DISSERTATION

THE ECOLOGY OF NATURAL CLIMATE SOLUTIONS: QUANTIFYING SOIL CARBON AND BIODIVERSITY BENEFITS

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Shelby C. McClelland

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Doctoral Committee:

Advisor: Meagan E. Schipanski

M. Francesca Cotrufo
Jasmine Dillon
Keith Paustian

ABSTRACT

THE ECOLOGY OF NATURAL CLIMATE SOLUTIONS: QUANTIFYING SOIL CARBON AND BIODIVERSITY BENEFITS

Achieving net zero greenhouse gas emission by 2050 will require simultaneous emissions reductions and carbon dioxide removal from the atmosphere. Natural climate solutions offer the most mature opportunities to remove atmospheric carbon and sequester it in woody biomass and soils but currently these options remain at low levels of adoption in the United States. To increase the uptake of these practices by growers, there needs to be greater confidence in the expected soil carbon benefits and improved understanding of potential environmental tradeoffs from these strategies across management and environmental contexts. This dissertation quantified the influence of management decisions and environmental variables on soil carbon responses under two proposed agricultural natural climate solutions: inclusion of cover crops and additions of organic amendments. The ecological and biodiversity co-benefits under these practices were also examined. Using a meta-analysis approach, the first chapter analyzed soil carbon responses to cover crop management decisions and environmental variables. Across 181 observations of 40 publications from temperate climates, inclusion of cover crops in cropping systems increased soil organic carbon stocks from 0-30 cm by twelve percent relative to a similarly managed system without cover crops. Management and environmental variables were responsible for variation in soil C responses across studies. The second chapter evaluated the application of organic amendments to improved and semi-native pastures at a semi-arid experimental site in northern Colorado. Over eight years and two applications of a high-quality organic amendment, soil organic carbon stocks as quantified by equivalent soil mass increased 0.7 Mg C ha^{-1} yr^{-1} from 0-20 cm under the organic amendment in the improved pasture relative to the control. After accounting for the additions of carbon from the two amendment applications, soil organic carbon stocks in the improved pasture increased by 0.46 Mg C ha^{-1} yr^{-1} from 0-20 cm. In contrast, there was no net change of soil carbon stocks in the semi-native pasture. The third chapter examined changes in plant and soil community composition and function after nitrogen application at the same experimental site. A single organic nitrogen addition to the improved pasture increased forage production, plant diversity, and soil microbial community composition and function. The stronger initial plant responses and the gradual change in microbial community composition and function suggests a plant-mediated response to organic nitrogen in this system, which likely impacted soil carbon cycling. Water-limited, semi-native pastures appear to be more resistant to change under one-time organic and inorganic nitrogen additions than irrigated, improved pastures. The final chapter of this dissertation compared two recommended approaches by the Food and Agriculture Organization of the United Nations for quantifying livestock production system impacts on biodiversity. The results illustrated how indicator selection and functional unit may result in discrepancies between the two methods. Together, these findings contribute to a growing body of scientific evidence in support of natural climate solutions for their climate and environmental co-benefits.

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DEDICATION

To the stewards of our natural and working lands.

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INTRODUCTION

To avoid catastrophic climate change, global anthropogenic greenhouse gas (GHG) emissions must be reduced by half in 2030 and reach net zero by 2050 (Rogelj et al. 2015, Fuss et al. 2020). The mitigation of GHG emissions alone will not be sufficient to reach to meet this target; according to the IPCC; scenarios that keep global warming at or well below 2°C will require carbon dioxide removal (CDR) from the atmosphere (O. Hoegh-Guldberg 2018, Fuss et al. 2020). Opportunities for CDR include a suite of biological, engineered, and hybrid approaches with varying capabilities to drawdown atmospheric CO_2. Carbon dioxide removal is not a substitution for fossil fuel emissions reductions, but it is a crucial strategy to help countries meet their nationally determined contributions (NDC) under the Paris Agreement (Holz et al. 2018) and it should be viewed as one component within a broader portfolio of strategies to mitigate climate change.

Natural climate solutions (NCS) are the most mature CDR strategies that exist today (National Academies of Sciences 2018). These include conservation, restoration, and improved management of natural and working lands to increase carbon (C) sequestration and mitigate CO_2 and non-CO_2 trace gas emissions (Griscom et al. 2017). Beyond climate mitigation and resiliency benefits, NCS also deliver other environmental co-benefits like increased soil health, air and water quality, and biodiversity conservation (Paustian et al. 2016, Fargione et al. 2018, Bossio et al. 2020). Globally, NCS can provide mitigation of almost 24 Gt CO_2-eq yr^{-1} or approximately 37% of the mitigation in GHG needed between now and 2030 (Griscom et al. 2017). In the United States, recent estimates indicate that the maximum mitigation potential of NCS is upwards of 21% of current net annual GHG emissions (Fargione et al. 2018). Soil C management contributes to about one-quarter of this potential with most atmospheric C removal opportunities stemming from the replenishment of depleted soil C stocks (Sanderman et al. 2017, Bossio et al. 2020).

The agriculture sector is a significant emitter of GHG (IPCC 2019) but also undoubtedly part of the solution to the climate crisis. In the United States, agriculture represents 10% of net annual GHG emissions (EPA 2019), and it is the leading contributor to N_2O emissions – a powerful, heat trapping GHG, 298 times more potent that CO_2 over a 100-year time horizon – and agriculture is the second largest contributor to CH_4 emissions, 28 times more potent than CO_2 (IPCC 2014). These trace gas emissions stem primarily from soil nutrient management and enteric methane production from livestock. Through improved soils management, cropland and grazing lands can act as a significant C sink (Paustian et al. 2019) offsetting a portion of these emissions.

Cropland and grazing land soil strategies to rebuild soil C stocks include a variety of crop, nutrient, and livestock best management practices (BMPs). In row and specialty crop production, cover cropping increases crop residue production and retention, enhancing plant C soil inputs critical to the formation of stable organic matter (Cotrufo et al. 2013, Haddix et al. 2016, Lavallee et al. 2018). Cover crops also provide additional ecological benefits by breaking pest cycles, improving soil fertility, and reducing nutrient losses. In the long-term, these ecosystem services can improve agroecosystem resilience by decreasing reliance on external nutrient inputs and through additional yield and economic returns (Schipanski et al. 2014).

Considerable attention over the past several years has centered on improved nitrogen (N) BMPs through the application of organic amendments to enhance grassland productivity and soil C stocks. In managed grasslands, N management can increase soil C sequestration upwards of 10% relative to unamended pastures corresponding to increased soil C stocks of 0.82 Mg C ha^{-1} yr^{-1} (Conant et al. 2017). In an annual California grassland, a composted organic amendment significantly increased net ecosystem C storage over three years and increased soil C and N in labile and protected soil organic matter (SOM) pools (Ryals and Silver 2013, Ryals et al. 2014). Similarly, application of composted biosolids to a semi-arid rangeland in Colorado increased total soil C with effects persisting 13 to 14 years after initial application (Ippolito et al. 2010). The application of organic matter to grassland and rangeland ecosystems also improves soil water holding capacity, runoff, and forage production and quality (Gravuer et al. 2019).

The growing global attention on agricultural soil BMPs to deliver climate benefits (Amelung et al. 2020) demands that researchers and decision-makers understand the variability in soil C responses within different management and environmental contexts. Previous research on cover cropping and organic amendments in cropland and grazing systems indicates that these practices can result in both climate benefits and tradeoffs (Basche et al. 2014, Poeplau and Don 2015, Basche et al. 2016, Gravuer et al. 2019). To better understand the impact of agricultural management decisions on GHG emissions and C cycling at the farm-scale and beyond, we need to quantify these responses and translate these findings for agricultural decision-support tools.

Other environmental benefits and tradeoffs must be evaluated when considering wider adoption of agricultural soil BMPs for CDR (Honegger et al. 2020). While the acceptability of tradeoffs will be determined by local and regional stakeholders, this information needs to be quantified and integrated into decision-making frameworks to support more holistic evaluations of climate and environmental impacts from agricultural production systems. Crop and livestock production are key drivers of biodiversity loss via land transformation and land occupation. Biodiversity is underrepresented in life cycle impact assessments (LCIA) of agricultural production (McClelland et al. 2018) because of data availability challenges and lack of scientific consensus for quantifying impacts in a life cycle framework.

The first two chapters of this dissertation will examine the influence of agricultural management and environmental constraints on soil C responses to NCS. In the third chapter, consideration will be given to the impacts of soil management on ecosystem services, including provisioning and supporting services, and consequences for biodiversity. The final chapter will present a novel method for integrating recent guidelines for biodiversity impact assessment in livestock production systems.

CHAPTER 1: MANAGEMENT OF COVER CROPS IN TEMPERATE CLIMATES INFLUENCES SOIL ORGANIC CARBON STOCKS – A META-ANALYSIS[1]

1. Summary

Increasing the quantity and quality of plant biomass production in space and time can improve the capacity of agroecosystems to capture and store atmospheric carbon (C) in the soil. Cover cropping is a key practice to increase system net primary productivity (NPP) and increase the quantity of high-quality plant residues available for integration into soil organic matter (SOM). Cover crop management and local environmental conditions, however, influence the magnitude of soil C stock change. Here, we used a comprehensive meta-analysis approach to quantify the effect of cover crops on soil C stocks from the 0-30 cm soil depth in temperate climates and to identify key management and ecological factors that impact variation in this response. A total of 40 publications with 181 observations were included in the meta-analysis representing six countries across three different continents. Overall, cover crops had a strong positive effect on soil C stocks ($p < 0.0001$) leading to a 12% increase, averaging 1.11 Mg C ha^{-1} more soil C relative to a no cover crop control. The strongest predictors of SOC response to cover cropping were planting and termination date (i.e., growing window), annual cover crop biomass production, and soil clay content. Cover crops planted as continuous cover or autumn planted and terminated led to 20-30% greater total soil C stocks relative to other cover crop growing windows. Likewise, high annual cover crop biomass production (> 7 Mg ha^{-1} yr^{-1}) resulted in 30% higher total soil C stocks than lower levels of biomass production. Managing for greater

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[1] McClelland, S. C., et al. (2020). "Management of cover crops in temperate climates influences soil organic carbon stocks - A meta-analysis." Ecological Applications. doi:10.1002/eap.2278

NPP by improving synchronization in cover crop growing windows and climate will enhance the capacity of this practice to drawdown carbon dioxide (CO_2) from the atmosphere across agroecosystems. The integration of growing window (potentially as a proxy for biomass growth), climate, and soil factors in decision-support tools are relevant for improving the quantification of soil C stock change under cover crops, particularly with the expansion of terrestrial soil C markets.

2. Introduction

The capacity of agroecosystems to capture and store atmospheric carbon dioxide (CO_2) depends on the balance between increasing carbon (C) inputs and reducing C losses. Land managers can increase system net primary productivity (NPP) and carbon sequestration of agricultural lands through shifts in plant diversity, often achieved through diversified crop rotations (McDaniel et al. 2014, Rosenzweig et al. 2018). Diverse species assemblages enhance spatial and temporal plant diversity, and the 'overyielding' of these assemblages relative to monoculture stands increases NPP (Tilman et al. 2001), and helps to sustain soil functions (McDaniel et al. 2014). The management of plant diversity in time and space within agroecosystems was highlighted in a recent National Academies of Sciences report that included diverse crop rotations and cover crops as key potential climate change negative emissions technologies for the agriculture, forestry, and other land use (AFOLU) sector because of their potential for widespread adoption, demonstrated increases in soil C, and ecosystem co-benefits (National Academies of Sciences 2018). Cover crops are a practical and cost-effective conservation agriculture practice that demonstrate a mitigation potential of 150 to almost 500 million tCO_2-eq in the U.S. as part of land-based negative emissions technologies under carbon prices ranging from 15 to 50 USD tCO_2-eq (Baker et al. 2010, Jackson and Baker 2010).

Cover crops can be planted in conjunction with the main cash crop (intercropping for enhanced spatial diversity) or grown during fallow periods between cash crops to enhance temporal diversity. Cover crops effectively represent an intensification of the agroecosystem, increasing NPP as the biomass produced from these crops is frequently unharvested, and the residues are left to decompose in the field as green manure. The quantity and quality of cover crop residues directly influences soil organic matter dynamics, nitrogen (N) supply, and other ecosystem services. By reducing erosion due to bare fallow, altering rooting depth distributions, and providing N rich plant residues (i.e., legumes), more organic matter inputs are available for decomposition and stabilization as soil organic matter (SOM) (Cotrufo et al. 2013, Cotrufo et al. 2015, Liang et al. 2017). It is estimated that improved cropping rotations can sequester 0.1-0.3 Mg C ha^{-1} yr^{-1} and approximately 0.3 Mg C ha^{-1} yr^{-1} under cover crops (Poeplau and Don 2015, National Academies of Sciences 2018).

While there is strong evidence that cover crops increase soil C (McDaniel et al. 2014, Poeplau and Don 2015, Abdalla et al. 2019), uncertainty surrounds the magnitude by which cover crop management and environmental conditions drive soil C response in cover cropping systems. Abiotic factors like climate and soil texture exert considerable influence on the NPP potential and the fate of these residues due to interactions with soil minerals for soil C stabilization (Wiesmeier et al. 2019). Differences in cover crop species assemblage and incorporation method can influence soil C storage, greenhouse gas (GHG) emissions, and performance of the cash crop (Schipanski et al. 2014). These sources of variability are a key limitation to including cover crop practices in policy and markets for negative emissions technologies due to the high degree of uncertainty in the outcomes.

Reducing uncertainty requires an improved understanding of the ecological and management factors that drive SOC response to cover crops. Management factors affect the quantity, quality, and decomposition of cover crop residues. The selection of individual cover crop species or functional types, and mixtures of these, results in variation of residue quantity and quality. Smith et al. (2014) found that diverse cover crop species assemblages yielded more biomass over component monoculture species in an oat (*Avena sativa* L.) rotation in the northeastern U.S. Similarly, in a study investigating the environmental advantages of bicultures over monocultures, Kramberger et al. (2013) found that bicultures supported greater biomass C and N accumulation, which are both important factors for increasing soil C. However, other studies have found that mixtures are equally, but not more productive as the most productive monoculture species (Finney et al. 2016, Wendling et al. 2019). In addition, the length of the cover crop growing window (i.e., planting and termination dates) can influence potential for NPP accumulation and design of cover crop species assemblages (Murrell et al. 2017).

Cover crop residue quality (i.e., C:N ratio) differs by species assemblage and functional type impacting the formation of stable soil C (Cotrufo et al. 2013). Legume cover crops like hairy vetch (*Vicia villosa* Roth) produce low C:N residues, while common non-legume cover crop species like cereal rye (*Secale cereal* L.), oats (*Avena sativa* L.), and annual ryegrass (*Lolium multiflorum* L.) have a higher biomass C:N (Kramberger et al. 2013, Finney et al. 2016). The quality and quantity of litter inputs can influence soil biota dynamics and, in turn, the efficiency with which crop residues are transformed into mineral-associated soil organic matter pools (Cotrufo et al. 2013, Kallenbach et al. 2019). For example, labile or low C:N residues are preferentially incorporated into microbial biomass, and the turnover of soil microbial biomass (i.e., necromass) and subsequent interaction with the soil matrix through physical protection and chemical bonding with soil minerals forms stable soil C (Cotrufo et al. 2013, Liang et al. 2017).

Residue management practices also influence the potential for cover crops to enhance soil C stocks. Residue incorporation through soil tillage stimulates soil C losses by disrupting soil aggregates and increasing soil microbial access to previously protected C pools (Six et al. 2000). In combination with increased residue retention no-till agriculture may improve soil health and the size of soil C stocks relative to conventional tillage agroecosystems (Hobbs et al. 2008). Other practices like seeding rate and timing of planting directly impact the amount and quality of residue returned to the soil at the end of the cover crop growing season (Clark et al. 1994, Brennan and Boyd 2012). In addition, while cover crops are rarely fertilized, background soil fertility levels can also influence cover crop residue quantity and quality (White et al. 2017).

Individual field experiments are insufficient for capturing the range of ecological and management drivers of SOC response to cover crops. Meta-analyses can aggregate and quantify the relative importance of different factors on response variables across a wide range of scientific studies. Previous meta-analyses have demonstrated that crop diversity increases soil microbial biomass and SOM (McDaniel et al. 2014) and crop rotation diversity increases SOC concentrations (King and Blesh 2018). Other data syntheses used regression modeling approaches to estimate cover crop contributions to soil C sequestration (Poeplau and Don 2015) and net GHG balance (Abdalla et al. 2019). While linear regression models are valuable for analyzing the relationships between variables, they make different assumptions about sampling variances relative to meta-regression techniques (i.e., the evaluation of individual moderator impacts on the overall effect size) (Huizenga et al. 2011). Linear regression models generally do not account for within study sources of variance such that applying regression to multi-study analyses can lead to an underestimation of standard errors of the regression coefficients resulting in incorrect conclusions about the importance of certain covariates (Thompson and Sharp 1999).

These previous publications demonstrated a strong positive response of SOC to cover crops (Poeplau and Don 2015, Abdalla et al. 2019) and the inclusion of cover crops in crop rotations (King and Blesh 2018) across a range of climates. Using linear regression approaches, cover crops did not increase SOC within temperate environments relative to tropical environments and cover crop functional type did not influence SOC stocks (Poeplau and Don 2015). We build upon these previous studies to apply a meta-analysis approach to specifically focus on other important cover crop management variables such as planting and termination date, the frequency of cover crops in a crop rotation, and cover crop species diversity effects on SOC within temperate climates. We focus on temperate climates due to greater consistency in management practices within these systems, which allowed us to analyze how specific cover crop management practices may drive differences in agroecosystem SOC dynamics. This management system information is essential for land managers interested in increasing SOC under cover crops to effectively implement this practice. Furthermore, many more cover crop publications have become available over the past several years, and there is a need to update and expand on previous findings using standard meta-analysis techniques in ecology (Harrison 2011). Our analysis of 40 studies included 15 additional publications not included in the recent analyses by Poeplau and Don (2015) and Abdalla et al. (2019), and our dataset only included six and five overlapping publications, respectively.

