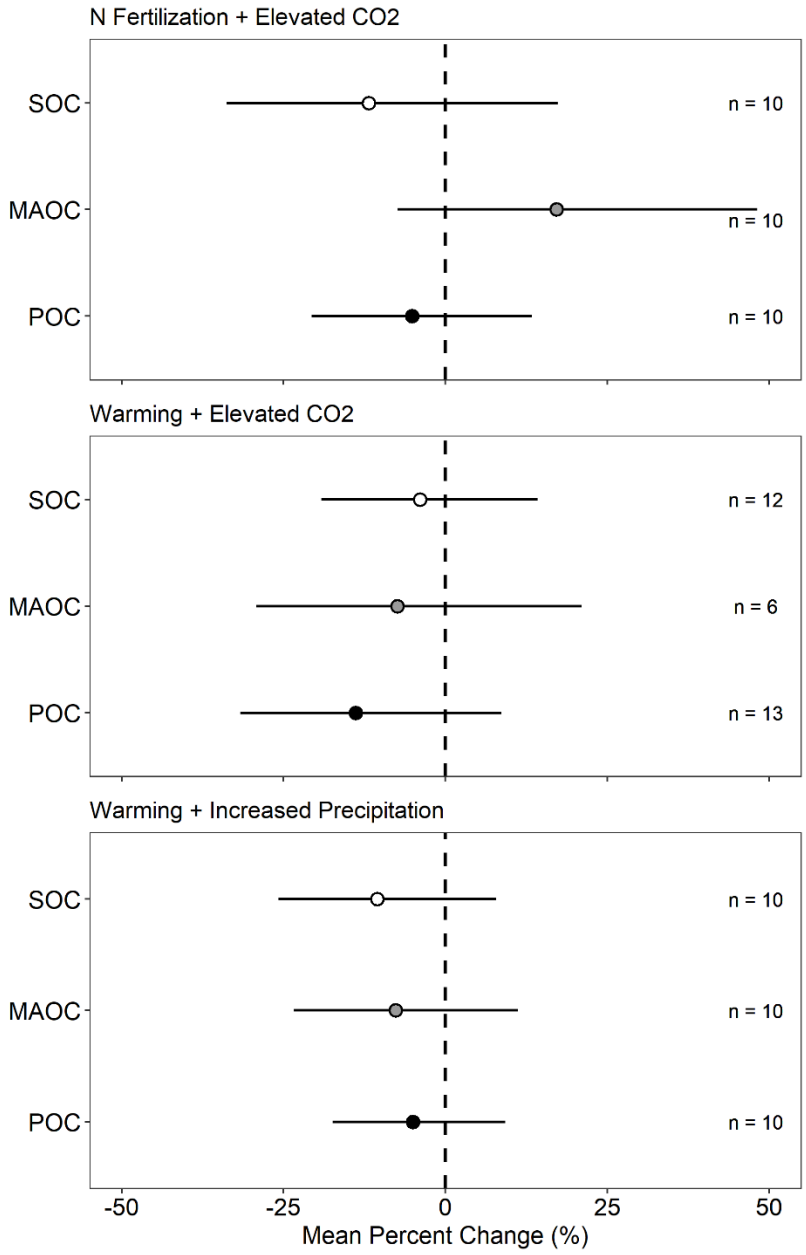
Supplementary Figure 1.4. Mean responses of carbon concentrations (g C kg soil^{-1}) in total soil organic matter (SOC, open symbol), mineral-associated organic matter (MAOC, grey symbol),

and particulate organic matter (POC, black symbol) to increased precipitation using published data with 95% confidence intervals and number of observations.

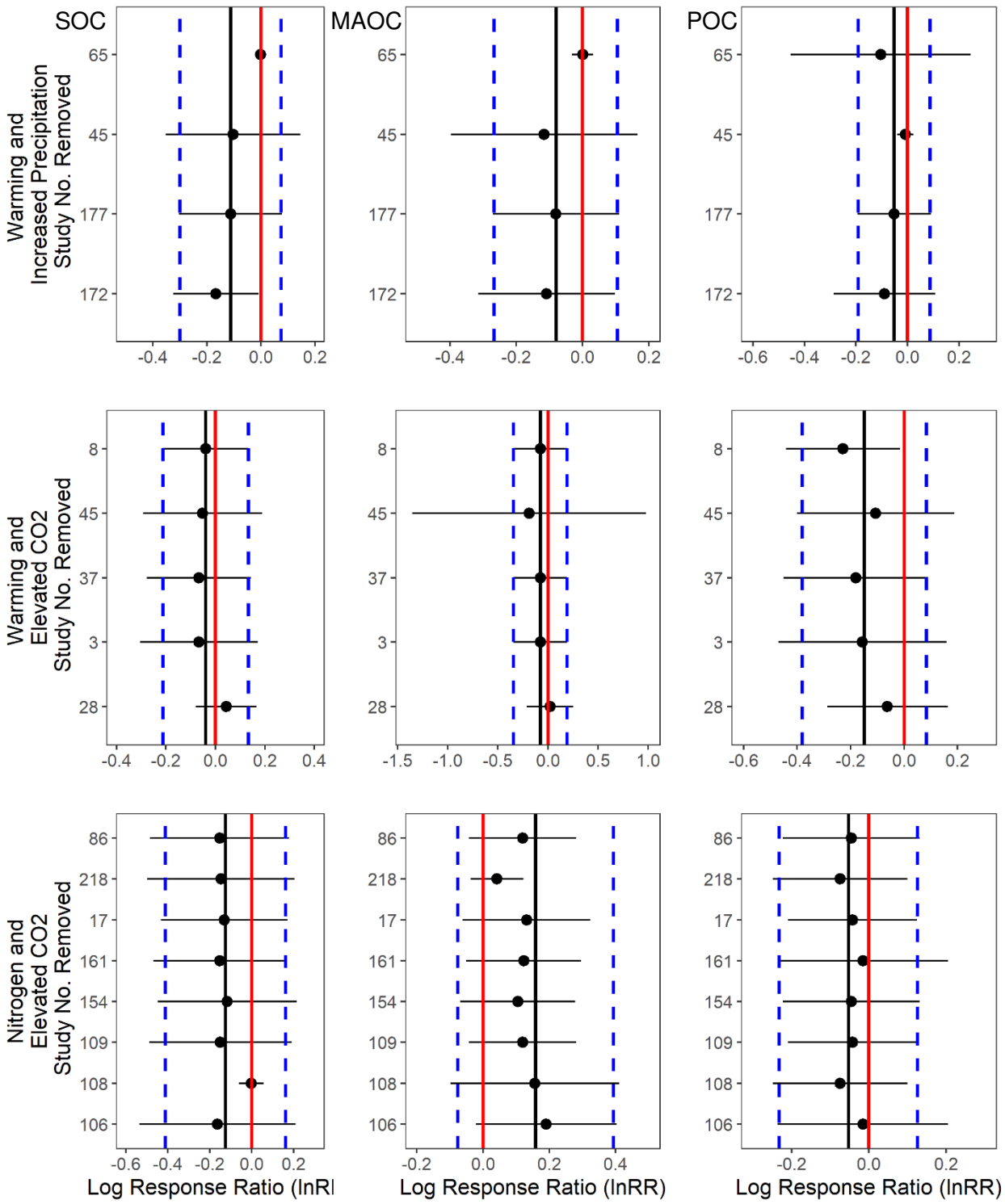
Additional Results

Multiple global changes

There were no clear effects of warming combined with elevated CO_2 on SOC (MPC = -3.92%; CI = -19.21 – 14.27%), MAOC (MPC = -7.43%; CI = -29.21 – 21.05%), or POC (MPC = -13.85%; CI = -31.71 – 8.69%), nor of warming combined with increased precipitation on SOC (MPC = -10.57%; CI = -25.80 – 7.80%), MAOC (MPC = -7.72%; CI = -23.42 – 11.20%), or POC (MPC = -5.02%; CI = -17.42 – 9.23%). There were also no clear effects of N fertilization combined with elevated CO_2 although these responses varied depending on fraction with SOC (MPC = -11.85%; CI = -33.79 – 17.36%) and POC (MPC = -5.17%; CI = -20.67 – 13.37%) responding negatively and MAOC (MPC = 17.17%; CI = -7.36 – 48.18%) responding positively (Supplementary Figure 1.5). Publication bias, as assessed by Egger's test, was not indicated for any fraction in response to any combined global changes. Histograms were difficult to interpret due to small sample sizes (Supplementary Figure 1.1). Sensitivity analysis indicated these results were generally robust (Supplementary Figure 1.6). Low study numbers prevented us from evaluating interactive effects of combined global changes and moderators.



Supplementary Figure 1.5. Mean responses of carbon concentrations (g C kg soil^{-1}) in total soil organic matter (SOC, open symbol), mineral-associated organic matter (MAOC, grey symbol), and particulate organic matter (POC, black symbol) to combined global change factors using published data with 95% confidence intervals and number of observations. Panels a, b, and c show the combined effects of nitrogen fertilization and elevated CO_2, warming and elevated CO_2, and warming and precipitation, respectively.



Supplementary Figure 1.6. Sensitivity analysis for each combined global change factor (row) and soil carbon pool (column). The plots show the mean effect size and confidence interval when the given study on the y-axis is removed. The red lines are at zero, the black lines are the mean

of all observations, and the blue dotted lines are the 95% confidence intervals using all observations.

Appendix 1 References

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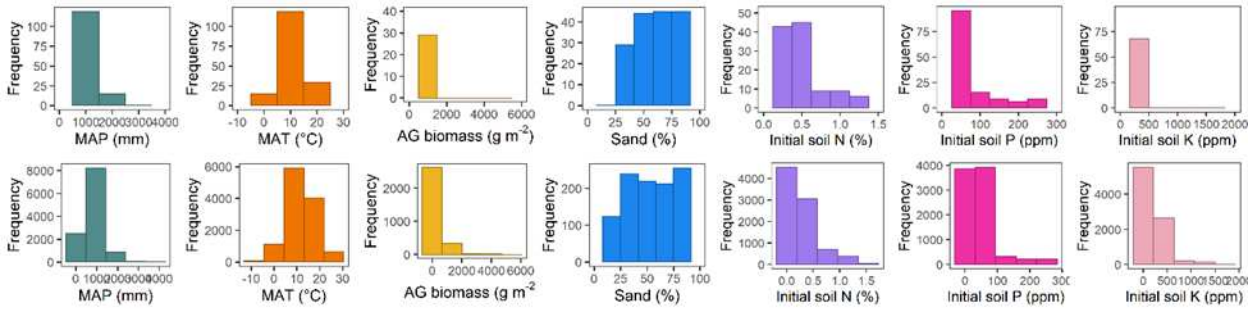
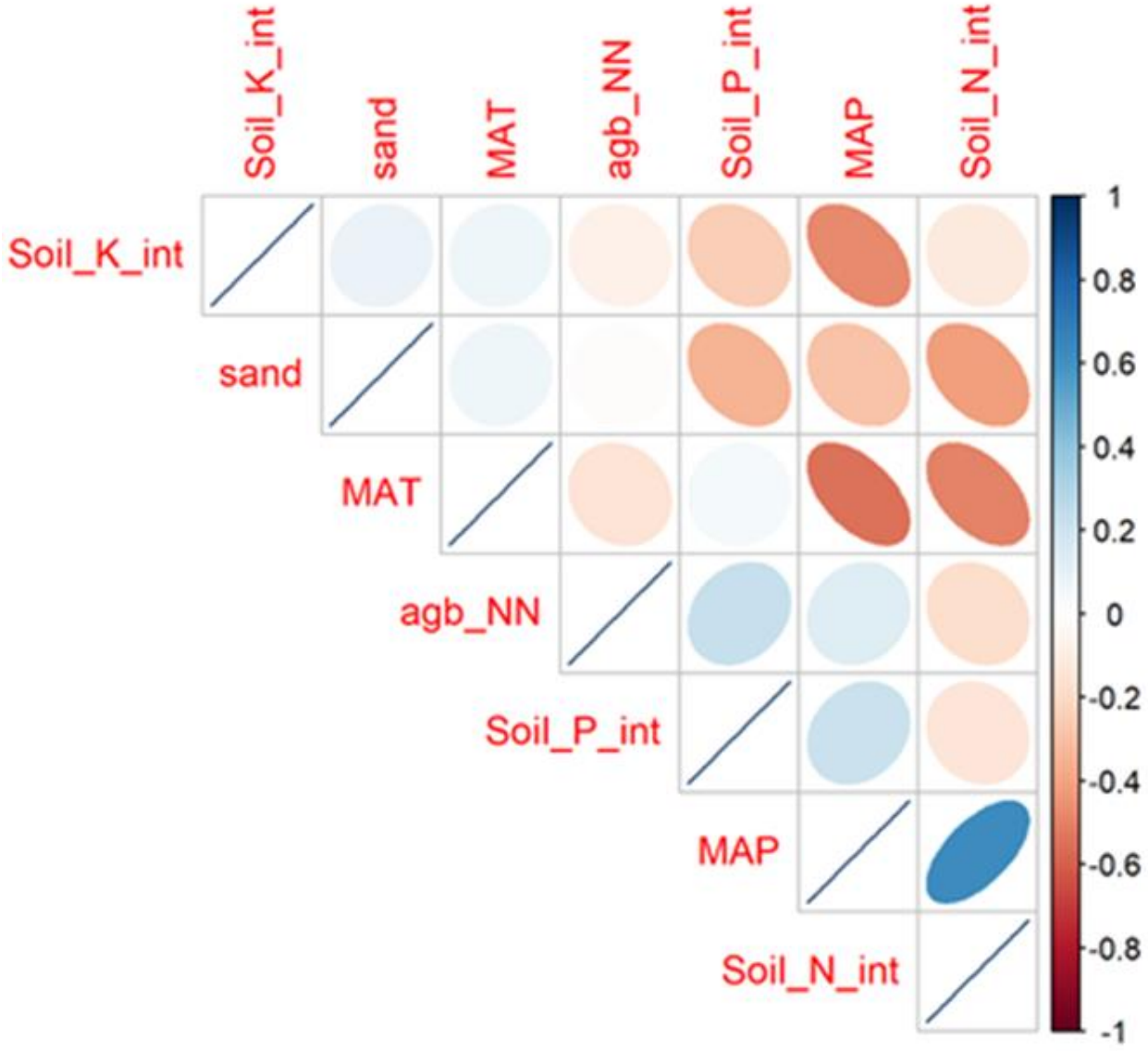
APPENDIX 2: SUPPLEMENTARY MATERIAL FOR CHAPTER 3



Supplementary Figure 2.1. Comparison of data distribution (individual observations) for the 11 sites used in this study (top) and all 130 Nutrient Network sites (bottom) for selected environmental variables used in this study.

Supplementary Table 2.1. Descriptions of response variables used in analysis of total soil, mineral-associated, and particulate organic matter carbon and nitrogen concentrations, stoichiometries and distributions at 11 grassland nutrient addition experiments.

Response Variable	Description
SOM, MAOM, and POM-C	Total soil, mineral-associated, and particulate organic matter carbon concentrations in g C kg^{-1} soil
SOM, MAOM, and POM-N	Total soil, mineral-associated, and particulate organic matter nitrogen concentrations in g N kg^{-1} soil
SOM C:N	The carbon-to-nitrogen ratio in soil organic matter; SOM-C/SOM-N
MAOM C:N	The carbon-to-nitrogen ratio in mineral-associated organic matter; MAOM-C/MAOM-N
POM C:N	The carbon-to-nitrogen ratio in particulate organic matter; POM-C/POM-N
fMAOM-C	The proportion of carbon in mineral-associated organic matter relative to particulate organic matter; MAOM-C/SOM-C
fMAOM-N	The proportion of nitrogen in mineral-associated organic matter relative to particulate organic matter; MAOM-N/SOM-N



Supplementary Figure 2.2. Correlation matrix for edaphic factors. Bigger and darker ellipses are stronger correlations and red and blue ellipses represent positive and negative relationships, respectively. "agb_NN" is averaged aboveground biomass, "MAT" and "MAP" are mean annual temperature and precipitation, respectively, "sand" is percent sand, and "Soil_N_int", "Soil_P_int", and "Soil_K_int" are ambient soil nitrogen, phosphorus, and potassium concentrations, respectively.

A. Methods for pre-treatment soil analyses (detailed in Seabloom et al., 2021)

All samples were processed in the Borer Seabloom lab at the University of Minnesota. A subsample (~80-100g) from each control plot was homogenized by grinding the soil with two

steel beads (Daisy Premium 3/8" steel slingshot ammo) with 90 minutes of vigorous shaking using a paint shaker. Approximately 20 (18-25) mg of ground, homogenized soils were then packed into 5 x 9 mm tin capsules for nitrogen analysis using dry combustion gas chromatography on an Elemental Analyzer (Costech ECS 4010 CHNSO Analyzer, Valencia, California, USA) calibrated with the analytical standard, atropine (C17H23NO3). Twenty grams of the ground, homogenized soils were sent to Waypoint Analytical (Memphis, TN, USA) to measure major nutrients and texture of the soil (percent sand, silt and clay). Phosphorus and potassium (in parts per million) were measured using the Mehlich-3 method. Texture was measured using the hydrometer method. In brief, the soil sample was shaken with Sodium Hexametaphosphate (HMP) solution, and then transferred to a settling cylinder and mixed. The percent sand, silt, and clay particles were calculated from hydrometer density readings taken at 40 seconds and two hours.