The purpose of our study was to quantify the effect of different management, climate, and soil factors on SOC response in cover crop systems. We used a comprehensive meta-analysis approach to address the following objectives: (1) estimate cover crop effects on SOC stocks (0-30 cm) in temperate climates; (2) estimate the relative importance of different cover crop management decisions and their interactions on SOC response; and (3) identify important environmental controls on SOC response under cover crops. We discuss the implications of our findings for cover crops as a negative emissions technology and how the integration of key management factors into decision support tools could improve confidence in SOC response estimates.

3. Methods

3.1 Literature search

We searched the Web of Science and CAB databases for articles containing "cover crop" or "green manure" or "catch crop" AND "greenhouse gas" or "carbon" or "trace gas" or "nitrous oxide" or "methane" or "carbon dioxide." The search in both databases included all papers published until July 06, 2017 (inclusive). After duplication removal, our search yielded 4,280 publications. However, our literature search did not produce a robust dataset for the evaluation of soil trace gas emissions response to cover crops. Thus, we only focus on the publications pertaining to soil C.

3.2 Systematic review

For this meta-analysis, we defined cover crops as plants not intended for harvest but grown for the benefit of enhancing agroecosystem quality. Catch crops and green manure studies were considered as cover crops if they met this definition criteria. We screened publications following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (Moher et al. 2009). After excluding results published in any language other than English and those that only included an abstract, we categorized publications as: (1) field-based experiments that contained a cover crop treatment, or (2) outside the scope of the review. Publications included in the first category were from peer-reviewed journals that contained original data from field experiments. These publications included a cover crop (or catch crop or green manure) treatment. Studies in the first category also met the following criteria:

1. The cover crop was not harvested and was grown during fallow periods between cash crops, or, in the case of orchard and vineyard systems, in the rows between trees/vines.
2. The control treatment was i) a treatment with no cover crop (fallow), or ii) a management system with no cover crop
3. All other management practices between the treatment and control were similar, e.g., tillage, cash crop rotation, soil amendments. However, there were a few cases where tillage was done in the control and not the experimental treatments to maintain bare ground. We included these studies in our analyses.
4. The experiment occurred in a country within temperate latitude zones, i.e., latitudes from 23.5° to 66.5° north and south of the equator
5. The response variable or variables measured was soil organic C or soil organic matter.

6. Means and sample size were reported for each treatment.
7. The publication included enough management details and data to be recorded.

We excluded studies that did not meet the criteria above. Other studies in the second category included publication types such as book chapters and fact sheets. We also omitted publications where the cover crop was grown and sampled in a lab or greenhouse including incubation, mesocosm, or pot experiments.

Given the criteria above, 40 of these publications were included in our final analysis (k = 181 observations). The analysis included publications from six countries and three continents (Figure 1). Most publications (n = 27) were from the United States, followed by Italy (n = 4), Denmark (n = 3), and others in North America, Europe and Oceania (n = 6). A complete list of the publications included in our analysis can be found in Appendix 1 Table 1.

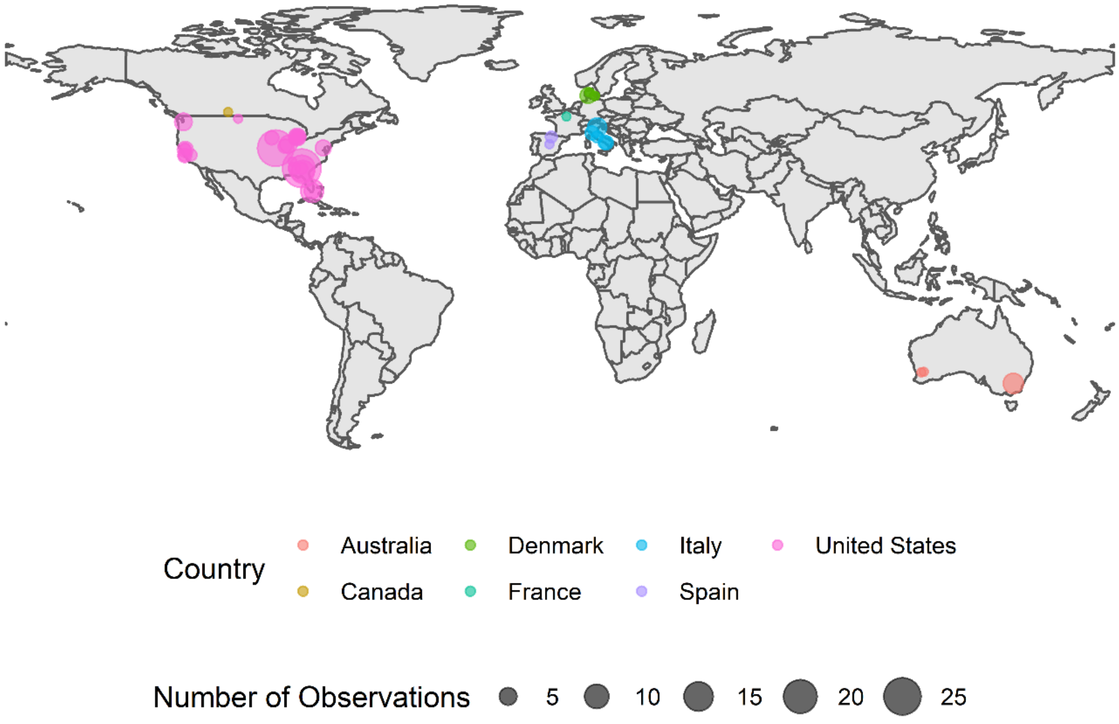


Figure 1. Map of publications included in our analysis of soil organic carbon (SOC) response to cover crop management. The analysis included six countries from three continents. The number of observations from each experimental site are indicated by the size of each circle on the map.

3.3 Data management and collection

For each publication we recorded environmental and management data expected to explain variation in SOC response (Table 1). If data were unavailable directly from the publication, we derived this information from other published databases or contacted authors for missing data (see below). When data were not provided in tables, we used the software program DataThief (DataThief III, v. 1.7; Tummers 2006) to extract values from figures.

Table 1. Environmental and management data included as covariates to analyze the variability of cover crop effects on soil organic carbon (SOC) response.

Category	Parameter	Factor levels or range	No. of observations
Climate	Agro-ecological Zone (AEZ)	Temperate – cool, subtropical –cool, subtropical – warm-moderately cool, tropical – warm	181
Cropping system	Cash crop rotation	Continuous annual, multi-crop annual, perennial	172
	Management system	Conventional, organic	181
	Tillage	Conventional, reduced, no-tillage	181
Cover crop management	Biomass (Mg ha^{-1} yr^{-1})	< 3 Mg ha^{-1}, 3-7 Mg ha^{-1}, > 7 Mg ha^{-1}	100
	C:N	≤ 25, > 25	70
	Functional type	Legume, grass, mixture, other	181
	Diversity	Monoculture, biculture, polyculture	181
	Growing window	Fall, overwinter, summer, continuous cover	170
	Termination method	Incorporated, non-incorporated	165
Soil characteristics	Percent Sand	< 50%, ≥ 50%	163
	Percent Clay	< 20%, ≥ 20%	163
Other	Time since introduction	1-2 years, 3-4 years, 5-9 years, > 10 years	181

Cover crop frequency	Annual, other	181
Original sampling depth	< 5 cm, 0-10 cm, 0-15 cm, 0-20 cm, 0-25 cm, 0-30 cm, > 30 cm	181

3.3.1 Climate

We collected the historical (i.e., 30-year mean) mean annual precipitation (MAP) and mean annual temperature (MAT) when reported in the publication. If these data were unavailable, we estimated historical MAP and MAT data from the PRISM Climate Data (PRISM Climate Group 2020) using site coordinates from U.S. publications. For international sites, we used the World Clim 2 dataset to derive historical MAP and MAT data at 4.5 km^2 resolution (Fick and Hijmans 2017). These data along with site coordinates were used to delineate each experimental site into a thermal zone under the FAO Agro-ecological Zone (AEZ) approach (Fischer et al. 2000) via the GAEZ database, version 3.0.1. All publications included in the analysis were from countries within the temperate zone (23.5° to 66.5° north and south of the equator). However, some experiments within these countries occurred in climates defined under FAO AEZ as subtropical or tropical.

3.3.2 Cropping system

Cropping system management data included main cash crop(s), system type, and tillage. Because of the diversity of cash crop types, we grouped them into three groups: continuous annual, multi-crop annual, or perennial. The continuous annual category included annual cash crops grown as the same monoculture crop each year (i.e., continuous corn rotation). Multi-crop annuals included a rotation of two or more cash crops, and perennial systems included vineyards and bioenergy crops. Cover crops in these systems were planted either between rows or between furrows of the perennial plants, respectively. Management system was defined as either conventional or organic. Tillage was divided into three intensity classes as defined by each publication: conventional, reduced, and no-tillage.

3.3.3 Cover crop management

The primary goal of this meta-analysis was to understand the influence of cover crop management on soil carbon response. Cover crop treatments were separated into groups of legume, non-legume, or a mixture of legumes and non-legumes. These typologies were further subdivided into functional types of grasses, legumes, mixtures (any combination of grass, legume, and brassica cover crops), or other (brassica or other functional type). Cover crops were also delineated into monoculture, biculture, or polyculture (three or more species). We treated cover crop biomass and C:N ratios as categorical variables. Cover crop biomass was divided into three groups: < 3 Mg ha^{-1} yr^{-1}, 3-7 Mg ha^{-1} yr^{-1}, > 7 Mg ha^{-1} yr^{-1}. The categories were determined by the ranges of the lower, middle, and upper quartiles of cover crop biomass data, respectively. When studies reported cover crop biomass in terms of carbon, these values were transformed to total biomass assuming a C content of 45%. Cover crop C:N was grouped as ≤ 25 or > 25 C:N. Additional cover crop management data included the cover crop growing window (summer, autumn, overwinter, or continuous cover) and termination method (incorporated or not incorporated). Growing window was defined by the planting and termination dates; summer included cover crops planted in the spring (March – June for northern latitudes, September – November for southern latitudes) and terminated in late summer or early autumn, autumn cover crops were planted in autumn (September – November for northern latitudes, March – June for southern latitudes) and winter-killed, overwinter cover crops were planted in autumn and terminated in spring, and, finally, continuous were cover crops that provided continuous cover throughout the year.

We also recorded information about the ‘time since introduction’ of the cover crop and cover crop frequency in the cropping rotation. The ‘time since introduction’ differs from experimental duration because in several studies experiments were conducted at sites with a history of cover cropping. Thus, we recorded the first year a cover crop occurred at the study site. Cover crop frequency was separated into annual and other (e.g., every other year, every four years, etc.) categories.

3.3.4 Soil characteristics

The reported values from each publication were recorded for soil texture class, bulk density, pH, and percentage of sand, silt, and clay. When these data were unavailable for U.S. publications, we used the latitudinal and longitudinal coordinates provided to locate the experimental site in the COMET-Farm™ tool (Paustian et al. 2018), which integrates the NRCS soil survey SSURGO database (Soil Survey Staff 2017) containing information about site soil texture and bulk density values. We also made efforts to contact publication authors to retrieve this data, particularly if the study occurred outside the U.S.

3.3.5 Soil carbon and soil carbon stock estimates

Soil carbon measurement frequency, type, and depth varied across the publications included in our analysis. We only reported the SOC value for the final year of the study or the last year reported. In some studies, only an average SOC value across study years was provided and we used this value. While most publications reported SOC, a few recorded values for total C or soil organic matter (SOM). We assumed given the pH values ($pH < 8$) reported by these studies that there was no inorganic C present in these soils, and, thus, the reported value could be considered as organic C (Izaurralde et al. 2001). When SOM was reported rather than SOC, we used a conversion factor of 0.58 to convert SOM values to SOC (Lunt 1931, Broadbent 1953, Howard 1965).

When SOC values were not presented as a C stock (Mg C ha^{-1}), we converted values reported as % SOC or C concentration using the following formulas from Guo and Gifford (2002):

% SOC to SOC stock (OC_{st}, Mg C ha^{-1})

$$OC_{st} = BD*OC_p*D \tag{1}$$

SOC concentration (g C kg^{-1} dry soil) to SOC stock (Mg C ha^{-1})

$$OC_{st} = BD*(OC_c/10)*D \tag{2}$$

where OC_p is soil organic C represented as a % value, OC_c is soil organic C represented as a concentration in g/kg, BD is bulk density (g soil cm^{-3}), and D is sampling depth (cm).

The reported SOC measurements in studies varied across soil depth intervals. We standardized the observed SOC measurements to a common depth of 0-30 cm to improve comparisons between studies. We followed the method outlined in Jobbágy and Jackson (2000) and described by (Abdalla et al. 2019) for normalizing SOC depth distribution:

$$SOC_{30} = ((1-\beta^{30})/(1-\beta^{d0}))*SOC_{d0} \quad (3)$$

where SOC_{30} is the value of SOC in Mg C ha^{-1} in the first 30 cm of the soil profile (Jobbágy and Jackson 2000), β is 0.9786, which is the relative rate of decrease in the SOC pool with soil depth. d_0 is the original soil depth (cm) reported in the study, and SOC_{d0} is the original SOC value. We examined the impact of using this depth normalization approach by evaluating original soil sampling depth as a moderator in our analysis.

3.4 Meta-analysis procedure

We estimated the effect size of SOC for each combination of cover crop (treatment) and no cover crop (control) within a study where the only variation across treatments was the presence or absence of a cover crop. All calculations were done using the *metafor* package (Viechtbauer 2010) in R version 3.5.2 (R Core Team 2013). The effect size was calculated as:

$$lnRR = ln\left(\frac{\overline{X_{CC}}}{\overline{X_{NCC}}}\right) \quad (4)$$

where lnRR is the natural log of the response ratio, $\overline{X_{CC}}$ is the mean value for SOC for the cover crop treatment, and $\overline{X_{NCC}}$ is the mean values of SOC for the no cover crop treatment. A natural log transformation normalizes the data when sample size is small and linearizes the response variable so that the deviations in the numerator and denominator are treated equally (Hedges et al. 1999). Because many studies also evaluated other management factors (e.g., tillage, fertilization, cover crop type), the number of observations differed among studies due to each study's experimental design. Thus, these different levels of management were also included as individual observations if the only difference between them was the presence of a cover crop.

However, the inclusion of multiple effect sizes from the same study often with the same no cover crop control introduces dependency among effect sizes. Non-independent effect sizes violate the traditional independency assumption of univariate meta-analyses (Rosenthal 1984). To account for the dependency of effect sizes, we applied a multivariate approach using the rma.mv function in the *metafor* package (Viechtbauer 2010). A three-level structure meta-analytic model accounts for three different levels of variance in the model: sampling variance of individual effect sizes, variance between effect sizes from the same study, and, finally, variance between studies (Assink and Wibbelink 2016). Models fit with this function are superior to linear mixed-effect model approaches because traditional linear mixed-effect models mix additive and multiplicative variance components, whereas meta-regression models use additive variance components (Thompson and Sharp 1999, Viechtbauer 2010). We also applied the Knapp and Hartung (2003) adjustment to each of the models. This approach is more conservative in its computation of p-values and confidence intervals because it evaluates models of individual coefficients based on the *t* distribution and multiple moderating variables based on the *F* distribution (Assink and Wibbelink 2016).

Individual effect size was weighted by the inverse of the sampling variance to account for differences in the level of precision between studies (Philibert et al. 2012, Hedges and Olkin

2014). Variance data were often not reported or could not be extracted from figures due to overlapping error bars. Rather than exclude these publications from analysis (16 publications, 119 observations), which can introduce bias into the meta-analysis, we used the method described in Wiebe et al. (2006) for imputing standard error data via arithmetic mean for studies that did not report variance estimates:

$$SD_I = \sqrt{\frac{\sum_{i=1}^{N} SD_i^2 (n_i - 1)}{\sum_{i=1}^{N} (n_i - 1)}} \tag{5}$$

where SD_I is the imputed standard deviation, and SD_i is the standard deviation and n_i is the sample size of the i^{th} study. This variant of the arithmetic mean calculation is weighted for sample size.

To evaluate the validity of meta-analysis assumptions, we examined the homogeneity of lnRR values across the dataset (Raudenbush and Bryk 2002, Hedges and Olkin 2014). The homogeneity test examines the variability in effect sizes across studies to test the assumption that the true effect of cover crops is homogenous across the observations. We evaluated homogeneity of effect sizes for within-study variance and between-study variance by performing separate log likelihood-ratio tests for each variance level (Assink and Wibbelink 2016). A significant test indicates that the within or between-study variance is heterogenous, and other moderators or controls may explain the variation in effect size (Basche et al. 2014).

Studies were weighted using an inverse weight factor (W_i), which assigns a greater weight to studies with smaller variance. This weighting scheme is a method to adjust for the assumption that the variances are equal across studies included in the meta-analysis (Hedges et al. 1999, Basche et al. 2014). The calculation of the inverse weighting factor is as follows:

$$W_i = \frac{1}{v_i} \tag{6}$$

where v_i is the standard deviation for the i^{th} study. Our homogeneity tests for within-study and between-study variance indicated significant variation across effect sizes. We used a mixed effects meta-regression with observation and publication as random effects and one of the categorical variables identified in Table 1 as a fixed effect to examine the impact of moderators on the overall lnRR. When a moderator contained fewer observations that the full dataset, we recalculated the overall lnRR in order to examine the impact of a given moderator. This resulted in slightly different overall lnRR and number of observations, which limits the statistical power of these models because of the reduced degrees of freedom (Del Re 2015).

To address this limitation, we fit a full model with each of the moderators identified as significant from the individual model analysis after examining each of the 13 moderators in Table 1. This simultaneous evaluation is a more robust approach to determine the true moderating effects of each of the moderators on SOC response under cover crops (Hox 2010, Assink and Wibbelink 2016). The full model resulted in a non-significant test for residual heterogeneity across the dataset ($n = 88$, $p = 0.980$) indicating that these moderators described the variation in SOC response across publications.