B. Results for SOM, MAOM, and POM-C and-N

B1. Response to nutrient addition

SOM-C was significantly affected by treatment (χ^2 (4, N = 163) = 12.84, p = 0.012), but no pairwise comparisons were significant. SOM-N was significantly affected by treatment (χ^2 (4, N = 163) = 21.4, p < 0.001) and had higher N under N and $NPK_{+\mu}$ addition as compared to $K_{+\mu}$ addition (p = 0.002 and 0.005, respectively). MAOM-C was not significantly affected by treatment (χ^2 (4, N = 163) = 6.4, p = 0.171), but MAOM-N was (χ^2 (4, N = 163) = 12.2, p=0.016). MAOM-N was higher under N addition than $K_{+\mu}$ addition (p = 0.014). POM-C was marginally affected by treatment (χ^2 (4, N = 163) = 9.0, p = 0.061), whereas POM-N was significantly affected by treatment (χ^2 (4, N = 163) = 17.0, p = 0.002). POM-N was higher under N and $NPK_{+\mu}$ addition than the control (p = 0.009 and 0.004, respectively).

B2. Responses to nutrient addition as modified by environmental factors

We evaluated how environmental factors affected the response of SOM fractions to nutrients by assessing relationships between mean percent change (MPC) in SOM components between treatment and control plots and environmental factors (equation 1). The response of SOM-C to nutrient addition was not related to any environmental factor (Supplementary Table 2). In contrast, the response of SOM-N to the addition of P was negatively related to MAT (Supplementary Table 3). Additionally, the response of SOM-N to the addition of $K_{+\mu}$ was positively related to ambient soil P and K (Supplementary Table 3). The responses of MAOM-C and -N under N addition were negatively related to ambient soil P (Supplementary Table 2 and 3). Similarly, POM-C and -N under N addition were also negatively related to ambient soil P (Supplementary Table 2 and 3). In addition, MPC in POM-C and -N in response to P additions were negatively related to soil P (Supplementary Table 2 and 3). MPC in POM-C under $K_{+\mu}$ was negatively related to ambient soil N (Supplementary Table 2), and, conversely, under N was negatively related to ambient soil K (Supplementary Table 3).

Supplementary Table 2.3. Chi-square estimate and (p-value) from type 3 Wald chi-squared tests for models of the response of total soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) carbon (C) concentrations to nutrient addition, as modified by environmental variables at 11 globally-distributed grasslands (Table 1). Columns for N, P, K, and NPK represent responses to nitrogen, phosphorus, potassium + micronutrients and their combined addition. Bolded relationships are significant at $p < 0.05$.

	SOM-C Df = 1; n = 31				MAOM-C Df = 1; n = 31				POM-C Df = 1; n = 31			
	N	P	K	NPK	N	P	K	NPK	N	P	K	NPK
Ambient soil N (%)	0.1 (0.803)	0.3 (0.584)	1.1 (0.297)	0.0 (0.896)	0.0 (0.992)	0.0 (0.966)	0.1 (0.784)	0.5 (0.483)	0.5 (0.468)	1.5 (0.216)	**6.8 (0.009)**	1.7 (0.189)
Ambient soil P (ppm)	3.8 (0.053)	2.7 (0.097)	0.4 (0.519)	0.8 (0.359)	**7.0 (0.008)**	1.1 (0.289)	0.2 (0.679)	0.2 (0.631)	**9.0 (0.003)**	**8.5 (0.004)**	0.8 (0.373)	2.9 (0.086)

Ambient soil K (ppm)	1.7 (0.190)	0.1 (0.813)	2.7 (0.102)	0.4 (0.531)	3.5 (0.061)	0.3 (0.586)	0.1 (0.813)	0.4 (0.507)	**3.9 (0.049)**	0.1 (0.788)	0.0 (0.947)	1.0 (0.309)
MAT (°C)	0.0 (0.875)	0.3 (0.568)	0.5 (0.477)	1.9 (0.172)	0.8 (0.386)	0.2 (0.631)	0.8 (0.369)	0.1 (0.775)	0.0 (0.966)	0.0 (0.856)	0.5 (0.466)	0.1 (0.713)
MAP (mm yr^{-1})	0.3 (0.586)	0.4 (0.552)	0.1 (0.720)	0.0 (0.990)	0.4 (0.537)	0.3 (0.616)	2.3 (0.128)	0.8 (0.383)	0.0 (0.86)	2.5 (0.114)	0.0 (0.929)	0.3 (0.558)
Sand content (%)	0.5 (0.490)	0.0 (0.969)	1.2 (0.283)	0.4 (0.530)	0.3 (0.595)	0.0 (0.843)	0.1 (0.784)	0.9 (0.333)	0.2 (0.620)	0.5 (0.478)	2.4 (0.117)	0.1 (0.795)
Aboveground biomass (g m^{-2})	0.0 (1.00)	0.4 (0.536)	0.1 (0.819)	0.2 (0.658)	0.6 (0.440)	0.9 (0.347)	0.0 (0.947)	2.0 (0.154)	0.0 (0.850)	0.1 (0.710)	0.2 (0.661)	0.0 (0.995)

Supplementary Table 2.4. Chi-square estimate and (p-value) from type 3 Wald chi-squared tests for models of the response of total soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) nitrogen (N) concentrations to nutrient addition, as modified by environmental variables at 11 globally-distributed grasslands (Table 1). Columns for N, P, K, and NPK represent responses to nitrogen, phosphorus, potassium +micronutrients and their combined addition. Bolded relationships are significant at $p < 0.05$.

	SOM-N				MAOM-N				POM-N			
	Df = 1; n = 31				Df = 1; n = 31				Df = 1; n = 31			
	N	P	K	NPK	N	P	K	NPK	N	P	K	NPK
Ambient soil N (%)	0.8 (0.360)	0.3 (0.609)	2.43 (0.119)	0.6 (0.455)	0.1 (0.699)	0.9 (0.339)	0.0 (0.917)	0.1 (0.725)	1.2 (0.283)	1.6 (0.209)	3.3 (0.068)	2.2 (0.139)
Ambient soil P (ppm)	1.2 (0.273)	0.1 (0.759)	4.4 (0.036)	0.4 (0.531)	**6.2 (0.013)**	1.1 (0.304)	0.1 (0.710)	0.0 (0.998)	**4.4 (0.035)**	**10.1 (0.001)**	0.8 (0.381)	1.3 (0.255)
Ambient soil K (ppm)	0.2 (0.679)	0.9 (0.355)	**6.6 (0.010)**	0.0 (0.857)	1.8 (0.176)	0.9 (0.333)	0.0 (0.904)	0.0 (0.844)	3.1 (0.078)	0.0 (0.846)	0.9 (0.331)	1.4 (0.241)
MAT (°C)	0.5 (0.484)	**4.3 (0.037)**	0.8 (0.380)	2.8 (0.097)	0.4 (0.513)	1.1 (0.305)	1.3 (0.262)	1.4 (0.231)	0.6 (0.443)	0.0 (0.853)	0.2 (0.627)	0.3 (0.602)
MAP (mm yr^{-1})	0.03 (0.853)	1.2 (0.277)	0.1 (0.745)	0.1 (0.748)	0.5 (0.472)	0.0 (0.859)	2.1 (0.148)	1.2 (0.272)	0.0 (0.841)	1.4 (0.232)	0.1 (0.699)	0.4 (0.510)
Sand content (%)	0.2 (0.643)	0.5 (0.469)	1.9 (0.168)	0.0 (0.945)	0.4 (0.516)	0.3 (0.616)	0.1 (0.814)	0.2 (0.662)	0.1 (0.817)	0.4 (0.527)	2.8 (0.096)	0.7 (0.408)

Aboveground biomass (g m^{-2})	0.0 (0.949)	0.2 (0.633)	0.1 (0.730)	0.3 (0.572)	0.3 (0.616)	0.1 (0.748)	0.0 (0.863)	1.2 (0.270)	0.0 (0.960)	0.0 (0.991)	0.0 (0.919)	0.3 (0.571)

B4. Relative importance of all variables

Supplementary Table 2.5. Top model (< 2 AICc) estimates and standard errors (in parentheses) from multi-model averaging for bulk soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) carbon (C) and nitrogen (N) concentrations with all environmental variables and the treatment at 11 globally-distributed grassland nutrient addition experiments (Table 1).