Among the individual moderators analyzed, growing window and biomass production had the largest effects on overall lnRR. We conducted a second series of meta-regressions testing the interaction between (1) growing window and other moderators, and (2) biomass and other moderators, individually, except for ‘time since introduction’ and cover crop frequency. For each of the moderators in the significant individual or interaction models, we applied an omnibus test of parameters to test for differences among the levels of a given moderator (e.g., incorporation versus no incorporation) using the general linear hypothesis test or glht function from the *multcomp* package (Hothorn et al. 2016). To determine the response of SOC over time, we used a linear mixed-effect regression model with change in SOC stock (difference between treatment and control) as the dependent variable, and ‘time since introduction’ as a single fixed effect and publication number as a random effect. The model was weighted by sample size and fit using maximum-likelihood in the *lme4* package in R (Bates et al. 2014).

We assessed a histogram of all lnRR to test for evidence of publication bias (Gurevitch et al. 2001, Basche and DeLonge 2019). Publication bias occurs when studies with minimal or non-significant effect sizes go unpublished, and this results in a non-normal distribution of lnRR. We applied the Jackknife approach (Philibert et al. 2012, Basche et al. 2014, Basche and DeLonge 2019) for our sensitivity analysis to determine the sensitivity of overall lnRR to individual studies. Under the Jackknife approach, one study is removed from the data set and the overall lnRR is recalculated to examine how lnRR is impacted by the absence of a given study. We also conducted a sensitivity analysis of our standard deviation imputation on the overall lnRR by testing the range of observed variances (0.1 to 10 Mg C ha^{-1}) from our dataset. These new overall lnRR estimates and their 95% confidence intervals were then compared to our original estimates, and we did not find an indication of bias based on our method of imputation (Appendix 1 Figure 2).

4. Results

4.1 Overall effect of cover crops on soil organic carbon

Across the entire dataset, cover crops demonstrated a strong, positive effect on SOC stock from 0-30 cm (Figure 2a; $p < 0.0001$). The average relative increase of SOC stocks under cover crops was 12% (95% CI: 7%, 16%), which is equal to an average increase in SOC under cover crops of 1.11 Mg C ha^{-1} (95% CI: 1.07, 1.16). Twenty-one percent of the dataset exhibited no change or a decrease in SOC under cover crops (negative or zero lnRR), while most of the dataset (79%) demonstrated positive lnRR indicating an increase in SOC under cover crops (Figure 2a). The approximately normal distribution of all lnRR in the histogram supported a lack of publication bias in the meta-analysis (Figure 2b). Observations across the different publications were neither skewed towards very negative nor towards very positive effect sizes.

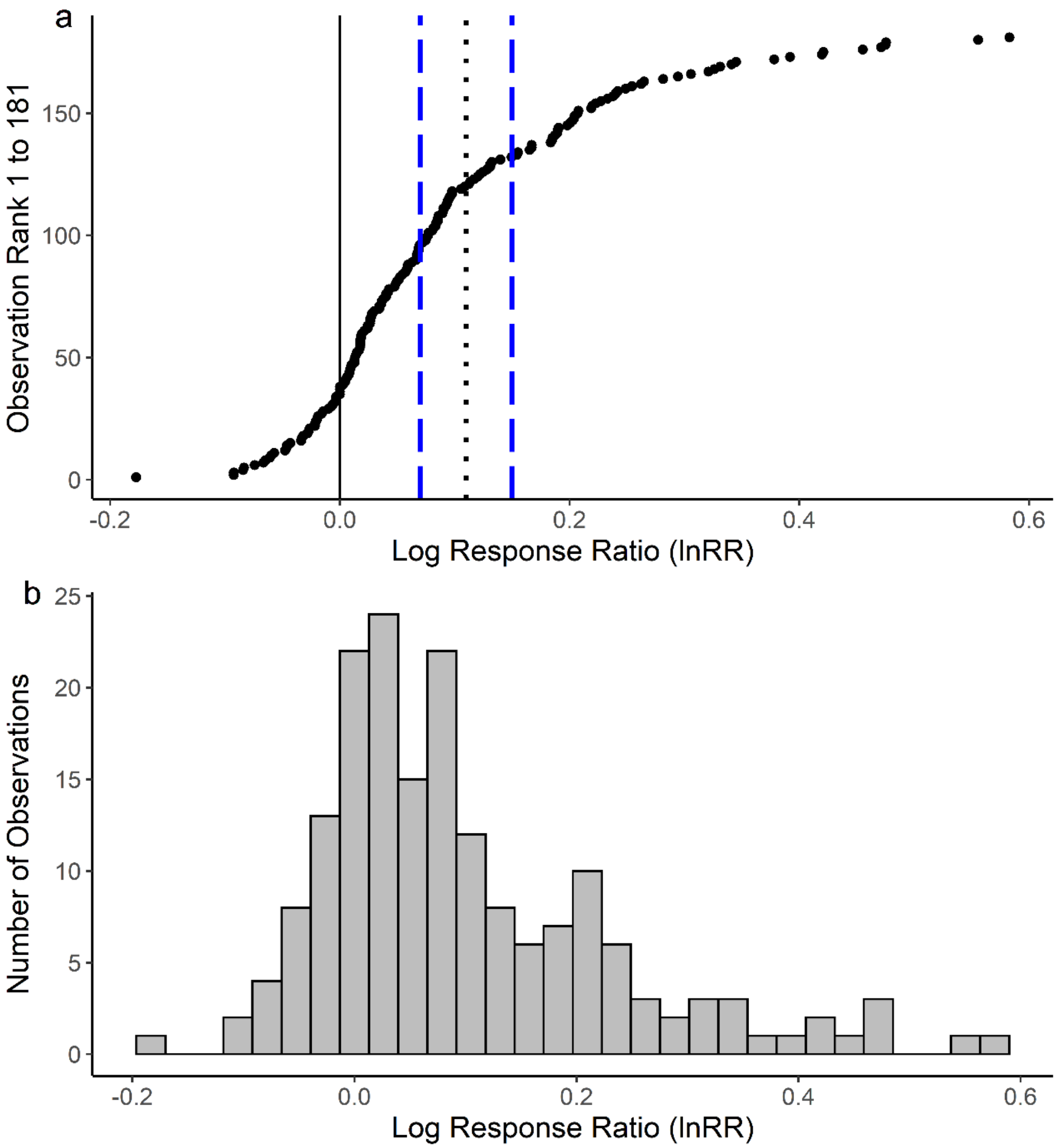


Figure 2. The (a) natural log of response ratios (lnRR) and (b) publication bias across the 181 observations in the dataset. A positive lnRR indicates that cover crops increased soil organic carbon (SOC) to a depth of 0-30 cm relative to a no cover crop control. The dotted black line represents the average lnRR across all studies, and the blue dashed lines represent the lower and upper 95% confidence interval of the average lnRR. A normally distributed histogram indicates a lack of publication bias in the dataset.

The tests of homogeneity for the overall dataset for within-study variance and between-study variance were both significant ($p < 0.0001$). These significant tests indicated a heterogeneous effect of cover crops across the dataset. Thus, other moderating factors explained the effect of cover crops on SOC response (Table 2).

Table 2. The effect of cover crops, cropping system, environmental factors, and 'time since introduction' on SOC response under cover cropping.

Category	Moderator	n[1]	DF[2]	p-value[3]	Levels	k[4]	lnRR[5]
Cover crop	Biomass	105	2	**0.009**	< 3 Mg ha^{-1}	17	0.11b
					3-7 Mg ha^{-1}	72	0.10b
					> 7 Mg ha^{-1}	16	0.26a
	C:N	70	1	0.972	≤ 25	49	0.08a
					> 26	21	0.08a
	Functional type	181	4	0.476	Grass	46	0.13a
					Legume	57	0.10a
					Mixture	65	0.11a
					Other	13	0.08a
	Diversity	181	2	0.957	Monoculture	132	0.11a
					Biculture	42	0.10a
					Polyculture	7	0.12a
	Growing window	170	3	**0.003**	Overwinter	120	0.08b
					Fall	11	0.24a
					Summer	25	0.07b
					Continuous cover	14	0.31a
	Termination method	165	1	0.079	Incorporated	92	0.08a
					Not incorporated	73	0.14a
Cropping system	Cash crop rotation	172	2	**0.036**	Continuous annual	93	0.08b
					Multi-crop annual	72	0.11b
					Perennial	7	0.30a
	Management	181	1	0.752	Conventional	140	0.11a
					Organic	41	0.10a
	Tillage	181	2	**0.051**	Conventional	94	0.09b
					Reduced	22	0.09ab
					No-till	65	0.15a
Climate & soil	AEZ	181	3	**0.030**	Temperate-cool	77	0.06b
					Subtropical-cool	7	0.24a
					Subtropical-warm	88	0.15a
					Tropical-warm	9	0.16ab
	Percent sand	163	1	0.976	< 50%	84	0.11a
					≥ 50%	79	0.11a
	Percent clay	163	1	**0.009**	< 20%	131	0.08b
					≥ 20%	32	0.19a
Other	Time since introduction	181	3	0.778	1-2 years	48	0.09a
					3-4 years	48	0.15a
					5-9 years	58	0.09a
					> 10 years	27	0.11a
	Cover crop frequency	181	1	0.411	Annual	163	0.12a
					Other	18	0.07a
	Original sampling depth	181	6	0.231	< 5 cm	14	0.19a
					0-10 cm	22	0.25a
					0-15 cm	38	0.09a
					0-20 cm	18	0.05a
					0-25 cm	18	0.08a
					0-30 cm	56	0.09a

	> 30 cm	15	0.07a

[1] n is the number of observations for each moderator
[2] DF are the degrees of freedom
[3] The p-values represent the significance level of the interaction terms in each mixed-effects meta-regression. Bolded p-values indicate a significant effect ($p \leq 0.05$)
[4] k is the number of observations for each level of a moderator
[5] Log response ratios (lnRR) followed by different letters indicate significant difference at $p \leq 0.05$

4.2 Controls on soil organic carbon response to cover crops

4.2.1 Individual moderators

Growing window and cover crop biomass production were strong predictors of SOC response to cover cropping (Table 2; Figure 3). The other cover crop management factors – C:N ratio, functional type, diversity, and termination method – did not explain the variance in the overall SOC response. However, termination method demonstrated a subtle effect on SOC response ($p = 0.08$), but the variation between the different methods was too great to detect a consistent response (Figure 3b).

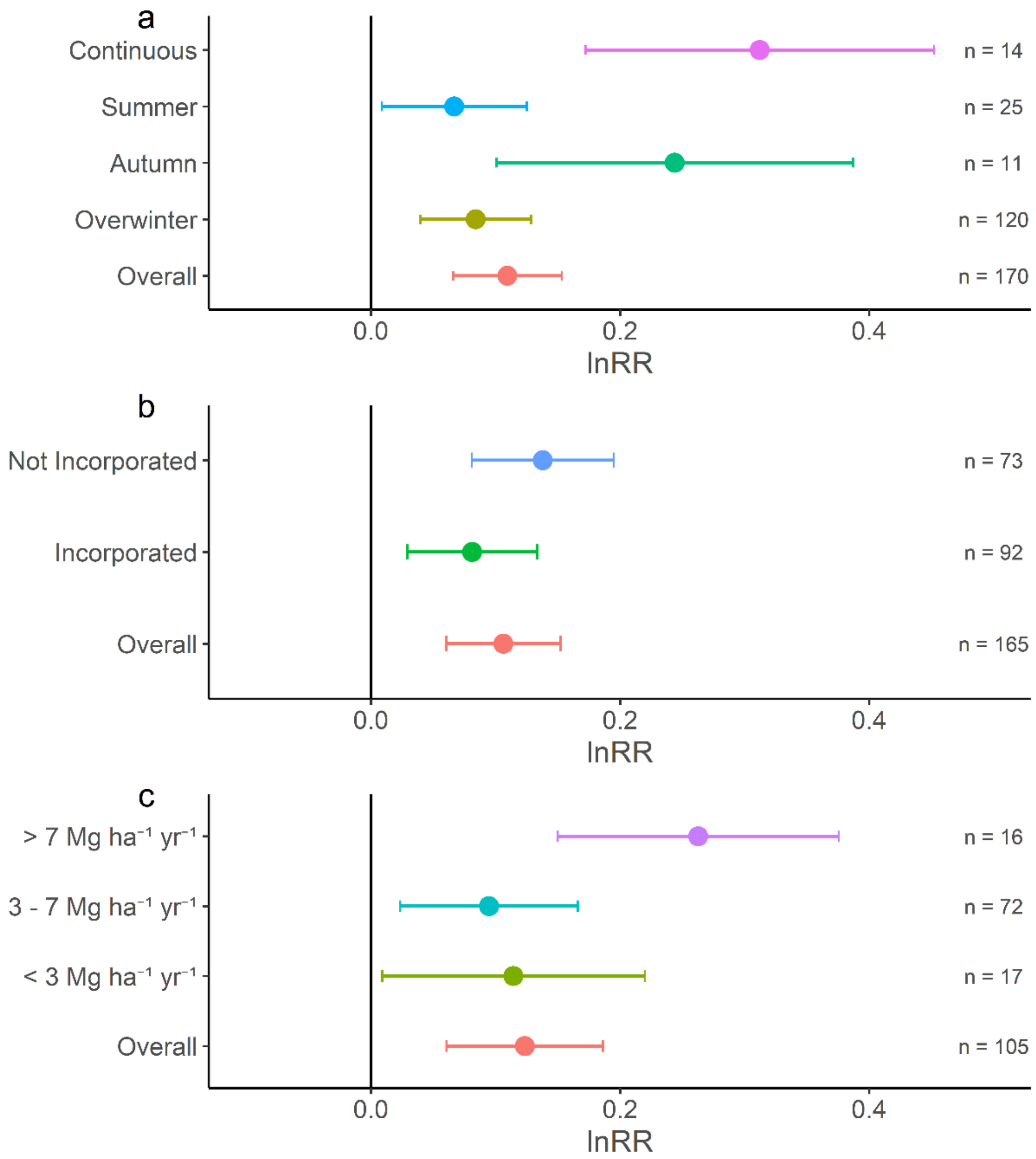


Figure 3. The effect of cover crop (a) growing window, (b) termination method, and (c) biomass production on the lnRR of soil carbon under cover crop treatments. The overall mean response ratio (lnRR) ± 95% CI under each moderator is given followed by the number of observations (n). Confidence intervals not overlapping zero were considered significant.

Cover crop planting and termination date, i.e., growing window, was the most significant predictor of SOC response across all the individual moderators ($p = 0.003$; Table 2). Continuous cover and autumn planted and terminated cover crops exhibited the highest influence on SOC response (36% and 27%, respectively) relative to overwintering (8%) and summer cover crops (7%). However, there were relatively few observations for all growing window categories except for overwinter, and, thus, these results should be interpreted with some caution.

Cover crop biomass production also had a significant influence on SOC response (p = 0.009). While all levels of cover crop biomass increased SOC, SOC response under high cover crop biomass production (> 7 Mg ha^{-1} yr^{-1}) was 30% (Figure 3c, Table 2), which was almost 20% greater than low (12%) or moderate (11%) cover crop biomass production. There were no differences in SOC response between the low and moderate cover crop biomass production categories.

Among the cropping system moderators, cash crop rotation (p = 0.035) and tillage (p = 0.051) predicted SOC response under cover cropping. Despite a small number of observations, perennial crop rotations (35% increase in SOC) had 27% and 23% higher SOC response to cover cropping relative to both continuous annual and multi-crop annual systems, respectively (Table 2). There was no difference between either of the annual cash crop rotations. Across the different levels of tillage, systems managed with no-till had higher mean percent change than conventional tillage (16% versus 9%, respectively), but there were no differences between no-till and reduced tillage or conventional tillage and reduced tillage (Table 2). Farm system type (conventional vs. organic) and ‘time since introduction’ (Figure 4) did not explain a significant proportion of the variation in SOC response.

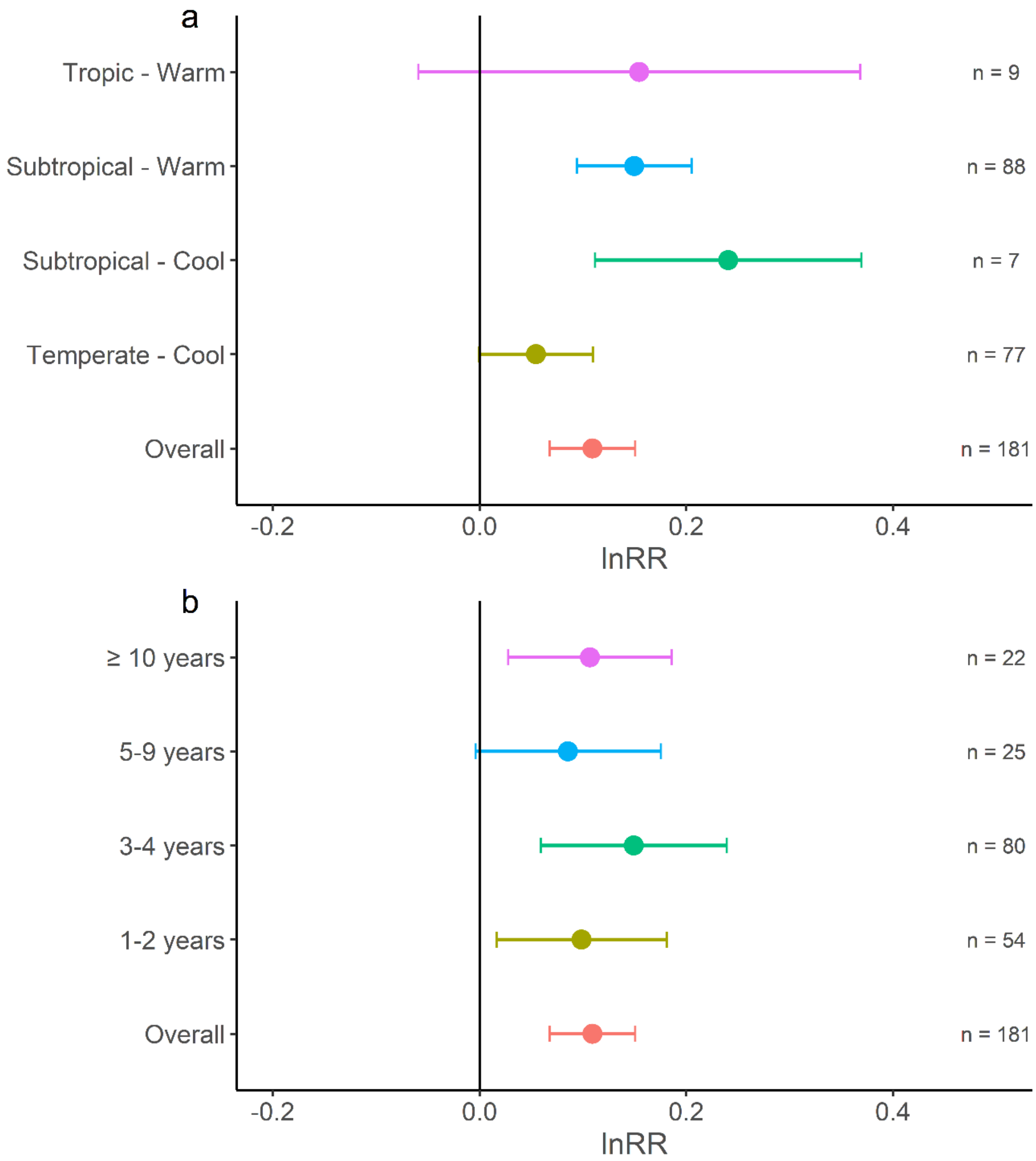


Figure 4. The effect of (a) agroecological zone, and (b) 'time since introduction' on the lnRR of soil carbon under cover crop treatments. The overall mean response ratio (lnRR) ± 95% CI under each moderator is given followed by the number of observations (n). Confidence intervals not overlapping zero were considered significant.