Soil component	Number of models	Percent sand	Aboveground biomass	MAT	MAP	Amb-ient soil N	Amb-ient soil P	Amb-ient soil K	Treatment
SOM-C	5	-0.38 (0.21)	0.10 (0.16)	-0.05 (0.12)		1.81 (0.19)		0.03 (0.09)	
SOM-N	5	-0.58 (0.22)	0.09 (0.18)	-0.18 (0.25)		1.42 (0.26)	0.04 (0.13)		
MAOM-C	5	-0.65 (0.21)	0.40 (0.20)		-0.45 (0.24)	1.94 (0.26)		0.31 (0.19)	
MAOM-N	5	-0.90 (0.23)	0.40 (0.22)		-0.42 (0.29)	1.58 (0.27)		0.34 (0.21)	
POM-C	2			-0.85 (0.16)		1.21 (0.16)		0.23 (0.23)	
POM-N	1			-1.02 (0.13)		1.00 (0.13)		0.39 (0.11)	

C. Response of fMAOM-C and -N to nutrient addition as modified by environment

Supplementary Table 2.6. Chi-square estimate and (p-value) from type 3 Wald chi-squared tests for models of the response of the proportion of carbon (C) and nitrogen (N) in mineral-associated organic matter (fMAOM-C and -N) to nutrient addition, as modified by environmental variables at 11 globally-distributed grasslands (Table 1). Columns for N, P, K, and NPK represent responses to nitrogen, phosphorus, potassium+micronutrients and their combined addition. Bolded relationships are significant at $p < 0.\ 05$.

	fMAOM-C df = 1; n = 31				fMAOM-N df = 1; n = 31			
	N	P	K	NPK	N	P	K	NPK
Ambient soil N (%)	0.0 (0.859)	1.1 (0.301)	1.7 (0.197)	0.3 (0.583)	0.3 (0.567)	0.5 (0.491)	1.8 (0.174)	0.0 (0.834)
Ambient soil P (ppm)	0.0 (0.857)	0.1 (0.712)	1.1 (0.285)	0.1 (0.718)	3.8 (0.051)	1.4 (0.231)	**4.8 (0.028)**	0.1 (0.703)
Ambient soil K (ppm)	0.1 (0.733)	0.3 (0.597)	1.6 (0.208)	0.0 (0.982)	1.3 (0.260)	0.0 (0.962)	3.7 (0.055)	0.0 (0.841)
MAT (°C)	0.6 (0.447)	0.0 (0.927)	0.1 (0.767)	0.4 (0.553)	**4.6 (0.032)**	0.4 (0.504)	0.1 (0.814)	0.2 (0.662)
MAP (mm yr^{-1})	0.2 (0.664)	0.0 (0.864)	1.2 (0.279)	0.4 (0.540)	0.7 (0.394)	0.9 (0.355)	0.8 (0.366)	0.5 (0.468)
Sand content (%)	0.4 (0.523)	0.1 (0.752)	1.4 (0.237)	0.2 (0.690)	0.3 (0.581)	2.0 (0.154)	1.2 (0.279)	0.1 (0.725)
Aboveground biomass (g m^{-2})	1.5 (0.215)	0.6 (0.430)	0.0 (0.892)	0.6 (0.454)	0.8 (0.383)	0.0 (0.916)	0.0 (0.906)	0.3 (0.566)

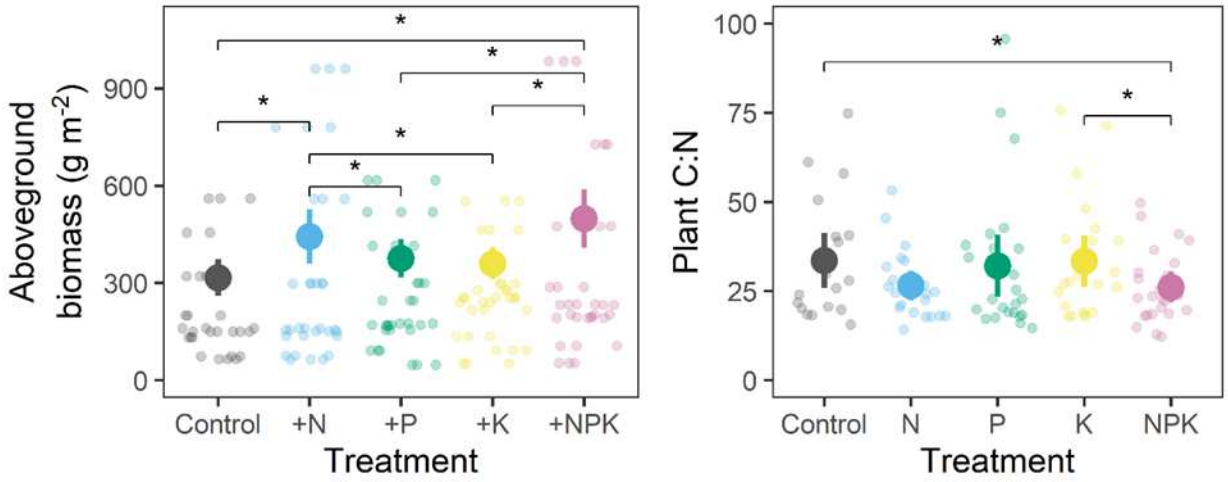
D. Responses of plant biomass and plant stoichiometry to nutrient treatment

Data on aboveground biomass and plant stoichiometry were analyzed to provide mechanistic understanding for SOM responses. We used plot-level aboveground plant C:N data, measured using near infrared spectroscopy, determined between 1-5 years post treatment (methods detailed in Anderson et al., 2018) and peak season aboveground biomass (measured as for aboveground biomass, in manuscript methods) averaged over the beginning of the treatment to the time of soil sampling for each site (except for Cereep, where only 2018 data were

available). Data for plant C:N were available for all but three sites (Cereep, Cedar Point, and Bogong), and aboveground biomass data were available for all sites. Plant data were used as response variables in mixed models for overall nutrient treatment (mirroring analysis for SOM component response to nutrient treatment). Significance was determined at $p < 0.05$.

Aboveground biomass was affected by treatment (χ^2 (4, N = 163) = 66.2, $p < 0.001$) with pairwise comparisons indicating significantly higher aboveground biomass under N and $NPK_{+\mu}$ as compared to the control, $K_{+\mu}$, and P (all $p < 0.05$; Supplementary Figure 2.2).

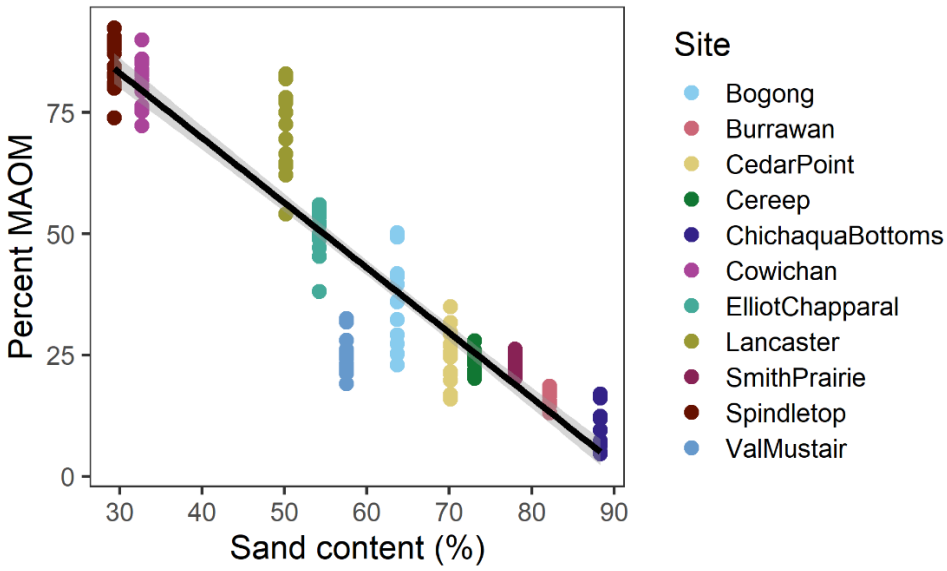
Plant C:N was significantly affected by nutrient treatment (χ^2 (1, N = 114) = 21.2, $p < 0.001$), with significantly higher C:N under $K_{+\mu}$ and the control as compared to $NPK_{+\mu}$ ($p < 0.001$ and $p = 0.015$, respectively; Supplementary Figure 2.2).



Supplementary Figure 2.3. Aboveground biomass and plant C:N under nutrient addition treatments (+N = nitrogen, +P = phosphorus, +K = potassium + micronutrients, +NPK = combined) at 11 globally-distributed grasslands (Table 1). Means with 95% confidence intervals (n=33 for treatments and n=31 for control) are shown as large dots with whiskers and individual observations are behind the large dots. Significantly different pairwise comparisons, at $p < 0.05$, are denoted with a "*"

E. Relative mass of POM and MAOM as related to soil texture

To assess the effect of soil texture on the relative mass of POM vs MAOM, we correlated sand content and percent recovery of POM and MAOM from our size fractionation. As expected based on the size cutoff for fractionation, percent POM was strongly positively correlated to percent sand ($R^2 = 0.86$, $p < 0.001$) and consequently, percent MAOM was strongly negative correlated to percent sand ($R^2 = 0.86$, $p < 0.001$; Supplementary Figure 4).