Several environmental moderators were also good predictors of SOC response. Higher clay content soils (≥ 20% clay) demonstrated greater SOC response relative to lower (< 20%) clay content soils ($p = 0.009$) (Table 2). Across the AEZ moderator ($p = 0.030$), temperate – cool climates exhibited between 10% and 21% lower mean SOC percent change relative to both levels of subtropical climate, but there were no differences across any of the other levels of the AEZ moderator (Table 2; Figure 4a). But, like some of the other moderators, these results should be interpreted cautiously given the small sample size of observations in subtropical – cool and tropical – warm levels of AEZ.

The individual moderators that were strong predictors of SOC response ($p < 0.05$), including growing window, cover crop biomass, cash crop rotation, tillage, AEZ, and percent clay, were integrated into a full model. Together, these moderators explained 69% of the total variation in SOC response ($p<0.0001$). In the full model, growing window, cover crop biomass, and clay texture were significant predictors of SOC response to cover cropping. However, there were important interactions between some of these predictors.

4.2.2 Interactions between moderators on soil organic carbon

Because cover crop growing window was the best predictor in the individual moderator analyses ($p = 0.003$), we tested interaction models including growing window plus one of the other individual moderators in Table 1. While cover crop species diversity and cover crop functional type were not good predictors in the individual models (Table 2), we found significant interactions between growing window and these moderators (Table 3; Figure 5). Grasses and mixtures had strong positive effects on SOC when grown as a continuous cover rather than as an overwinter or summer cover crop, respectively (Table 3; Figure 5a). The effect of legume cover crops on SOC response was greater under fall, summer, and continuous cover growing windows relative to overwinter. The response of cover crops classified as “other” was highly variable and we did not find a consistent effect on SOC across different growing windows. The effect of cover crop species diversity also varied across the levels of growing window. For example, monoculture cover crops had a greater positive effect on SOC under the autumn and continuous cover growing windows relative to overwinter and summer and this effect was similar for species grown as bicultures (Table 3; Figure 5b).

Table 3. The interaction between growing window and other cover crop, cropping system, and environmental parameters on SOC response under cover cropping

	Growing Window x Moderator			
Category	Moderator	n[1]	DF[2]	p-value[3]
Cover crop	Biomass	100	8	0.155
	C:N	70	3	0.838
	Functional type	170	12	**0.041**
	Diversity	170	7	**< 0.001**
	Termination method	158	5	0.551
Cropping system	Cash crop rotation	161	8	0.265
	Management	170	6	**0.048**
	Tillage	170	7	0.177
Climate & soil	AEZ	170	10	0.647
	Percent sand	153	6	0.066
	Percent clay	153	6	**0.030**

[1] n is the number of observations for each moderator

[2] DF are the degrees of freedom

[3] The p-values represent the significance level of the interaction terms in each mixed-effects meta-regression. Bolded p-values indicate a significant effect ($p \leq 0.05$)

The only significant interaction between biomass production and another moderator in the biomass interaction models occurred between percent sand (Table 4; Figure 5c). Soil organic carbon stocks increased as annual cover biomass production increased under soils with less than 50% sand content.

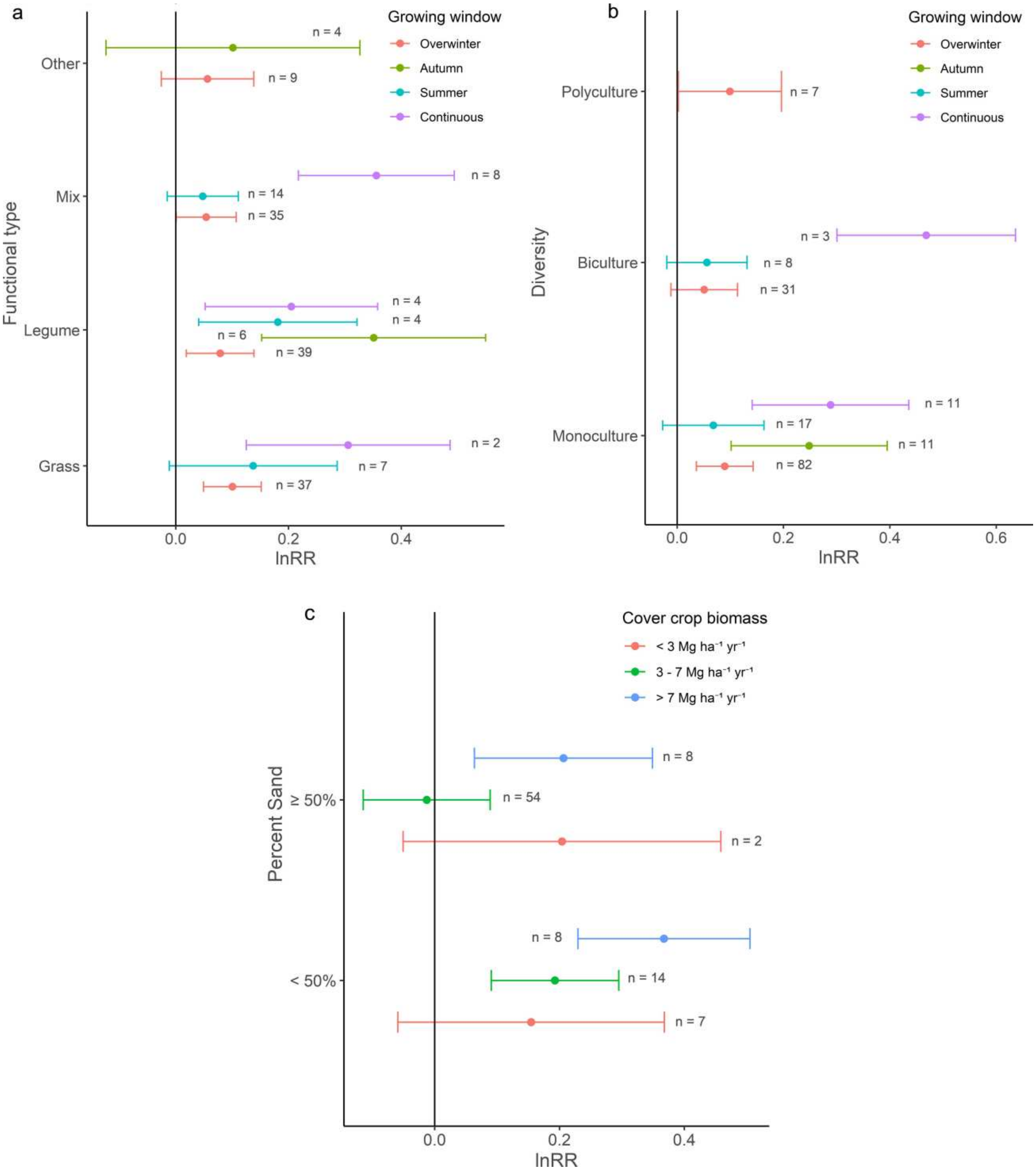


Figure 5. The effect of cover crop (a) functional type, (b) diversity type on the lnRR of soil carbon under different planting and termination periods (i.e., growing window), and (c) percent sand on the lnRR of soil carbon under different levels of annual cover crop biomass production. The overall mean response ratio (lnRR) ± 95% CI under each moderator is given. Confidence intervals not overlapping zero were considered significant.

Table 4. The interaction between cover crop biomass and other cover crop, cropping system, and environmental parameters on SOC response under cover cropping.

	Biomass x Moderator			
Category	Moderator	n[1]	DF[2]	p-value[3]
Cover crop	Growing window	100	8	0.147
	C:N	70	5	0.858
	Functional type	105	11	0.554
	Diversity	105	9	0.184
	Termination method	98	5	0.603
Cropping system	Cash crop rotation	105	6	0.518
	Tillage	105	7	0.451
Climate & soil	AEZ	105	7	0.391
	Percent sand	93	5	**0.021**
	Percent clay	93	4	0.429

[1] n is the number of observations for each moderator

[2] DF are the degrees of freedom

[3] The p-values represent the significance level of the interaction terms in each mixed-effects meta-regression. Bolded p-values indicate a significant effect ($p \leq 0.05$)

4.3 Response of soil carbon under cover crops over time

‘Time since introduction’ of a cover crop was a poor predictor of SOC response even after controlling for outliers ($p = 0.23$; marginal $R^2 = 0.004$ and conditional $R^2 = 0.083$). Thus, we were unable to make a robust estimate of the average annual SOC stock change from 0-30 cm under cover crops. A simple calculation dividing the overall mean (1.11 Mg C ha^{-1}) by the average ‘time since introduction’ across the observations in the dataset (5.2 years) indicates an average annual SOC stock change of 0.21 Mg C ha^{-1} yr^{-1} (95% CI: 0.20, 0.22). While not robust, this estimate provides a general indication of the expected annual SOC stock change with cover crops.

4.4 Sensitivity analysis

The stepwise removal of individual publications from the overall analysis using the Jacknife approach indicated that no particular publication drove the overall lnRR of the dataset (Appendix

1 Figure 1). Nearly all the recomputed mean lnRR and 95% confidence intervals were within the bounds of the original estimates and there were only a few publications whose removal shifted the mean lnRR slightly above or below the original estimate.

5. Discussion

5.1 Cover crop management drives differences in soil carbon response

Our meta-analysis confirms the findings of previous reviews and analyses that cover crops positively impact SOC (McDaniel et al. 2014, Poeplau and Don 2015, King and Blesh 2018, Abdalla et al. 2019). By focusing on temperate systems and integrating studies published after these earlier analyses, we demonstrate the importance of certain cover crop management and environmental conditions on the magnitude of SOC stock response under cover crops, using a statistical meta-analysis approach. In particular, the factors with potential to increase SOC response to cover crops included increased biomass production, perennial or continuous cash crop rotations, no-till management systems, and adoption in warmer climate regions and soils with higher clay content (Figure 6).

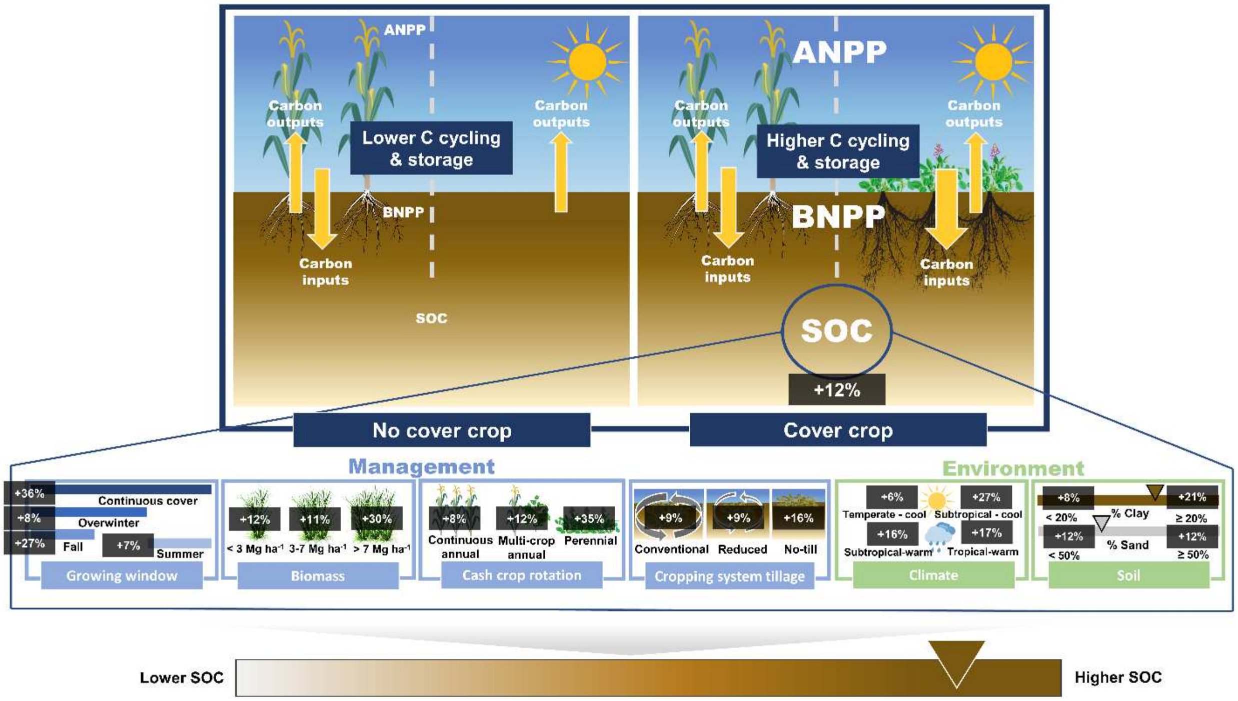


Figure 6. Diagram of key cover crop management decisions that influenced cover crop effects on soil organic carbon (SOC) response relative to a no cover crop control. Residue management is a critical factor in building and maintaining healthy soils. Conventional cropping systems (first panel) are represented by a continuous corn monoculture with limited above and belowground residue inputs leading to higher C outputs relative to C inputs. The alternative cover crop system (second panel) is similar to the conventional cropping system but also includes a legume cover crop, which provides high quality residues (low C:N) and continuous plant roots. Management decisions (lower panel) demonstrate the variety of approaches available for maintaining cover crops in a cropping rotation. These management decisions interact with environmental factors (climate, soil type) to influence SOC response through altered decomposition patterns (growing window and tillage), residue quantity (biomass), and productivity (biomass and cash crop rotation). The variability in these individual drivers and their interactions influences total SOC stocks in cover crop systems. The grey boxes indicate overall percent change under each of these moderators (this study) relative to a no cover crop system.

Soil C stocks increased substantially with biomass above 7 Mg ha^{-1} yr^{-1} relative to lower biomass categories. This level of cover crop biomass production, however, is unrealistic to expect in many climates and growing windows. Across the entire dataset average annual cover crop biomass production was 4.9 Mg ha^{-1} yr^{-1} (n = 105). While higher biomass production led to higher SOC response, it is important to note that all levels of biomass production increased SOC stocks.

The high biomass production category (> 7 Mg ha^{-1} yr^{-1}) contained only 16 observations from six publications. But, this level of biomass production is consistent with estimated yields of winter and summer cover crop varieties across USDA Hardiness Zones (Snapp et al. 2005). Nearly all the high biomass observations were from studies in subtropical AEZ (n = 14) (Sainju et al. 2002, Wang et al. 2009, Hubbard et al. 2013, Mancinelli et al. 2013, Morra et al. 2017), and more than half were grown as summer cover crops (Wang et al. 2009, Morra et al. 2017). Similarly, almost all these cover crops were grown as monocultures of grass or legume species. Despite the few observations, the strong effect of cover crop biomass production on SOC is likely due to increased NPP making greater above and belowground residues available for decomposition and stabilization as SOC (Sainju and Singh 1997).

Contrary to our expectations, cover crop growing window was a better predictor of SOC stock response than cover crop aboveground biomass production or termination method. As a moderator, cover crop growing window integrates multiple factors that control SOC stock response like cover crop establishment, biomass production, local temperature and moisture conditions as well as cover crop species in some cases. This integration likely contributed to the robustness of growing window as a predictor over the other individual moderators in our analysis (Table 1). In modeling efforts, the use of a single integrated predictor can reduce model complexity and user inputs to help facilitate easier computation of SOC response.

The cover crop systems planted as continuous cover produced some of the highest increases in SOC stocks across the entire dataset despite relatively low amount of aboveground biomass production (Wang et al. 2012, Gentry et al. 2013, Peregrina et al. 2014a). These increases were consistent across climate types, agroecosystems, and times since introduction. This may be due the continuous production of root biomass, which was not measured in these publications. Root biomass can be preferentially retained as SOC (Kong and Six 2010, Austin et al. 2017) relative to aboveground biomass via two mechanisms: (1) physical protection of root inputs in macroaggregates, and/or (2) continuous inputs of C from root exudates and the turnover of root biomass (Puget and Drinkwater 2001, Sokol et al. 2019). Thus, a decrease in soil disturbance during the cover cropping period (Grandy and Robertson 2007) and reduction in bare fallow (Paustian et al. 2000) across these studies likely contributed to high SOC stock response.

There are both climatic and management factors that likely influenced the larger SOC stock response for cover crops in the autumn growing window. Across the observations within the autumn growing window, aboveground biomass production was large, and the cover crops grown included legumes and other crops (i.e., forage radish) (Hubbard et al. 2013, Wang et al. 2017a). Other factors that may have contributed to the larger effect of cover crops grown in the autumn include improved cover crop establishment and the interaction of high residue quality and low temperatures after cover crop termination that can influence the greater conversion of biomass into SOC relative to other growing windows (Kuzyakov et al. 2000, Wang et al. 2017a).