Supplementary Figure 2.4. Relationship of percent MAOM with percent sand with points color-coded to sites.

F. Site level responses of SOM C and N to nutrients

Supplementary Table 2.7. Effect of treatment on soil, mineral-associated, and particulate organic matter (SOM, MAOM, and POM) carbon and nitrogen (C and N) concentrations and stoichiometries at each site used in study of grassland responses to nutrient addition. Significant ($p < 0.1$) pairwise comparisons denoted for overall significant ($p < 0.05$) relationships. N = nitrogen, P = phosphorus, K = potassium + micronutrients, NPK = combined, and C = control.

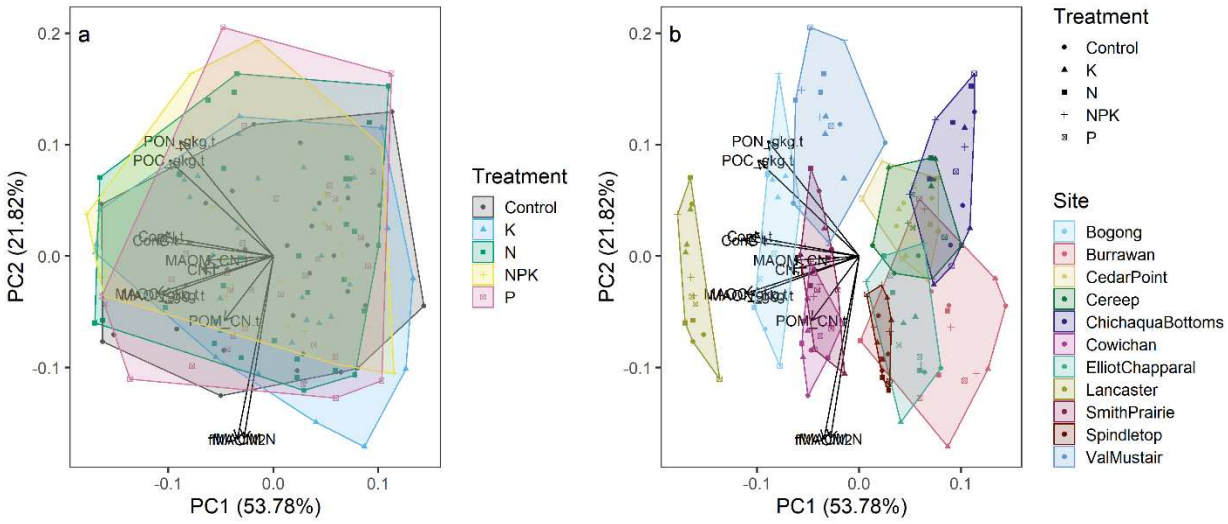
SOM component	SOM C:N	SOM-C	SOM-N	MAOM C:N	MAOM-C	MAOM-N	POM C:N	POM-C	POM-N
Site									

Bogong		C > P	P, N, C > NPK; N > K; P > K	P > NPK; N > K		
Burrawan		NPK > N, K	N > K	N > K, NPK		
Cedar Point						
Cereep			C, NPK > N, K	NPK, C > K, N	C > P	
Chichaqua Bottoms						NPK > K
Cowichan	K > P		C> NPK, P			
Elliot Chapparal						
Lancaster	K > N, P, NPK	K > NPK				
Smith Prairie		C > N, NPK, P				
Spindletop					P > K, NPK	
Val Musair						

G. Principal components analysis for SOM components

All SOM components were combined in principal components analysis (PCA). Principle component 1 and 2 (PC1 and PC2) explained 55.4% and 21.6% of the variance, respectively. SOM, MAOM, and POM-C and -N all loaded negatively on PC1, fMAOM-C and -N loaded negatively on PC2 (Supplementary Figure 2.2), and SOM, MAOM, POM C:N loaded negatively on PC3 (not shown). We performed permutational multivariate analysis of variance

(PERMANOVA) using the vegan package (Oksanen et al., 2019) in R to determine how treatment versus site groupings explained variance in the PCA. Treatment significantly explained the data distribution in PCA space (p = 0.008) but only explained 1% of the variation. Site also significantly explained the data distribution in PCA space (p = 0.001) and explained 81% of the variation.



Supplementary Figure 2.5. Principal component analysis of soil organic matter (SOM) components with groupings based on (a) treatment (colors and shape) and (b) treatment (shape) and site (colors). N = nitrogen, P = phosphorus, K = potassium + micronutrients, and NPK = combined

Supplementary Table 2.8. Author contributions to the manuscript.

Co-author name	Developed & framed research questions	Analyzed data	Contributed to data analyses	Wrote the paper	Contributed to paper writing	Site Coordinator	Nutrient Network Coord-inator	Soils Coord-inator
Katherine S Rocci	x	x		x				
M. Francesca Cotrufo	x				x			
Rebecca L. McCulley					x	x		

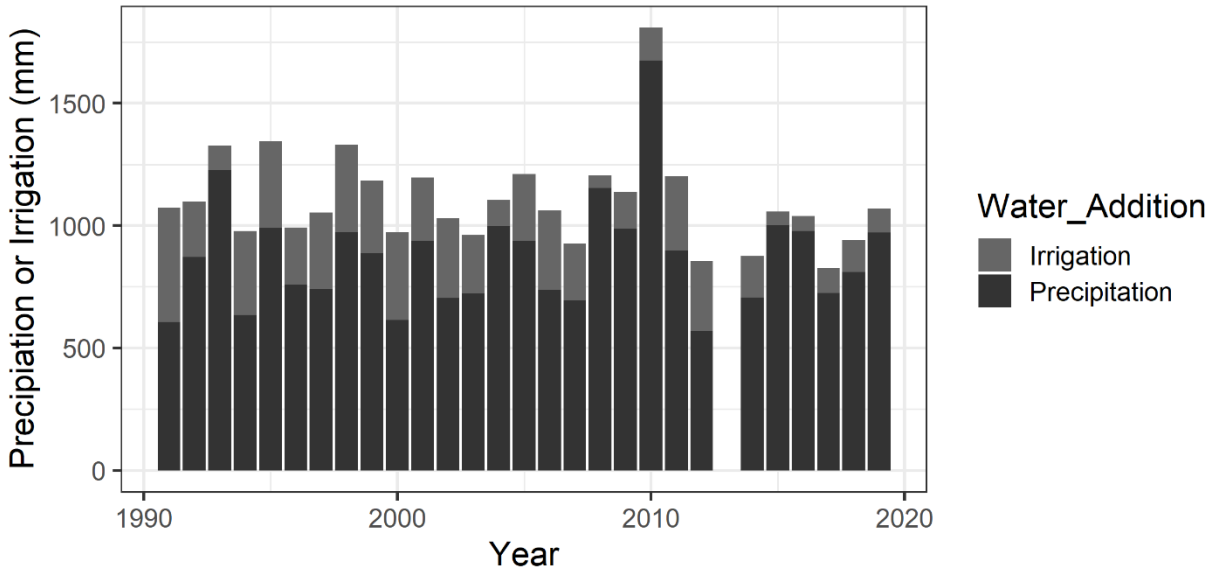
Kaydee S. Barker	x	x			
Eric W. Seabloom		x		x	
Elizabeth T. Borer		x		x	
Andrew S MacDougall		x	x		
Carly Stevens		x	x		
Xavier Raynaud		x	x		
Joslin L. Moore		x	x		
Jonathan D. Bakker		x	x		
Sarah E. Hobbie		x			x