As individual predictors, cover crop functional type and diversity did not predict SOC stock response. This finding is consistent with past analyses that did not observe a difference between legume and non-legume species (Poeplau and Don 2015, Abdalla et al. 2019). When we controlled for growing window, however, cover crop functional type and diversity exhibited significant differences in SOC stocks. While we did not evaluate pairwise comparisons for each level of growing window and cover crop functional type or diversity categorization, legume cover crops, for example, showed a greater SOC stock response if they were planted and terminated in the autumn (42% increase) rather than allowed to overwinter (8% increase). Poor legume establishment in the previous autumn may inhibit growth in the spring prior to termination leading to low residue inputs to the soil (Sainju and Singh 1997). Different types of legume cover crops also exhibit varying abilities to overwinter, which is why it is critical to select species well-adapted to local climatic conditions (Sainju and Singh 1997, Snapp et al. 2005).

Approximately one-fifth of our dataset exhibited no change or a decline of SOC stocks under crop crops. Of the studies that led to a decrease in SOC stock greater than 3 Mg C ha^{-1}, all but one occurred in a temperate-cool AEZ (Wang et al. 2012). There was little consistency in cover crop functional type or diversity, but several of the observations were characterized by low aboveground biomass production, which could be due to growing conditions or poor stand establishment (Kuo et al. 1997a, Kaspar et al. 2006, Ladoni et al. 2016). The combination of low amounts of residue and termination by tillage across many of these observations may have led to SOC stock declines. We found that 63% of these observations used conventional tillage management. The repeated disturbance of soil macroaggregates can increase exposure of microaggregate C to oxidation (Six et al. 2002), and, together with exposure to new plant residues can increase SOC decomposition (Poeplau and Don 2015).

5.2 Climate and soil clay content are key environmental drivers

Cover crops grown in subtropical AEZ increased SOC stock 10-21% more than those grown in temperate cool climates. The higher MAP in these categories likely resulted in more favorable growing conditions for cover crops, which was supported by the highest rates of aboveground biomass production occurring in these climatic zones. This finding is generally consistent with the expectation that warmer temperatures and reduced moisture limitations support higher rates of biomass production and residue return to the soil (Snapp et al. 2005). Global estimates of SOC distribution and controls on SOC accumulation find that soil C increases with increasing precipitation, but tends to decrease with increasing temperature (Jobbágy and Jackson 2000, Wiesmeier et al. 2019). The interaction between moisture and temperature is more difficult to decipher particularly at local scales, which was part of the rationale behind our selection of the AEZ approach because it integrates climate, soil, and terrain conditions.

Consistent with the literature, a decrease in soil sand and the corresponding increase in soil clay content played a significant role in SOC stock response with soils containing 20% or more clay exhibiting 13% higher SOC stocks than soil with less than 20% . Soil clay content is important for the chemical stabilization of soil organic matter (Six et al. 2002). It can also be important for the physical protection of POM in soil aggregates, particularly microaggregates (Franzluebbers and Arshad 1996, Six et al. 2002). The dominant role soil clay mineralogy plays in soil C stabilization makes it one of the best predictors of SOC storage potential followed by soil carbon input (Wiesmeier et al. 2019). The strong interaction between cover crop biomass and percent sand as predictors of SOC stock changes also suggest that texture influenced overall cover crop biomass production. Increased soil clay content, and the corresponding decrease in sand content, is positively correlated with soil water holding capacity and nutrient availability.

5.3 Lack of relationship between SOC response and experimental factors

‘Time since introduction’ was a poor explanatory variable of SOC stock response under cover crops. This finding is contrary to the results of Poeplau and Don (2015) who found that cover crops increase soil C at a rate of 0.32 ± 0.08 Mg C ha^{-1} yr^{-1}. In addition to methodological differences, our analyses focus on different scales (global vs. temperate) and the average length of treatment time in their dataset was more than twice as long as our dataset (11.9 years vs. 5.2 years). More than two-thirds of our observations came from experiments that were in place for five years or less. And, based on our inclusion criteria, some publications only included cover crops once in a cropping rotation over multiple cropping years. However, we did not detect an effect of cover crop rotational frequency on soil C stock, which was likely due to small sample size and a lack of specific management descriptions in some publications.

The benefits of cover crops are not always realized in the short-term (Snapp et al. 2005), and it may take several years to detect an effect of new residue inputs especially if soils are near or approaching C saturation (Castellano et al. 2015). Interestingly, our analysis suggests SOC improves even with shorter-term studies and our general finding did not change when we re-analyzed the dataset excluding one and two-year studies. We need more empirical data from long-term studies in temperate climates with multiple occurrences of cover crops in a rotation to increase our understanding of SOC stock response over time.

The initial depth of soil C measurement may have also impacted our results. In our moderator analysis, we did not find a significant difference in SOC stocks when we controlled for original sampling depth. However, we note that publications with very shallow sampling depths (i.e., less than 5 cm; Kaspar et al. 2006, Hubbard et al. 2013) did exhibit higher overall lnRR than other depth intervals, but the upper and lower 95% confidence intervals were quite wide for these estimates. Thus, the original sampling depths may, in some cases, over or under estimate the true effect of cover crops in the topsoil (Poeplau and Don 2015). Standardizing soil C stocks across observations is important for understanding the absolute soil C stock response to cover crops.

5.4 Potential as a negative emission technology requires full accounting

Sustainability metrics and evaluation tools can encourage more accurate quantification of the relative impact of different agroecosystems on the environment. Improved, data-driven decision support tools that can evaluate the benefits and impacts of various agricultural management practices are essential to meeting consumer demands for ecologically-oriented agriculture systems. The empirical data supporting these tools need to reflect the complexity of management and environmental conditions across agroecosystems. Our meta-analysis provides new insights into how cover crop management and environmental conditions influence soil C stock response under cover crops. We found strong evidence that cover crops can increase soil C storage. Our results suggest that estimates of cover crop practice effects on soil C could be improved by integrating information on cover crop biomass production, growing window, climate, and soil type while information about cover crop type was less important.

However, cover crops can also influence other GHG emissions, which may offset the capacity of cover crop systems to mitigate climate change. In their meta-analysis on the impact of cover crops on nitrous oxide (N_2O) emissions, Basche et al. (2014) found that on average cover crops did not increase N_2O emissions relative to a no cover crop control. But, cover crop management, specifically the selection of legumes and incorporation of cover crop residues into the soil, resulted in higher N_2O flux relative to non-legume species and no residue incorporation, respectively (Basche et al. 2014). This uncertainty in N_2O emissions and net GHG balance is a result of few, high resolution empirical studies from long-term experiments. More frequent, long-term measurements throughout and across growing seasons from a variety of regions and climates can help reduce uncertainty around N_2O emissions and C sequestration. While cover crops are not expected to have a large impact on methane (CH_4) emissions, few studies have measured these emissions. Our literature search identified only three publications (Lauren et al. 1994, Fronning et al. 2008, Sanz-Cobena et al. 2014), which were too few to include as part of our meta-analysis. These publications found conflicting responses of soil CH_4 emissions to cover crops; cover crops resulted in a net CH_4 sink (Sanz-Cobena et al. 2014), no change (Fronning et al. 2008), or a positive CH_4 flux (Lauren et al. 1994).We also found few, if any, new or additional publications to supplement the N_2O emissions estimates from Basche et al. (2014). Thus, a fuller accounting of cover crops as a negative emission technology will require additional quantification of N_2O and CH_4 emissions.

Looking at the overall GHG balance of cover cropping systems, Abdalla et al. (2019) found that cover crops can mitigate around 2 Mg CO_2-eq ha^{-1} yr^{-1}, but with a high degree of uncertainty (±

2.1 Mg CO_2-eq ha^{-1} yr^{-1}). However, when considering the net GHG effect of cover crops, it is also critical to consider overall agroecosystem management impacts such as those on fertilizer application rates and cash crop yields. Both legume and non-legume cover crops can result in lower N fertilizer application rates after long-term implementation (Kaye and Quemada 2017), which can contribute to climate change mitigation at the farm-scale. Additional research suggests that overwintering cover crops do not result in a yield penalty when proper agronomic management is applied (Marcillo and Miguez 2017), and they can modestly increase maize and soybean yields when controlling for soil quality (Seifert et al. 2019).

5.5 Knowledge gaps and data limitations

One key knowledge gap that future field studies and analyses need to address is the impact of cover crop on subsoil C dynamics. Only five of the 40 publications included in our synthesis sampled soil C below 30 cm (Appendix 1 Table 1). This analysis along with others (McDaniel et al. 2014, Poeplau and Don 2015, Abdalla et al. 2019) finds strong evidence that cover crops increases soil C in the topsoil (0-30 cm). This bias in sampling depth may overestimate the potential of cover crop systems to increase soil C storage. A nineteen-year study on winter crops in wheat-fallow and tomato-maize in California found that while cover crops led to an increase of 1.44 Mg C ha^{-1} to a depth of 30 cm, they decreased soil C stocks by almost 15 Mg C ha^{-1} from 30-200 cm (Tautges et al. 2019). An increase in subsoil C priming and decreased sorption capacity of the soil could have contributed to these increased SOC losses at depth. These findings have consequences for carbon markets and other policy mechanisms that encourage the use of cover crops for soil C storage (Tautges et al. 2019). Additional research across AEZ will help inform researchers if this finding is unique to Mediterranean climates or part of a broader pattern under cover cropping.

The limited number of observations within certain categories of growing window, climate, cover crop species diversity, and functional type reduces the generalizability of our findings. Cover crop growing window as a key management predictor of soil C stock response demonstrates promise, but there are too few observations in the autumn and continuous cover categories to robustly evaluate mechanisms that contribute to higher SOC in these systems. In general, most publications provided poor descriptions of cover crop management. While we could usually ascertain data about the type of cover crop species or functional group planted, numerous studies omitted data about termination date and termination method. Other sparse data included soil characteristics like bulk density, soil texture, and pH. A little more than one-half of our dataset measured aboveground cover crop biomass production, and even fewer measured cover crop C:N ratio. Better data reporting and measurements can reduce knowledge gaps and uncertainty around optimal cover crop management for improved selection of cover crop species, planting, and termination dates. This knowledge can help to improve cover crop establishment across growing conditions and maximize biomass cover to increase soil C and provide other environmental co-benefits (Strock et al. 2004, Vincent-Caboud et al. 2017).

6. Conclusions

Cover cropping is part of a suite of low-cost, ready-to-implement agricultural practices to draw down atmospheric CO_2 for combatting the climate crisis (National Academies of Sciences 2018, Bossio et al. 2020). The annual rate of C sequestration under cover crops is on par with shifts in annual cropland to grassland or forest (Guo and Gifford 2002), alleycropping (Cardinael et al. 2017), and improved grazing practices (Conant et al. 2017). And, when accounting for other GHG emissions on-farm, the climate mitigation potential from cover crops is greater than the transition from conventional to no-till (Kaye and Quemada 2017). Our work demonstrates that decisions pertaining to the management of cover crops and their residues, as well as regional variation in climate and soil influence the magnitude of SOC stock change under cover cropping. On average, cover crops increase SOC stocks by 12% or 1.11 Mg C ha^{-1}. This finding aligns with estimates from past reviews and analyses (McDaniel et al. 2014, Poeplau and Don 2015, King and Blesh 2018, Abdalla et al. 2019).

Across climates and management systems in temperate zones, cover crops provide a clear benefit to agroecosystem C balances by enhancing soil C sequestration. Land managers looking to increase soil C sequestration with cover crops should aim for high biomass production. This can best be achieved by increasing the complementarity between cover crop growing window and climate to maximize cover crop growth. Likewise, combining cover crops with other conservation agriculture practices such as no-till and perennial crops provides additional C sequestration benefits relative to cover crops in conventional tillage and continuous annual cropping systems. In the future, better data reporting and explicit examination of these management drivers will help reduce uncertainty in the response of soil C stocks to these practices. The integration of growing window (potentially as a proxy for biomass growth), climate, and soil factors in decision-support tools are relevant for improving the quantification of soil C stock change under cover crops, particularly with the expansion of terrestrial soil C markets.

CHAPTER 2: INFREQUENT ORGANIC AMENDMENT APPLICATIONS INCREASE PLANT PRODUCTIVITY AND SOIL ORGANIC CARBON IN IRRIGATED BUT NOT DRYLAND PERENNIAL PASTURES

1. Summary

Improved agricultural soil management can facilitate the removal of carbon dioxide from the atmosphere to help keep planetary warming at or well-below 2°C as outlined in the Paris Agreement. The application of organic amendments to agricultural soils increases soil carbon by directly fertilizing the soil with carbon (no net carbon dioxide removal), and by stimulating plant productivity and plant-derived carbon inputs (net carbon dioxide removal). In semi-arid managed grasslands, it is unclear how organic amendment application may impact net primary production and total soil carbon stocks to depth. We conducted a three-year experiment measuring plant and soil carbon and nitrogen responses following additions of organic and inorganic nitrogen applications in two representative semi-arid pasture management systems: an irrigated, improved perennial pasture and non-irrigated, semi-native rangeland. The application of organic nitrogen in the form of compost altered plant production and soil carbon and nitrogen dynamics in the improved, but not the semi-native pasture. Aboveground biomass increased approximately 40% under the organic amendment in the improved pasture, while belowground biomass only differed between treatments in the first experimental year. Soil organic carbon stocks as quantified by equivalent soil mass increased 1.5 Mg C ha^{-1} yr^{-1} from 0-20 cm, which was a net increase of 1.3 Mg C ha^{-1} yr^{-1} after accounting for the C supplied by the organic amendment. Soil inorganic carbon was highly variable in both experiments contributing to about half or more of total soil carbon at depth. This variability reduced our capacity to detect total soil C stock changes under

the organic amendment below 10 cm, demonstrating the importance of considering soil inorganic carbon in carbon stock change estimates under improved soil management practices in semi-arid systems. We conclude that moderate, infrequent applications of high-quality composted amendments to irrigated pastures can plant productivity and soil fertility in agricultural soils, but dryland pastures may be less responsive to this practice.

2. Introduction

By 2030, global greenhouse gas (GHG) emissions must peak and decline to below current emissions levels if humanity is to meet the goals of the Paris Agreement (Millar et al. 2017). To meet the assumptions outlined in most Intergovernmental Panel on Climate Change (IPCC) mitigation scenarios, humanity must simultaneously reduce anthropogenic greenhouse gas (GHG) emissions while removing large amounts of carbon dioxide (CO_2) from the atmosphere (O. Hoegh-Guldberg 2018). Carbon dioxide removal strategies via natural climate solutions (NCS) will be critical to keep planetary warming at or well-below 2°C (Griscom et al. 2017, Bossio et al. 2020).

Improved management of natural and working lands provides numerous opportunities for CO_2 removal in the near-term (National Academies of Sciences 2018), primarily through increased carbon (C) capture and storage in soils (Amelung et al. 2020). Soils contain more C than biomass and atmospheric C reservoirs combined (Batjes 1996), but historical agricultural cultivation has resulted in an estimated loss of 133 Pg of C from soils (Sanderman et al. 2017). The restoration of C in agricultural soils can be achieved by increasing the inputs of organic C to the soil, and protecting this carbon from losses, mainly as CO_2, back to the atmosphere.

Grassland and rangeland ecosystems represent half of the land area in the U.S. offering considerable potential to capture and store atmospheric C through improved soil management (Reeder and Schuman 2001). The application of organic amendments in the form of compost to managed grasslands may augment the C storage potential of these ecosystems (Ryals and Silver 2013, Ryals et al. 2014, Gravuer et al. 2019), while also improving soil health (Stott and Moebius-Clune 2017, Acharya et al. 2019) and economic benefits to land managers (Bowman et al. 2016). Grassland fertilization with organic nitrogen (N) can increase soil organic C (SOC) stocks upwards of 0.54 Mg C ha^{-1} yr^{-1} (Conant et al. 2017). In an annual California grassland, a composted organic amendment (C:N 11) significantly increased net ecosystem C storage over three years by increasing above and belowground net primary production (NPP) (Ryals and Silver 2013). The increases in SOC exceeded the amount of C input from the amendment itself indicating a net soil C gain through greater annual plant C inputs.

However, more than half of U.S. grassland and rangeland ecosystems occur in water-limited arid and semi-arid environments (Sobecki et al. 2001). Just a fraction of these pasture-based systems receive irrigation – approximately three percent of all permanent pasture and rangeland acres in the U.S. (USDA NASS 2019). There is evidence to suggest that the application of exogenous N to semi-arid grasslands may not stimulate aboveground NPP without concurrent applications of water (Lü et al. 2014). In a long-term study of chronic N deposition at three semi-arid grassland sites in the Colorado Plateau with ambient precipitation, plant communities were not sensitive to N additions indicating resistance of these communities to change (Phillips et al. 2020). But, these communities are also vulnerable to annual invasive species, like *Bromus tectorum*, that can substantially modify plant communities by altering fire regimes and ecosystem C cycling

(Bishop et al. 2019). The application of organic N to irrigated and non-irrigated semi-arid grasslands requires careful consideration of these challenges because nitrogen availability can favor *B. tectorum* relative to native perennial bunch grasses (Vasquez et al. 2008).

For organic amendments to be considered a scalable NCS, more research is required under different environmental conditions to understand the capacity of this management practice to increase soil C storage in the short and medium-term in semi-arid regions. While previous research has shown increased plant cover and soil organic matter levels in the top 8 cm of the soil following biosolid applications to a Colorado semi-arid system (Ippolito et al. 2010), it remains unclear how organic amendment applications may impact soil C cycling at depth. Increases in root biomass and rooting depth stimulated by amendment applications may have consequences for deeper soil C pools. Greater inputs of organic substrates at depth (i.e., root exudates) can stimulate microbial activity and lead to priming of SOC (Bernal et al. 2016). Ignoring deeper soil depths (> 30 cm) jeopardizes the conclusions about the impacts of agricultural management strategies as NCS (Tautges et al. 2019). Improved carbon accounting and consideration of ecosystem C balance under organic amendments will help clarify the feasibility of this practice for adoption in semi-arid regions (Garcia-Franco et al. 2018, National Academies of Sciences 2018) and increase confidence in incentives and policies for soil C management (Paustian et al. 2016, Amelung et al. 2020).