References for Appendix 2

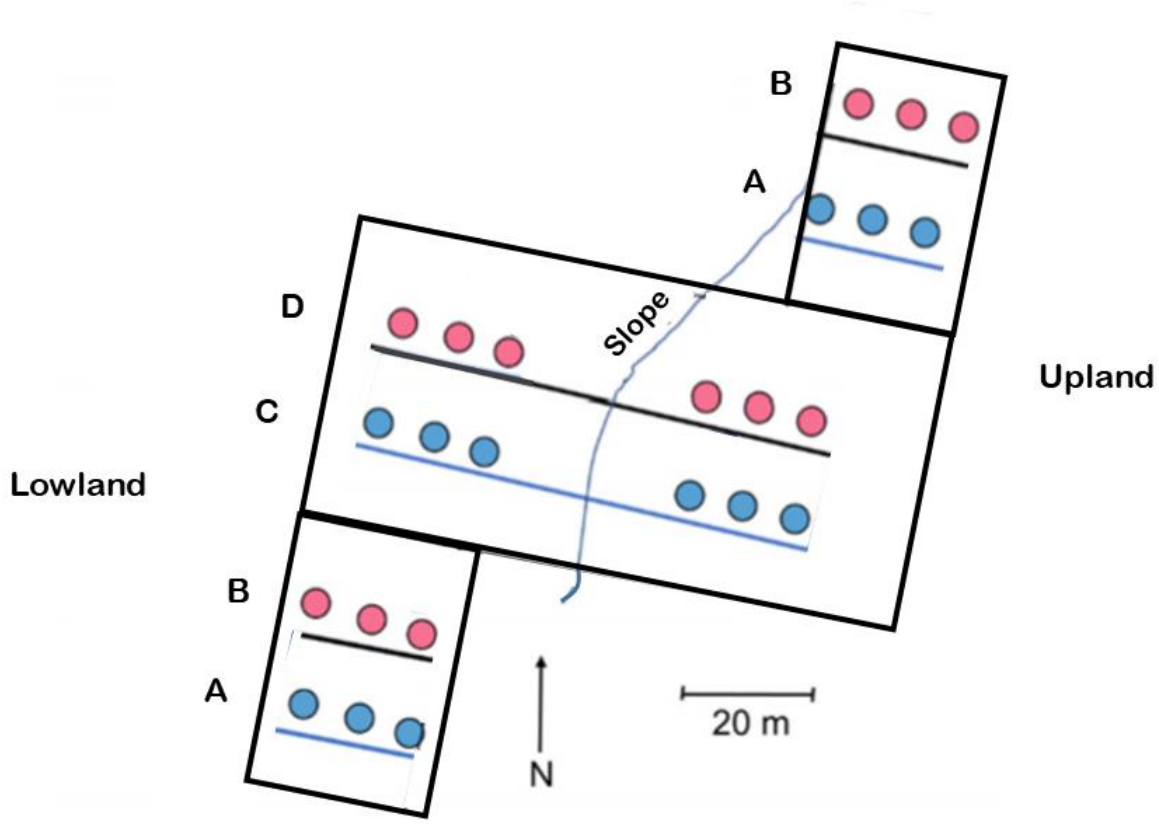
Oksanen FJ, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, M. Stevens MHH, Szoecs E and Wagner H (2019) vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Seabloom EW et al. (2021) Increasing effects of chronic nutrient enrichment on plant diversity loss and ecosystem productivity over time Ecology 102:e03218

APPENDIX 3: SUPPLEMENTARY MATERIAL FOR CHAPTER 4



Supplementary Figure 3.1. Water addition (gray) and annual precipitation (black) since 1991 at Irrigation Transect Experiment at Konza Prairie Biological Station. Data for 2013 were not available.



Supplementary Figure 3.2. Diagram of Irrigation Transect Experiment at Konza Prairie Biological Station adapted from Broderick et al. (2022). Pink plots represent controls with no irrigation and blue circles represent treatment plots with irrigation to mimic increased precipitation. Blue lines represent irrigation lines and black lines are piping with no irrigation. The AB transect is split and the CD transect is continuous.

Supplementary Table 3.1. F-values and p-values (in parentheses) from ANOVA output for analysis of soil C and N stocks in response to increased precipitation in two landscape positions across soil depths. Responses with $p < 0.05$ are bolded to indicate significance. LP = land position; T = treatment; SD = soil depth.

Response Variable	Land position	Treatment	LP*T	LP*SD	T*SD	LP*T*SD
SOM C	0.07 (0.796)	0.33 (0.570)	2.93 (0.092)	**86.0 (<0.001)**	0.02 (0.977)	2.63 (0.081)
SOM N	0.08 (0.785)	0.04 (0.837)	2.55 (0.116)	**90.43 (<0.001)**	0.06 (0.937)	2.08 (0.135)
fPOM C	0.89 (0.413)	0.88 (0.354)	0.80 (0.376)	**8.62 (<0.001)**	0.28 (0.597)	0.32 (0.572)

fPOM N	0.03 (0.865)	1.53 (0.224)	1.74 (0.195)	1.03 (0.368)	0.76 (0.390)	0.38 (0.542)
oPOM+ hcOM C	0.27 (0.626)	0.00 (0.956)	1.81 (0.187)	1.21 (0.309)	3.02 (0.091)	0.47 (0.496)
oPOM+ hcOM N	0.53 (0.498)	0.19 (0.666)	0.00 (0.995)	**8.11 (0.001)**	1.85 (0.182)	0.40 (0.533)
MAOM C	**12.79 (<0.001)**	1.04 (0.308)	2.88 (0.090)	**274.70 (<0.001)**	0.13 (0.718)	0.07 (0.788)
MAOM N	**8.23 (0.032)**	0.16 (0.693)	**5.51 (0.024)**	**243.53 (<0.001)**	0.14 (0.713)	0.78 (0.384)

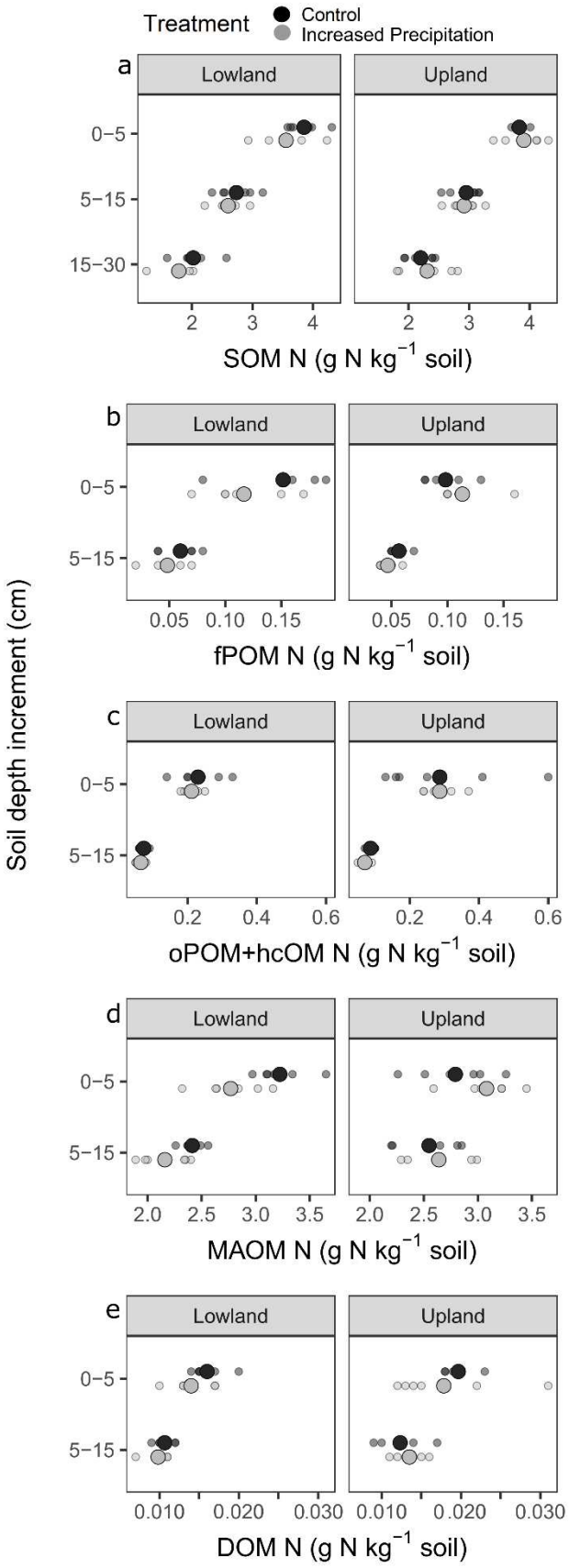
Supplementary Table 3.2. F-values and p-values (in parentheses) from ANOVA output for analysis of soil bulk density (g cm^{-3}) in response to increased precipitation in two landscape positions across soil depths. Responses with $p < 0.05$ are bolded to indicate significance. LP = land position; T = treatment; SD = soil depth.

Response Variable	Land position	Treatment	Soil depth	LP*T	LP*SD	T*SD	LP*T*SD
Bulk density	0.07 (0.831)	2.48 (0.121)	**174.40 (<0.001)**	3.20 (0.079)	**4.60 (0.014)**	0.446 (0.643)	0.703 (0.499)

Supplementary Table 3.3. F-values and p-values (in parentheses) from ANOVA output for analysis of soil C and N concentrations and stoichiometry in response to increased precipitation in two landscape positions across soil depths. Responses with $p < 0.05$ are bolded to indicate significance. LP = land position; T = treatment; SD = soil depth.