The objectives of our study were 1) to quantify the impacts of organic N applications on ecosystem-level C and N dynamics, and 2) determine if infrequent organic N applications are a viable short-term strategy for building soil C in low input semi-arid perennial pasture systems under different types of management. We hypothesized that moderate, infrequent applications of organic N in the form of compost would result in a net ecosystem C sink through the stimulation of aboveground biomass and root production leading to greater plant C inputs and subsequent increases in SOC stocks. We also hypothesized that the increases in soil C would be greater than the contributions from the organic amendment, indicating a net capture of atmospheric CO_2. We conducted a three-year experiment (2018-2020) in an irrigated pasture with a history of amendment application and an adjacent non-irrigated pasture with no history of N management in northern Colorado.

3. Methods

3.1 Study site

Two experiments were conducted on a working ranch located 34 km southwest of Fort Collins, Colorado at an elevation of 1574 m above sea-level (Loveland, Colorado, USA; 40° 24' 26.7156" N, 105° 11' 16.6272" W). One experiment was on an irrigated, improved pasture and a second experiment was on semi-native rangeland. Field data were collected for three growing seasons across both experiments from 2018 to 2020. The climate in this region is semi-arid characterized by cool winters and warm summers. Precipitation is episodic with most of the precipitation falling during the growing season. The growing season typically begins in early spring (March-April) and ends in the late summer to early autumn months (August-October). The mean annual precipitation (MAP) at the site is 421 mm (30-yr mean), the mean annual temperature (MAT) is 9.5 °C, and the mean maximum and minimum temperatures are 18°C and 1.2°C, respectively (30-yr mean, 4 km resolution) (PRISM Climate Group 2020). Local daily precipitation and temperature data were collected from an on-site weather station installed in August 2018 (Figure 7).

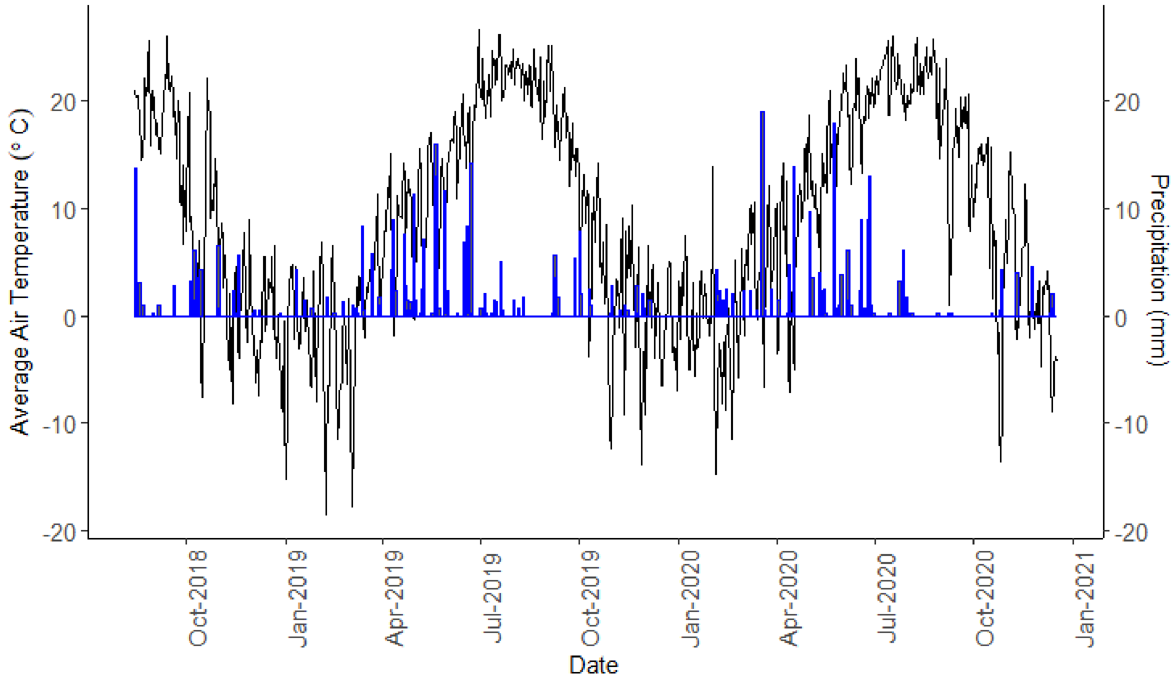


Figure 7. The daily average air temperature (°C) in black and b) daily precipitation (mm) in blue from August 2018 to December 2020 at the experimental site.

Prior to the start of the field experiments, low lying areas of the site were maintained as a perennial pasture comprised of improved pasture that consisted of alfalfa and cool season grasses harvested for hay in the summer and continuously grazed in the winter months. Higher elevation areas at the site were grazed but never seeded or harvested for hay. From the mid-1970s to late 1990s the site was continuously grazed by horses until cattle were introduced around the year 2000. Continuous grazing led to bare areas and invasion by *Ambrosia artemisifolia* and *Kochia scoparia* in sections of the pasture. Rotational grazing was introduced in 2005 to combat these issues. Irrigation at site began in the 1970s with side roll irrigation and a center pivot was installed in 2007. The irrigated and adjacent dryland pastures are harrowed every three to four years for soil aeration purposes. The entire site is also part of a corridor of seasonal grazing by Rocky Mountain elk (*Cervus canadensis nelson*) in the early spring and late autumn.

The soils in the study area are mesic Aridic Arguistolls. The primary soil in the irrigated pasture is a Nunn clay loam with three to nine percent slopes (Soil Survey Staff 2017). For the dryland pastures, the soil is described as a Connerton-Sylvan Dale complex and a Kirtley loam with three to nine percent slopes (Soil Survey Staff 2017). The parent material of soil in both pastures is alluvium derived from sandstone and shale. A complete description of baseline soil characteristics and textures for each experiment can be found in Table 5.

Table 5. Baseline mean ± SE soil properties of each field experiment measured in 2012 (IP) and 2018 (SNP). Soils were sampled to bedrock, which was around 50 cm in IP and 80 cm in SNP. The reported soil carbon (C) and nitrogen (N) stocks were quantified using equivalent soil mass. IP: improved pasture; SNP: semi-native pasture.

Field experiment	Depth (cm)	Clay (%)	Silt (%)	Sand (%)	pH	Bulk Density (g cm^{-3})	Soil organic C (Mg C ha^{-1})	Soil inorganic C (Mg C ha^{-1})	Soil N (Mg N ha^{-1})
IP	0-10	34.6	19.9	45.4	7.8	1.01 ± 0.01	18.9 ± 0.8	10.5 ± 1.9	2.3 ± 0.05
	10-20	34.3	19.3	46.5	8.0	1.14 ± 0.01	12.3 ± 0.5	11.1 ± 1.9	1.8 ± 0.04
	20-50	35.0	24.2	40.8	8.0	1.09 ± 0.02	14.5 ± 1.7	57.2 ± 7.6	3.2 ± 0.11
SNP	0-10	44.7	23.0	32.3	8.1	1.33 ± 0.05	19.5 ± 1.0	7.0 ± 0.8	2.1 ± 0.1
	10-20	45.2	21.2	33.7	8.3	1.40 ± 0.04	13.7 ± 1.0	10.8 ± 0.8	1.7 ± 0.1
	20-50	34.0	20.7	45.3	8.1	1.39 ± 0.03	25.6 ± 2.3	40.8 ± 4.0	2.9 ± 0.1
	50-80	32.3	25.3	42.3	8.2	1.42 ± 0.03	19.0 ± 1.2	53.0 ± 2.9	3.7 ± 0.1

Adjacent to IP was the second experimental site, SNP. These pastures were non-irrigated, semi-native rangeland comprised of a mixture of cool and warm-season grasses, legumes, forbs, and shrubs (e.g., *Thinopyrum intermedium*, *M. sativa*, *Lepidium virginicum, Artemisia frigida*). Exotic invasive annuals like *Bromus tectorum* and *Bromus secalinus* are also found within these pastures. Beef cattle were rotated through these pastures year-round, and grazing varied in timing and frequency.

3.1.1 Experiment design – Improved pasture (IP)

The improved pasture experiment was established in 2012 with two levels of treatment – organic N and unamended control with eight replicated blocks (Figure 12). The plots measure 10 x 10 meters. Organic N in the form of commercially available composted livestock manure (Humalfa; Iliff, Colorado, USA) was applied twice; once in March 2012 and again in March 2018 at the discretion of land manager. The main focus of this experiment was on the years 2018 – 2020 after the second application, but differences in soil C and total N stocks were evaluated from 2012 to 2020. At both applications, the compost was applied at a rate of 11.2 Mg ha^{-1} (C:N ratio 8:1) by compost spreader, which was equivalent to 123 kg N ha^{-1}, 974 kg organic C ha^{-1}, 171 kg P ha^{-1}, and 212 kg K ha^{-1}. The unamended control plots were carefully marked and given a five-meter buffer to avoid contamination by wind. A single hay cutting occurred in June or July each experimental year, and the pasture was occasionally grazed by beef in the winter months. A description of management and sampling dates can be found in Table 6.

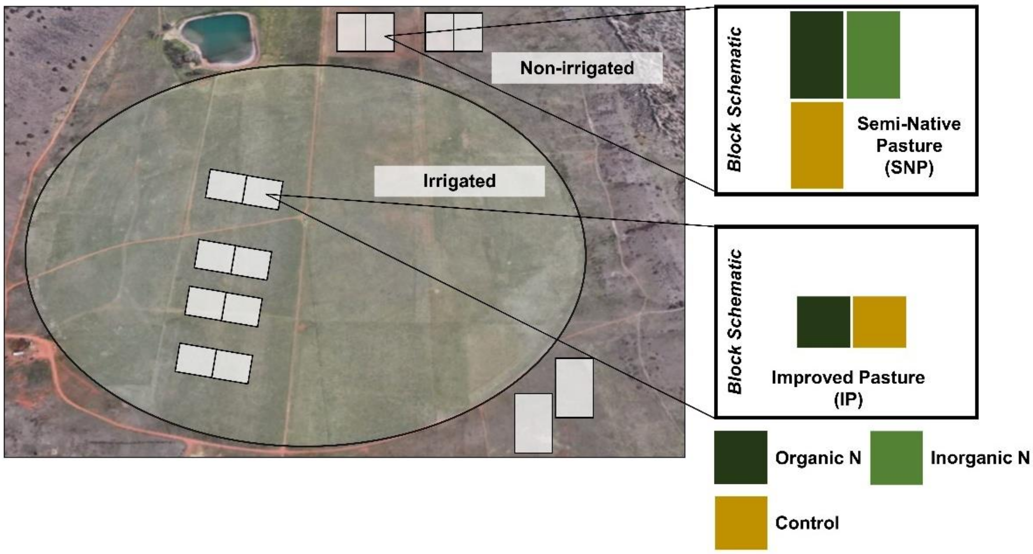


Figure 8. Replicated experimental plot design for the improved pasture (IP) with 8 blocks and the semi-native pasture (SNP) with 6 blocks.

3.1.2 Experiment design – Semi-native pasture (SNP)

The semi-native pasture experiment was established in March 2018 as a randomized complete block design. To further examine the effects of C and N additions on net ecosystem C, we added an additional inorganic N treatment within this new study. This was not possible within IP due to the previous experimental design established in 2012. There were three levels of treatment: organic N, inorganic N, and an unamended control with six replicated blocks. The organic N treatment was the same compost material applied in IP, but in these pastures, it was applied at lower rate to better reflect potential investment a rancher might make on semi-native rangeland and the lower potential productivity of SNP. The compost was applied at a rate of 6.7 Mg ha^{-1} (C:N ratio 8:1) by compost spreader on March 25, 2018, which was equivalent to 74 kg of total N ha^{-1}, 583 kg organic C ha^{-1}, 103 kg P ha^{-1}, and 127 kg K ha^{-1}. The inorganic N treatment was applied at rate to reflect the amount of available mineral N in the compost that would be bioavailable (approximately 25%; Colorado State University Extension 2015) during the first growing season. We applied urea N by hand on April 03 and 05, 2018 at a rate of 20 kg N ha^{-1}. More management details are available in Table 6.

Grazing by beef cattle was managed by a different cattle leasee over the three growing seasons. The stocking rate ranged from 15.8 – 23.8 head ha^{-1} and followed rotational grazing management. In 2018 and 2019, the beef cattle were rotated once through two blocks of SNP (approximately 1.26 ha) beginning in September for a period of 10-14 days until all plots were grazed. During the final growing season, beef stockers rotated through only four of the replicate blocks of SNP from late April to early May for upwards of seven days (Table 6).

Table 6. Management history and operations timeline for field experiments. IP: improved pasture; SNP: semi-native pasture.

	IP				SNP		
	2012	2018	2019	2020	2018	2019	2020
Management							
Organic N							
Amount applied (Mg ha^{-1})	11.2	11.2	—	—	6.7	—	—
N rate (kg N ha^{-1})	137	137	—	—	82	—	—
Date applied	March 27	March 23			March 26		
Inorganic N							
N rate (kg N ha^{-1})	—	—	—	—	20	—	—
Date applied					April 03, 05		
Annual Irrigation Amount (mm ha^{-1} yr^{-1})	—	408	292	775	—	—	—
Hay cutting	—	July 10	July 21	June 18	—	—	—
Grazing	—	Continuous (winter)	Continuous (winter)	Continuous (winter)	Rotational (September-December)	Rotational (September-January)	Rotational (April, May)[a]
Sampling							
Baseline soil samples	March 26	—	—	—	March 13, April 26[b]	—	—
Root biomass	March 26	March 12, August 06	August 01	March, July 01	March, September-November	—	September 17
Soil measurements	—	March 12, August 06	August 01	March 25, July 01	March, September-November	—	September 17
Aboveground biomass	—	June 12	July 02	June 17	August 06	July 29	July 20
Soil CO_2 Flux	—	—	—	—	April 12 – October 19	April 08 – October 09	April 15 – October 01

[a] As a result of grazing management decisions, only four of six replicate plots were grazed in 2020.

[b] Bulk density samples

3.2 Above and belowground biomass

We collected both aboveground and belowground biomass over the experimental period. Aboveground biomass estimates were made during peak standing biomass in IP and SNP. In IP, these samples were collected prior to haying in June or July of each year, and in SNP they were collected in late July or early August prior to cattle grazing. We collected two 0.25 m^2 quadrats from each plot. We clipped biomass a few centimeters above the soil surface to avoid soil or manure contamination. Biomass samples were dried at 55°C immediately after collection for at least 48 hours, and then weighed for mass on an oven-dried basis. All samples were ground with a ball mill and analyzed for C and N concentration on a LECO Tru-Spec CN analyzer (Leco Corporation; St. Joseph, Minnesota, USA).

Standing root biomass estimates were made to 20 cm in IP in SNP using the soil core method (Milchunas 2009). Previous analyses of the proportion of roots from 0-50 cm in IP indicated that less than 20% of roots occurred below a 20 cm depth. Thus, we only collected roots to 20 cm depth during the growing season for both experiments. In SNP, belowground biomass was collected in 2018 during the first growing season two weeks after grazing by beef cattle (September – December; 0-20 cm) and again in September 2020 (0-20 cm).

Soil cores were bulked in the field and stored at 4°C until processing. After obtaining sample mass and removing a subsample for gravimetric water content analysis, roots were extracted by hand. We partitioned roots into two different fractions. The first fraction (coarse roots) was obtained by collecting all visible roots from the sample before passing through an 8mm sieve. For the second fraction, a 150 g air dried soil subsample was used to collect fine roots before passing the soil subsample through a 2mm sieve. All soil was carefully removed from the roots before drying at 55°C to obtain oven-dried mass. Consistency in the efficiency of root extraction was difficult to maintain across year, thus, we primarily focus on differences between treatments within year in our analyses. To estimate belowground standing biomass, we multiplied the oven dried mass of the root sample by a 45% C concentration value (Ma et al. 2018). The sum of the two root fractions represented total belowground standing biomass per depth interval. .

3.3 Grazed biomass removal

To estimate biomass removal by grazers, we used the moveable cage method to determine total grazed biomass as described by McNaughton et al. (1996). In early April of each experimental year, four metal cages (1.1 m diameter, 0.6 m high) were randomly installed in each SNP plot. After each grazing event, a 0.25 m^2 quadrat was placed inside the cage to determine the amount of pre-grazed biomass, and another quadrat was placed immediately adjacent to the cage to estimate post-grazed biomass. Samples were immediately returned to the lab, dried at 55°C for a minimum of 48 hours before obtaining mass on an oven dried basis. The difference between pre- and post-grazed samples after multiplying oven dried mass by the C concentration represented the estimated biomass removal (Mg C ha^{-1}) by the cattle. Our estimates of grazed biomass C removal are likely high because we did not estimate C returned to the soil through cattle excrement.

3.4 Soil carbon, nitrogen, and physical properties

We quantified field bulk density from cores (4 cm diameter) collected with a truck mounted Giddings hydraulic soil probe Giddings Machine Company; Windsor, Colorado, USA). Three cores were collected per plot at three depth intervals (0-10, 10-20, and 20-50 cm) in IP in 2012 prior to first organic amendment application. In IP, we collected five (0-10, 10-20 cm) and three soil cores (20-50 cm). In SNP, we collected five soil cores (4 cm diameter) per depth interval (0-

10, 10-20, 20-50, 50-80 cm) per plot due to the larger plot size. Bulk density estimates were mass corrected for coarse particles > 2mm diameter following the recommended approach for dryland soils in Throop et al. (2012). We quantified total soil C and N concentrations on 2mm sieved soils that were ground and dried at 55°C using a LECO Tru-Spec CN analyzer (Leco Corporation; St. Joseph, Minnesota, USA).

We quantified total soil organic C as the difference between total soil C and soil inorganic C (SIC). We measured SIC concentration on all soil samples (Table 1) using the pressure-calcimeter method (Sherrod et al. 2002). Due to the high variability and large concentrations of SIC, particularly at deeper soil depths (Table 5), we also quantified SIC for a subset of samples by acid fumigation (Harris et al. 2001) and dry combustion on a CN analyzer. The results from both methods were highly correlated ($R^2 = 0.93$, Slope = 0.97), but three of 48 samples in IP fell well outside the 1:1 correlation when comparing methods. We elected to remove these outlier samples from our analyses of soil C stocks. Soil C and N concentrations were converted to C and N stocks using the equivalent soil mass (ESM) method described in (Lee et al. 2009). Soil texture and soil pH were analyzed by Ward Laboratories, Inc. (Lincoln, Nebraska, USA) by hydrometer (Gee and Bauder 1986) and a 1:1 slurry of soil and deionized water, respectively.