Response Variable	Land position	Treatment	Soil depth	LP*T	LP*SD	T*SD	LP*T*SD
SOM C	9.40 (0.141)	0.60 (0.441)	**312.57 (<0.001)**	3.39 (0.071)	**3.48 (0.038)**	0.03 (0.967)	0.18 (0.837)
SOM N	9.47 (0.140)	1.20 (0.278)	**181.55 (<0.001)**	3.07 (0.085)	0.43 (0.653)	0.06 (0.946)	0.27 (0.765)
SOM C:N	0.00 (0.991)	0.97 (0.328)	**41.20 (<0.001)**	0.07 (0.796)	**5.23 (0.008)**	0.26 (0.770)	0.17 (0.843)
DOM C	1.69 (0.328)	3.19 (0.082)	**8.49 (0.006)**	2.10 (0.156)	3.68 (0.063)	**5.21 (0.028)**	1.71 (0.199)

DOM N	11.06 (0.127)	1.44 (0.238)	**43.16 (<0.001)**	0.60 (0.444)	0.03 (0.855)	2.00 (0.166)	0.84 (0.366)
DOM C:N	0.07 (0.826)	**5.73 (0.022)**	**6.86 (0.013)**	0.39 (0.538)	1.97 (0.168)	1.23 (0.274)	0.54 (0.467)
fPOM C	2.26 (0.277)	2.60 (0.116)	**104.62 (<0.001)**	2.11 (0.155)	2.80 (0.103)	0.64 (0.430)	1.07 (0.308)
fPOM N	0.15 (0.741)	**4.29 (0.045)**	**149.70 (<0.001)**	2.22 (0.145)	2.34 (0.135)	1.45 (0.236)	1.81 (0.187)
fPOM C:N	4.70 (0.175)	0.25 (0.617)	**43.88 (<0.001)**	0.95 (0.335)	0.30 (0.587)	0.89 (0.352)	0.013 (0.911)
oPOM+ hcOM C	1.19 (0.429)	0.65 (0.426)	**268.31 (<0.001)**	0.82 (0.371)	0.00 (0.986)	3.74 (0.061)	1.41 (0.242)
oPOM+ hcOM N	3.47 (0.255)	0.99 (0.327)	**255.35 (<0.001)**	0.10 (0.754)	0.28 (0.602)	2.17 (0.149)	0.97 (0.332)
oPOM+ hcOM C:N	4.02 (0.235)	0.86 (0.361)	**38.60 (<0.001)**	**6.33 (0.016)**	0.84 (0.365)	0.01 (0.918)	0.00 (0.997)
MAOM C	5.04 (0.206)	0.64 (0.429)	**64.91 (<0.001)**	**9.12 (0.005)**	**7.59 (0.009)**	0.19 (0.662)	1.40 (0.245)
MAOM N	1.99 (0.340)	1.44 (0.238)	**48.30 (<0.001)**	**12.05 (0.001)**	**5.87 (0.021)**	0.0 (0.991)	1.75 (0.194)
MAOM C:N	2.82 (0.246)	0.50 (0.486)	**9.40 (0.004)**	1.91 (0.175)	1.55 (0.221)	1.73 (0.197)	0.23 (0.636)


Treatment
Control
Increased Precipitation
a
Lowland
Upland
0–5
5–15
15–30
2
3
4
SOM N (g N kg^{-1} soil)
b
0.05
0.10
0.15
fPOM N (g N kg^{-1} soil)
c
0.2
0.4
0.6
oPOM+hcOM N (g N kg^{-1} soil)
d
2.0
2.5
3.0
3.5
MAOM N (g N kg^{-1} soil)
e
0.010
0.020
0.030
DOM N (g N kg^{-1} soil)
Soil depth increment (cm)

Supplemental Figure 3.3. Soil organic matter (SOM) nitrogen (N; a), free particulate OM (fPOM) N (b), occluded POM and heavy, coarse OM (oPOM+hcOM) N (c), mineral-associated OM (MAOM) N (d), and dissolved OM (DOM N (e) in response to increased precipitation (teal) *versus* a control (yellow) at two depths and landscape positions.

Supplementary Table 3.3. F-values and p-values (in parentheses) from ANOVA output for analysis of root biomass and quality metrics in response to increased precipitation in two landscape positions across soil depths. Responses with $p < 0.05$ are bolded to indicate significance. AUR = acid unhydrolyzable residues; HWE = hot water extractable; C = carbon; N = nitrogen; LP = land position; T = treatment; SD = soil depth.

Response Variable	Land position	Treatment	Soil depth	LP*T	LP*SD	T*SD	LP*T*SD
Root biomass (mg cm^{-3})	**5.43 (0.039)**	**11.78 (0.001)**	N/A	0.01 (0.919)	**179.67 (<0.001)**	0.17 (0.840)	1.02 (0.367)
Root AUR (%)	0.00 (0.996)	2.68 (0.110)	2.36 (0.134)	**4.53 (0.040)**	0.02 (0.879)	0.20 (0.660)	0.02 (0.900)
Root cellulose (%)	0.86 (0.498)	1.82 (0.186)	**7.17 (0.011)**	0.02 (0.890)	**5.58 (0.024)**	2.53 (0.121)	0.39 (0.537)
HWE C (mg C g^{-1} root)	0.70 (0.494)	0.00 (0.960)	0.00 (0.985)	0.50 (0.482)	0.34 (0.564)	0.25 (0.624)	0.05 (0.821)
HWE N (mg N g^{-1} root)	0.24 (0.675)	0.57 (0.453)	1.31 (0.260)	1.27 (0.267)	0.39 (0.537)	0.11 (0.745)	0.77 (0.386)
HWE C:N	0.01 (0.928)	0.96 (0.334)	3.25 (0.080)	0.08 (0.781)	0.02 (0.892)	0.07 (0.795)	0.54 (0.469)

Appendix 3 References

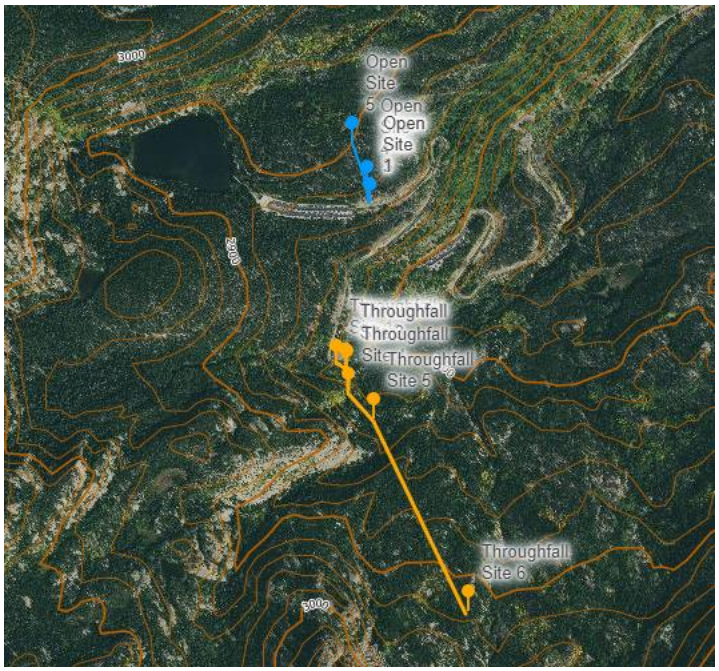
Broderick CM, Wilkins K, Smith MD, Blair JM 2022. Climate legacies determine grassland responses to future rainfall regimes. Global Change Biology, 28, 8, 2639-2656.