3.5 Soil respiration

Because we expected changes in SOC to be difficult to detect during the three-year experimental period, particularly with the low compost application rates in SNP, we also measured soil respiration in SNP plots. Soil CO_2 flux can be a more responsive measurement to treatment effects on soil carbon dynamics. The measurements were collected twice weekly during the first growing season (2018) beginning in April unless a rain event prevented data collection. We collected measurements at this frequency to better capture the anticipated increase in CO_2 fluxes immediately after amendment application. The sampling frequency was reduced to once weekly in second growing season (2019) and bi-monthly in the third growing season (2020). Briefly, at least 24 hours prior to the first measurement date, PVC collars were installed 5 cm into the soil surface and allowed to equilibrate. One PVC collar was installed per plot for a total of 6 observations per treatment. Soil CO_2 flux was measured with a LI-8100 infrared gas analyzer and survey chamber system (~ 2-minute flux intervals [LI-COR Biosciences, Lincoln, Nebraska, USA]). Soils within the collars were kept free of plant shoots to provide an estimate of soil respiration only. To scale to an approximation on seasonal soil CO_2 flux, we applied a linear interpolation (Bremer et al. 1998, Ryals and Silver 2013) method between sampling time points and summed the estimated daily fluxes over the entire sampling period (Table 2).

3.6 Statistical analyses

We analyzed all data using linear mixed models using the *lme4* package (Bates et al. 2014) in R version 3.6.3 (R Core Team 2013). The IP and SNP experiments were analyzed separately. To assess change over time we used repeated measure multivariate analysis of variance (MANOVA) where there were multiple years of observations. Treatment and year were fixed effects and block and plot were random effects. When we had observations at a single time point, we applied one-way analysis of variance (ANOVA) to test for statistically significant treatment effects; block was included as a random effect. Degrees of freedom were corrected according to the method recommended by Kenward and Roger (1997). We included baseline 2012 root biomass, soil carbon, soil nitrogen, and bulk density responses at the plot level in IP as covariates due to initial differences between treatment and control plots for these variables. Linear interpolation of daily soil CO_2 flux rates to seasonal cumulative soil CO_2 flux yr^{-1} was performed using the *PKNCA* package in R (Buckeridge et al. 2015). Soil C and N concentrations were converted to ESM using the *SimpleESM* R script (Ferchaud 2020). Outliers were removed in rare instances when they exceeded the 25 or 75% quartile ranges impacting less than 6% of data points for any given variable. Significant differences between treatments and treatment by year were assessed with pairwise comparisons using the *emmeans* package (Lenth 2020) in R to estimate least-squares means. Data were reported as least squares means followed by standard error, and we determined statistical significance at $P \leq 0.05$ unless otherwise noted.

4. Results

4.1 Annual weather and irrigation

Because the onsite weather station was installed in August 2018, we summarized weather patterns for the first experimental year using additional data from PRISM Climate Group (2020) for 2018 at a 4 km resolution. Using the 2018 PRISM data, mean annual precipitation was 370 mm. Using the onsite weather station, the mean annual precipitation was 235 mm in 2019, and in 2020 it was 196 mm. The mean annual temperature was lower in 2019 relative to 2018 and 2020 by about 1.5°C. In IP, annual irrigation rates were 47% and 62% lower in 2018 and 2019, respectively, relative to 2020 (Table 6). Irrigation in 2019 was lower than 2018 and 2020 due to equipment malfunction.

4.2 Aboveground biomass carbon and nitrogen responses

The organic N treatment increased aboveground biomass production during the experimental period in IP (Figure 9). In 2018 and 2020, aboveground biomass C and N were 37% and 42% greater, respectively, where compost had been applied than the unamended control ($P < 0.01$;

Figure 9a, Figure 13a). While this trend persisted in 2019, overall biomass C was 30% lower in 2019 across all treatments than the other years and the treatment means did not differ (*P* > 0.05).

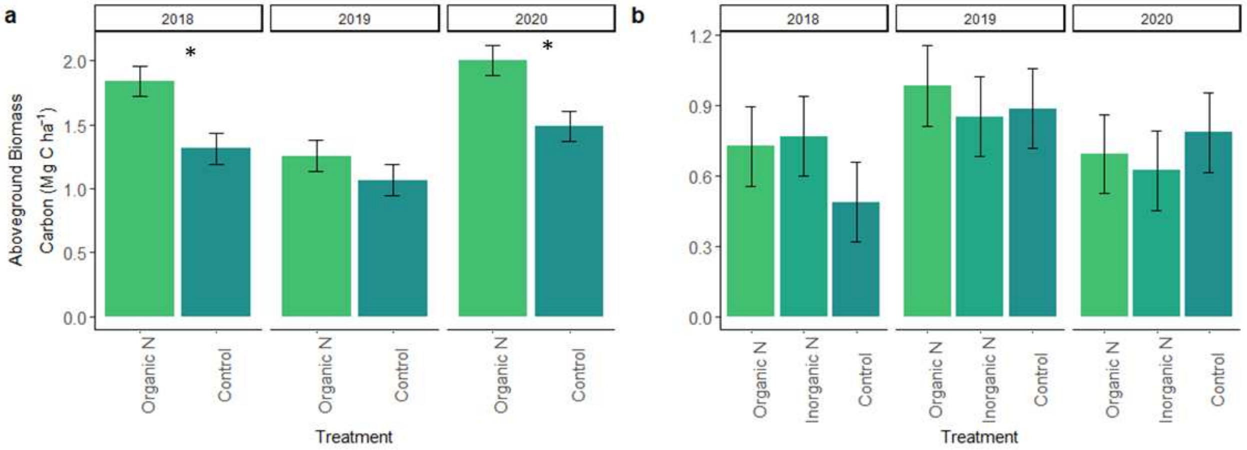


Figure 9. Annual least-squares means of aboveground biomass carbon (Mg C ha^{-1}) responses for a) improved pasture (IP) and b) semi-native pasture (SNP) during a three-year experiment near Loveland, CO. Significant p-values for treatments within year; $P > 0.1$ (non-significant; NS), $P \leq 0.05$ (*), and $P \leq 0.01$ (**). Error bars represent standard error; IP (n=8) and SNP (n=6).

In SNP the inorganic N treatment resulted in marginally higher aboveground biomass during the first and second experimental years relative to the control, suggesting that the system was N limited, but this relationship was reversed in the third experimental year (Appendix 2 Table 1). In general, the relationships between treatments for aboveground biomass were similar for aboveground biomass C and N.

4.3 Root standing biomass

Standing root biomass varied by season in IP. Estimates of root biomass collected during peak standing aboveground biomass differed between treatments in IP in the first study year (Figure 10a). The organic N treatment had 23% greater standing root biomass from 0-20 cm in 2018 (Appendix 2 Table 1). However, there were no other differences between treatments from 0-20 cm in 2019 or 2020. Root biomass was notably lower in summer 2020 relative to other years during the experimental period.

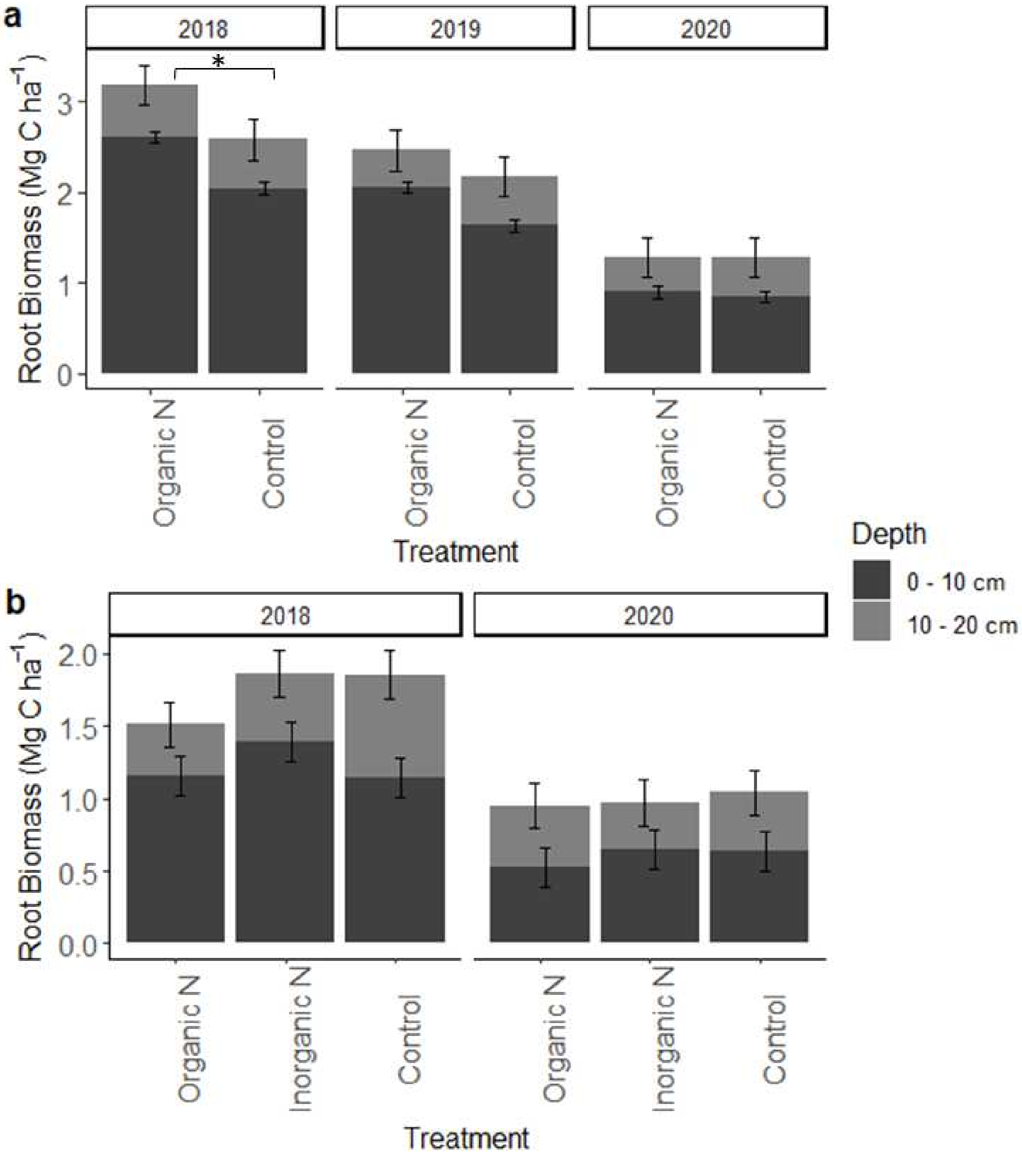


Figure 10. Annual least-squares means of standing root biomass carbon (Mg C ha^{-1}) responses for a) improved pasture (IP) and b) semi-native pasture (SNP) during a three-year experiment near Loveland, CO. Significant p-values for treatments within year; $P > 0.05$ (non-significant; NS), $P \leq 0.05$ (*). Error bars represent standard error; IP (n=8) and SNP (n=6).

There were no differences in root biomass between treatments when estimated from 0-20 cm in SNP (Appendix 2 Table 1). Estimates of root biomass also varied annually, particularly for the 0-

10 and 10-20 cm depth intervals, which were sampled twice over the three-year experiment (Figure 10b). In 2018, root biomass measured from 0-20 cm was 60% higher than in 2020.

4.4 Biomass removal

We estimated hay removal at 90% of aboveground biomass production in IP (Figure 11a, Appendix 2 Table 3), which is typical for single cuttings in the region (Brummer 2021). Following the trends in aboveground biomass C for each treatment, the harvested biomass C differed by treatment ($P = 0.002$). Overall, biomass C removed from the organic N treatment was 32% greater than the unamended control. There were also differences in biomass C removed annually; in 2019 harvested biomass C across treatments was 26 and 33% lower than 2018 and 2020, respectively. The amount of biomass removed annually by grazers did not differ between treatments or years (Figure 11b). However, grazed biomass tended to be lower in 2020, which was likely attributable to shorter grazing duration times (Appendix 2 Table 3).

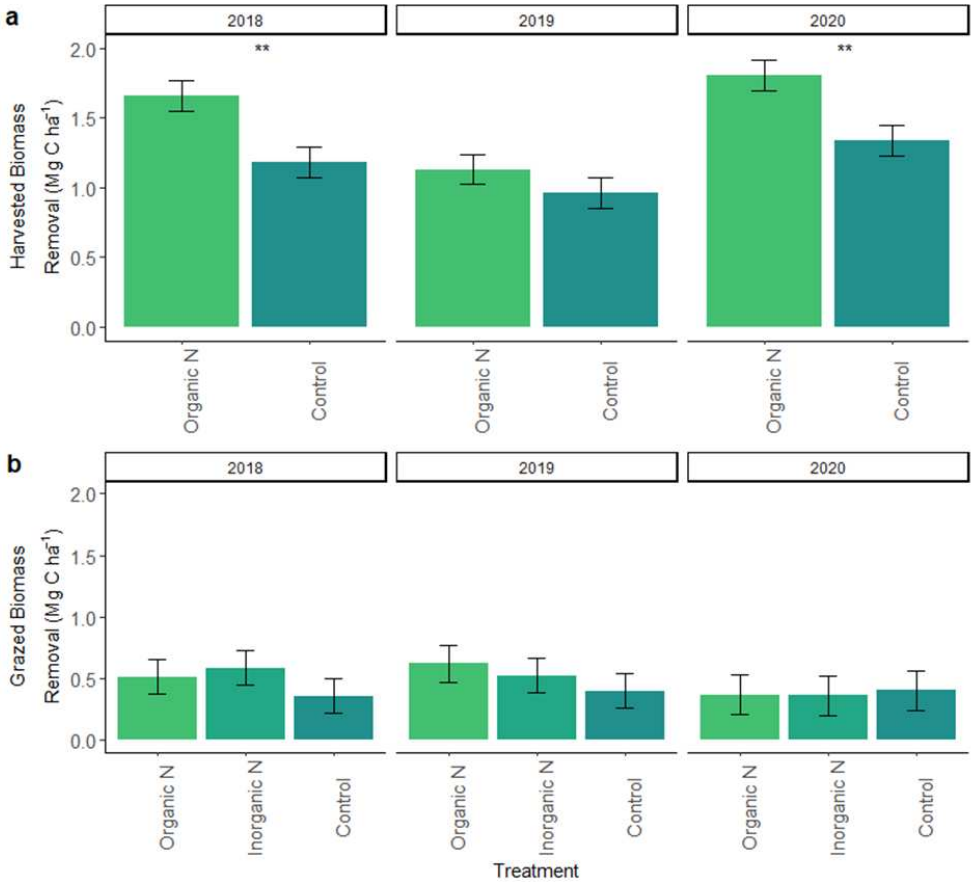


Figure 11. Annual least-squares means of annual aboveground biomass carbon removed by a) hay harvest in the improved pasture (IP) and b) beef cattle grazing (Mg C ha^{-1}) in the semi-native pasture (SNP) during a three-year experiment near Loveland, CO. Significant p-values for treatments within year; $P > 0.05$ (non-significant; NS), $P \leq 0.05$ (*), and $P \leq 0.01$ (**). Error bars represent standard error; IP (n = 8), SNP, 2018 and 2019 (n=5) and 2020 (n=3).

4.5 Bulk density and soil carbon and nitrogen stocks

Soil bulk density did not differ between treatments at any depth interval over the three-year experiment in IP. All depth intervals showed decreases in bulk density from 2018 to 2020 (Table 7). Similarly, in SNP, there were no differences between treatments in bulk density at any depth interval.

Table 7. Annual least-squares means ± standard error (SE) of bulk density for control and treatment plots from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant treatment differences within year indicated by different letters (P < 0.05).

Variable	Depth	Year	IP[1]		SNP[2]		
			Organic N	Control	Organic N	Inorganic N	Control
Soil bulk density (g cm^{-3})	0-10 cm	2012	1.01 ± 0.01	1.01 ± 0.01	__	__	__
		2018	1.40 ± 0.02a	1.43 ± 0.02a	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05
		2020	1.22 ± 0.03a	1.22 ± 0.03a	1.38 ± 0.03a	1.34 ± 0.03a	1.32 ± 0.04a
	10-20 cm	2012	1.14 ± 0.01	1.14 ± 0.01	__	__	__
		2018	1.52 ± 0.03a	1.56 ± 0.03a	1.40 ± 0.04	1.40 ± 0.04	1.40 ± 0.04
		2020	1.41 ± 0.03a	1.39 ± 0.03a	1.43 ± 0.03a	1.40 ± 0.03a	1.41 ± 0.03a
	20-50 cm	2012	1.09 ± 0.02	1.09 ± 0.02	__	__	__
		2018	1.55 ± 0.03a	1.52 ± 0.03a	1.39 ± 0.03	1.39 ± 0.03	1.39 ± 0.03
		2020	1.39 ± 0.03a	1.27 ± 0.03a	1.42 ± 0.02a	1.41 ± 0.02a	1.36 ± 0.02a
	50-80 cm	2012	__	__			
		2018	__	__	1.42 ± 0.03	1.42 ± 0.03	1.42 ± 0.03
		2020			1.46 ± 0.03a	1.44 ± 0.03a	1.44 ± 0.03a

[1] Reported bulk density estimates in 2012 are means ± SE

[2] Reported ESM and bulk density estimates in 2018 are means ± SE at the block level

Soil organic C and N stocks were greater in amended treatments relative to controls in IP and not SNP. Soil C stock estimates were highly variable across all treatments, particularly for SIC, while total soil N showed less variability. We quantified equivalent soil mass SOC, SIC, total soil C (the sum of SOC and SIC) and total N stocks in the topsoil and to bedrock; we quantified stocks from 0-20 cm to align with commonly measured topsoil depth intervals, and we also quantified total stocks from 0-50 cm and 0-80 cm in IP and SNP, respectively. In SNP, there were no differences between treatments in any soil C or total N stocks from 0-20 cm or 0-80 cm (Table 8) or at any of the individual depth increments (Appendix 2 Table 2).