APPENDIX 4: SUPPLEMENTARY MATERIAL FOR CHAPTER 5

A. Full summer data for throughfall transect

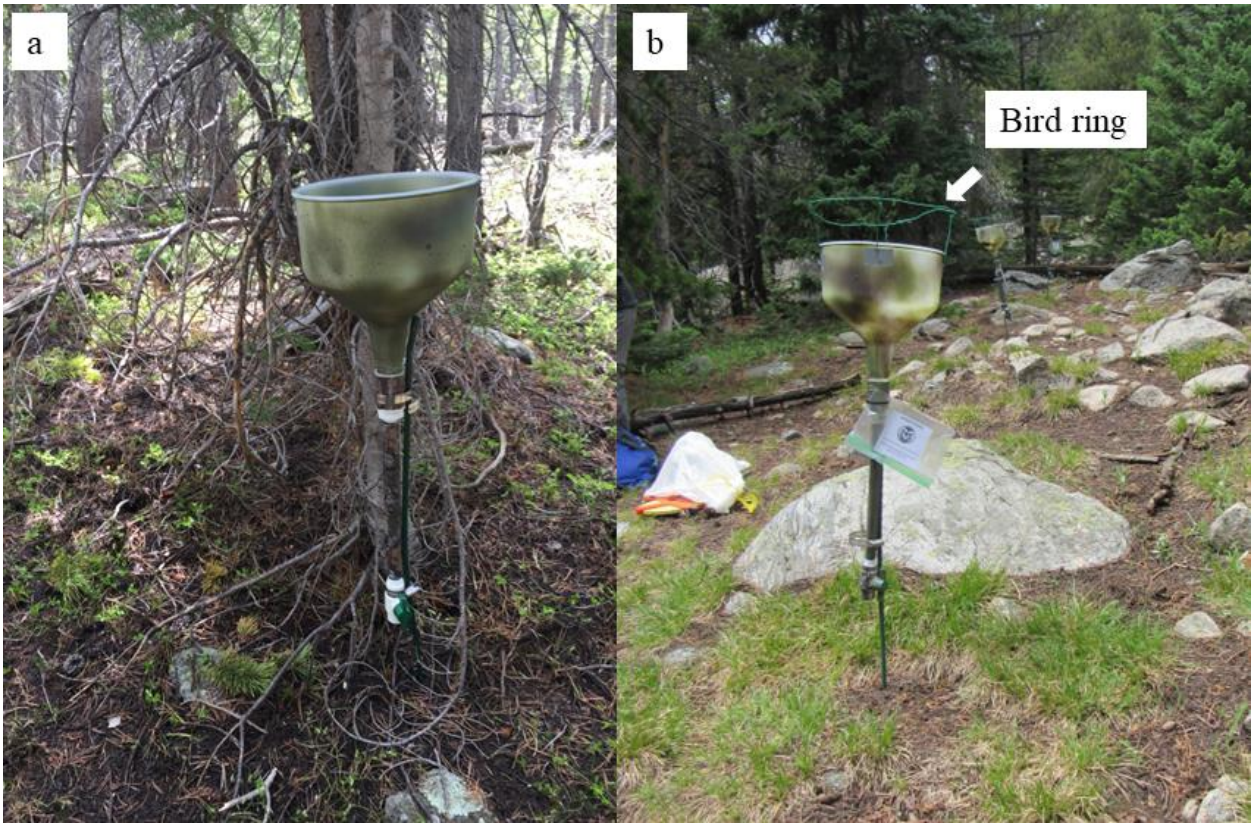
Data for the full summer (May 25th – October 7th) were collected for the throughfall sites only. Using the full summer dataset, throughfall deposition of NO_3^- ($F = 1.211$; $p = 0.362$) and TIN ($F = 1.326$; $p = 0.318$) did not vary between sites. Site was marginally significant for NH_4^+ ($F = 2.806$; $p = 0.066$) and NH_4^+: NO_3^- ($F = 2.698$; $p = 0.074$) with no significant pairwise comparisons. With respect to relationships between throughfall deposition and DBH, only NO_3^- ($r^2 = 0.272$; $p = 0.026$) and TIN ($r^2 = 0.281$; $p = 0.024$) deposition were significantly positively related to DBH and NH_4^+ ($r^2 = 0.120$; $p = 0.160$) and NH_4^+:NO_3^- ($r^2 = 0.059$; $p = 0.331$) were not.

B. Topographic complexity of area



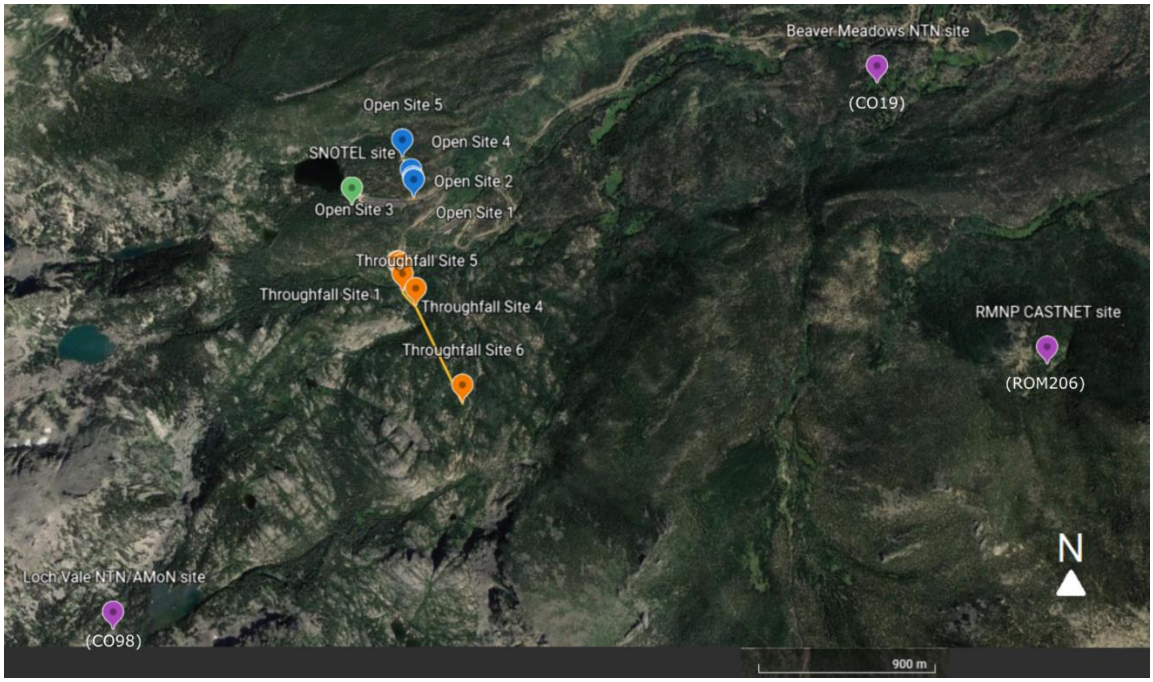
Supplementary Figure 4.1. Image showing topographic complexity surrounding the two transects used in a study of nitrogen deposition at Rocky Mountain National Park. Image created with CalTopo.

C. Images of collectors



Supplementary Figure 4.2. (a) Throughfall ion exchange resin (IER) collector and (b) open IER collector with arrow pointing to bird ring that is meant to discourage birds from landing on the funnel.

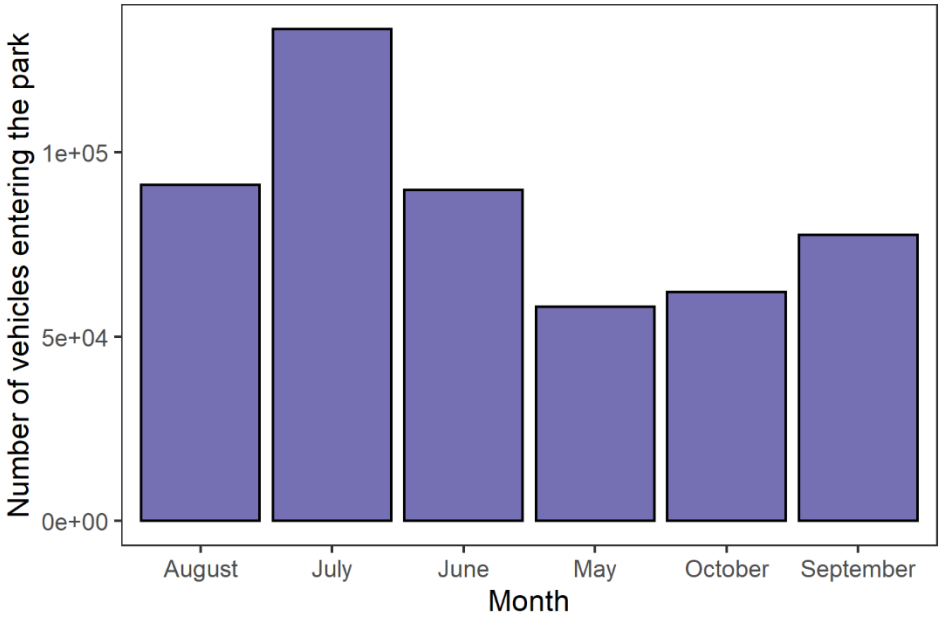
Placement of ion exchange resin sites relative to National Network sites



Supplementary Figure 4.3. Location of open (blue) and throughfall (orange) ion exchange resin sampler transects relative to nearby National Trends Network (NTN) and Clean Air Status and Trends (CASTNET) sites (purple) and a SNOTEL site (green). Image from Google Earth.

Supplementary Table 4.1. Average blank values (n = 7 for throughfall and n = 5 for open) for each N species for throughfall and open sites in a study of nitrogen deposition in Rocky Mountain National Park in Colorado, USA.

Site Type	Nitrogen Species	Average blank N (mg N)
Throughfall	NH_4^+	-0.039
	NO_3^-	0.010
Open	NH_4^+	0.006
	NO_3^-	0.009



Supplementary Figure 4.4. Traffic count during experimental period at the Beaver Meadows entrance, commonly used to access the Bear Lake area where the study took place.