In IP, total soil C stocks from 0-20 cm did not differ between treatments in 2018 or 2020 (Table 8), while SOC and soil N differed by treatment. Soil organic carbon stocks from 0-20 cm did not differ between treatments in 2018, but in 2020 the organic amendment had 12% higher SOC stocks from 0-20 cm in IP. Soil inorganic C stocks were 23% higher in the unamended control in 2018 when measured from 0-20 cm relative to the amended treatment, but we did not observe

SIC differences between treatments in 2020. Total N stocks in 2018 and 2020 were 8% and 15% higher in the organic N treatment, respectively, than the unamended control in IP.

From 0-50 cm in IP, we observed differences in SOC and soil N, but not in total C stocks between treatments. Soil organic carbon stocks throughout the profile were not different between treatments in 2018, but they were 10% higher in the organic N treatment compared to the control in 2020 (Table 8). For SIC, there were no differences between treatments from 0-50 cm in 2018 or 2020. Total N stocks from 0-50 cm in the organic N treatment were 4% and 12% higher than the control in 2018 and 2020, respectively (Table 8).

The treatment differences in soil C and N stocks in IP were concentrated primarily in the topsoil. Total C stocks in 2018 (i.e., six years after the first amendment application) were not different when measured from 0-10 cm, but were 10% greater from 10-20 cm in the unamended control relative to the organic N treatment (Appendix 2 Table 2). Soil organic C stocks were 8% higher under the organic amendment in 2018 and there were no differences between treatments when measured from 10-20 cm. In 2018, unamended control plots had 30% greater SIC stocks from 0-10 cm relative to the organic N treatment (Appendix 2 Table 2), but there were no differences from 10-20 cm.

In 2020, the organic N treatment contained 10% higher total soil C stocks compared to the control when measured from 0-10 cm and we did not observe differences in total C from 10-20 cm. Soil organic C stocks were 16% higher under the organic amendment relative to the unamended control from 0-10 cm and there were no differences from 10-20 cm (Appendix 2

Table 2). There were no differences in SIC stocks between treatments in IP in 2020 at either depth interval. We also did not observe any differences between treatments in total C, SOC, or SIC in 2018 or 2020 from 20-50 cm.

Total N stocks were consistently higher under the organic amendment in IP; from 0-10 cm in 2018 and 2020 they were 14% and 23% greater, respectively, compared to the control (Appendix 2 Table 2). We did not observe any differences in total N from 10-20 cm between treatments in either year. And, while there were no differences between treatments for total N stock from 20-50 cm in 2018, total N stocks in 2020 were 7% higher in the organic amendment compared to the unamended control (Appendix 2 Table 2).

Table 8. Annual least-squares means ± standard error (SE) of soil equivalent soil mass (ESM) carbon and nitrogen stocks for control and treatment plots from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values for treatments within year indicated by different letters; $P \geq 0.05$ (non-significant; NS), $P \leq 0.05$ (*), and $P \leq 0.01$ (**).

Variable	Depth	Year	IP[1]		*P*	SNP[2]			*P*
			Organic N	Control		Organic N	Inorganic N	Control	
Soil organic carbon (Mg C ha-1)	0-20 cm	2018	39.4 ± 1.2a	37.9 ± 1.2a	NS	33.1 ± 1.8	33.1 ± 1.8	33.1 ± 1.8	
		2020	43.0 ± 1.2a	38.5 ± 1.2b	**	37.1 ± 2.4a	36.4 ± 2.4a	35.0 ± 2.4a	NS
	0-50 cm	2018	68.8 ± 2.2a	67.5 ± 2.0a	NS	___	___	___	
		2020	72.9 ± 2.2a	66.1 ± 2.0b	*	___	___	___	
	0-80 cm	2018	___	___		77.8 ± 4.3	77.8 ± 4.3	77.8 ± 4.3	NS
		2020	___	___		80.7 ± 6.2a	73.2 ± 5.6a	73.4 ± 5.6a	
Soil inorganic carbon (Mg C ha-1)	0-20 cm	2018	18.3 ± 1.7b	23.8 ± 1.7a	*	17.8 ± 1.4	17.8 ± 1.4	17.8 ± 1.4	
		2020	18.9 ± 1.7a	21.5 ± 1.7a	NS	10.0 ± 3.7a	20.9 ± 3.7a	20.7 ± 3.7a	NS
	0-50 cm	2018	55.7 ± 4.4a	61.0 ± 3.8a	NS	___	___	___	
		2020	48.9 ± 4.4a	59.2 ± 3.8a	NS	___	___	___	
	0-80 cm	2018	___	___		112 ± 6.9	112 ± 6.9	112 ± 6.9	
		2020	___	___		91.9 ± 19.2a	136.7 ± 17.1a	128.1 ± 17.1a	NS
Total soil carbon (Mg C ha-1)	0-20 cm	2018	55.8 ± 1.7a	59.9 ± 1.5a	NS	50.9 ± 2.3	50.9 ± 2.3	50.9 ± 2.3	
		2020	60.8 ± 1.7a	58.2 ± 1.5a	NS	47.1 ± 3.7	57.3 ± 3.7	55.6 ± 3.7	NS
	0-50 cm	2018	124.0 ± 4.9a	128.0 ± 4.3a	NS	___	___	___	
		2020	121.0 ± 4.9a	125.0 ± 4.3a	NS	___	___	___	
	0-80 cm	2018	___	___		189 ± 6.1	189 ± 6.1	189 ± 6.1	
		2020	___	___		173.0 ± 16.0	210.0 ± 14.3	201.0 ± 14.3	NS
Total soil nitrogen (Mg N ha-1)	0-20 cm	2018	4.2 ± 0.1a	3.9 ± 0.1b	*	3.7 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	
		2020	4.5 ± 0.1a	3.9 ± 0.1b	**	3.8 ± 0.3a	3.7 ± 0.3a	3.6 ± 0.3a	NS
	0-50 cm	2018	7.3 ± 0.2a	7.0 ± 0.2b	*	___	___	___	
		2020	7.6 ± 0.2a	6.8 ± 0.2b	**	___	___	___	
	0-80 cm	2018	___	___		9.0 ± 0.3	9.0 ± 0.3	9.0 ± 0.3	
		2020	___	___		8.6 ± 0.5a	8.3 ± 0.5a	8.0 ± 0.5a	NS

[1] Values from 2018 represent stocks measured over the period of 2012-2018, and values from 2020 represent stocks measured over the period of 2018-2020. These values include 2012 estimates as a covariate due to initial treatment differences at the plot level.

[2] Values from 2018 represent baseline stock measurements at the block level, and values from 2020 represent stocks measured over the period of 2018-2020.

4.6 Changes in soil carbon and nitrogen stocks

There were no changes in soil C or N stocks over the short experimental duration (2018-2020) or differences between treatments in SNP. To estimate change in soil C and total N stocks over time in IP, we compared baseline measurements collected in 2012 to our final year of measurements in 2020. We examined the differences in stocks between treatments and whether changes in stocks were different from baseline measurements (Table 9). Over eight years since the first application, total soil C gains in 0-10 cm were 3.5 ± 0.7 Mg C ha^{-1} greater in the organic N treatment compared to the unamended control (data not shown). Both treatments gained soil C between 2012 and 2020. Total C stocks increased 6.3 ± 0.8 Mg C ha^{-1} under the organic amendment and 2.8 ± 0.8 Mg C ha^{-1} in the unamended control resulting in annual change of 0.8 and 0.4 Mg C ha^{-1} yr^{-1}, respectively. However, there were no differences in the change in total C between treatments when estimating stocks in the topsoil (0-20 cm) or subsoil (0-50 cm) (Table 9). Both treatments also increased total C in the top 20 cm over this time (Table 9), but there were no differences from baseline in total soil C stocks when measured to 50 cm (Table 5).

Between 2012 and 2020, we measured significant increases in SOC stocks. Soil organic carbon stocks increased by 5.6 Mg OC ha^{-1} more over eight years in the organic N treatment from 0-20 cm than in the unamended control (Table 9). This increase exceeds the total amount of carbon added to the system by the organic amendment over two applications (1.95 Mg OC ha^{-1} total). On an annual basis, the organic amendment resulted in an increase of 0.7 Mg OC ha^{-1} yr^{-1} more than the control. After subtracting the added amendment carbon from the difference between total change in SOC under the organic amendment versus the control, SOC stocks increased by

3.7 Mg C ha^{-1} or 0.46 Mg C ha^{-1} yr^{-1} from 0-20 cm in the organic N treatment. However, when accounting for SOC stocks to bedrock (0-50 cm), we did not observe any differences between treatments, but both treatments gained SOC over the period. While there were no differences in SIC stocks between treatments, the organic amendment lost SIC from 0-50 cm over the eight years since initial application. These losses were driven by decreases in SIC at depth (20-50 cm).

Total soil N stocks were less variable and demonstrated consistent differences in stock changes between treatments in the top and full soil profile (Table 9). In the topsoil, total N had a larger increase in the organic amendment treatment relative to the unamended control. The difference between treatments from 20-50 cm appeared to come from a marginal loss of total soil N at this depth in the control rather than an increase in total soil N in the organic N treatment. From 0-50 cm, soil N in 2020 did not differ from baseline measurements.

Table 9. Annual least-squares means ± standard error (SE) of carbon and nitrogen stock changes in soil equivalent soil mass for control and treatment plots from baseline values for two field experiments (changes from 2012 to 2020 for IP and 2018 to 2020 for SNP). IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values for treatments within year indicated by different letters. Bolded values indicate significant change from baseline ($P \leq 0.05$). $P \geq 0.05$ (non-significant; NS), $P \leq 0.05$ (*), and $P \leq 0.01$ (**).

Variable	Depth	IP		*P*	SNP[1]			*P*
		Organic N	Control		Organic N	Inorganic N	Control	
Δ Soil organic carbon (Mg C ha-1)	0-20 cm	**12.3 ± 1.3**a	**6.7 ± 1.3**b	**	-1.3 ± 5.3a	-0.84 ± 5.3a	-1.9 ± 5.3a	NS
	0-50 cm	**21.1 ± 3.3**a	**19.3 ± 2.6**a	NS	___	___	___	
	0-80 cm	___	___		1.5 ± 6.8a	-4.6 ± 6.2a	-4.4 ± 6.2a	NS
Δ Soil inorgani	0-20 cm	-2.8 ± 1.5a	-2.2 ± 1.6a	NS	-21.2 ± 11.5a	13.6 ± 11.5a	13.7 ± 11.5a	NS

c carbon (Mg C ha-1)	0-50 cm	**-18.2 ± 5.7**a	-7.6 ± 5.3a	NS	___	___	___	
	0-80 cm	___	___		-15.4 ± 16.1a	25.0 ± 14.4a	16.4 ± 14.4a	NS
Δ Total soil carbon (Mg C ha-1)	0-20 cm	**9.5 ± 1.5**a	**6.7 ± 1.5**a	NS	-22.6 ± 11.3a	12.8 ± 11.3a	11.8 ± 11.3a	NS
	0-50 cm	7.8 ± 4.8a	9.0 ± 4.2a	NS	___	___	___	
	0-80 cm	___	___		-14.0 ± 13.4a	20.4 ± 11.9a	12.0 ± 11.9a	NS
Δ Total soil nitrogen (Mg N ha-1)	0-20 cm	**0.4 ± 0.1**a	-0.1 ± 0.1b	**	0.0 ± 0.1a	0.1 ± 0.1a	0.2 ± 0.1a	NS
	0-50 cm	0.3 ± 0.2a	-0.5 ± 0.2b	**	___	___	___	
	0-80 cm	___	___		-0.1 ± 0.2a	-0.2 ± 0.2a	-0.3 ± 0.2a	NS

[1] Values from 2018 were baseline stock measurements estimated at the block level.

4.7 Soil carbon respiration

There were no differences in seasonal cumulative soil respiration between treatments in SNP (Figure 11; Appendix 2 Table 4). However, soil respiration averaged across treatments was highest during the first experimental year when the N treatments were applied, and lowest in 2020. On average, cumulative soil CO_2 flux was 14% and 45% higher than 2019 and 2020, respectively, when averaged across treatments. Daily CO_2 flux rates were highest earlier in the growing season (April-July) and then steadily declined during late summer and early fall (Figure 11). These trends generally follow the annual temperature and precipitation patterns during each experimental year (Figure 7).

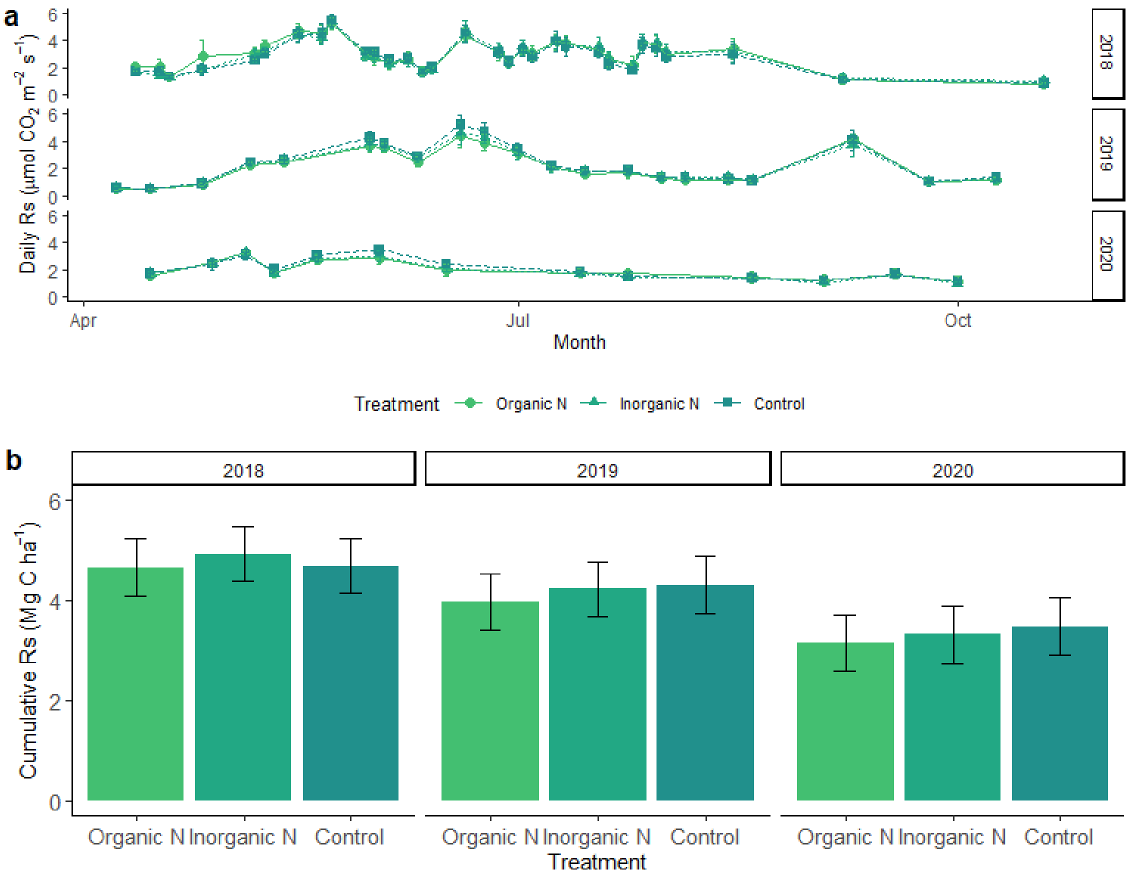


Figure 12. The mean a) daily soil respiration (Rs) (μmol CO_2 m^{-2} s^{-1}) and b) least squares means of cumulative soil Rs (Mg C ha^{-1}) in the semi-native dryland pasture (SNP) during a three-year experiment near Loveland, CO. Significant p-values for treatments within year; $P > 0.05$ (non-significant; NS), $P \leq 0.05$ (*), and $P \leq 0.01$ (**). Error bars represent standard error; n = 6.

5. Discussion

The application of organic N in the form of compost altered plant productivity and soil C and N dynamics in an irrigated, improved pasture (IP), but not in a neighboring semi-arid native pasture (SNP). The increased above and belowground plant C inputs in IP contributed to increased SOC stocks in the topsoil (0-20 cm), but total soil C only differed between treatments when measured from 0-10 cm over eight years. Otherwise, total soil C stocks did not differ between treatments or over time when measured over the entire soil profile (0-50 cm). However, total N stocks were consistently higher under the organic amendment in the top and full soil profile over time, and these persistent differences in total soil N may be a better indicator of change in soil organic matter in soils with highly variable inorganic C stocks.

Overall, the increases in soil organic C stocks in IP beyond the initial C inputs from the compost indicate that infrequent organic amendment applications in irrigated perennial pastures can increase SOC sequestration and total N stocks in the topsoil. Organic amendment applications to pastures also increase forage yields and offer potential as an additional revenue stream via carbon markets or soil health incentive programs (Basche et al. 2020). However, the lack of change in total soil C stocks below 10 cm and the high variability in SIC below 20 cm merits careful consideration of how carbon sequestration is accounted for in semi-arid ecosystems. In soils with significant contributions of SIC to total C, the attention given to SOC sequestration under NCS practices should expand to include total C sequestration impacts.

5.1 Organic amendments increase biomass production

We observed large, sustained increases in aboveground biomass after a single organic N application in IP despite interannual variation in temperature and irrigation rates. These findings are consistent with other experiments that measured pasture forage responses to organic N applications; in a global meta-analysis of 92 studies, Gravuer et al. (2019) found organic amendments in arid, semi-arid, and Mediterranean climates consistently increased aboveground biomass. However, the response in IP to N fertilization was stronger than SNP, which was likely a result of water limitation. While we observed initial productivity increases immediately after inorganic N application, these increases were not observed in the following years. Plant growth in arid and semi-arid climates is often limited by nitrogen availability (Hooper and Johnson

1999), but other co-limiting resources like water and phosphorus can impact plant responses (Chapin et al. 1987). Multiple, interacting resource limitations can inhibit growth of plant communities in grassland (Harpole et al. 2016) and shrubland ecosystems (James et al. 2005). In their global meta-analysis of grassland communities, DeMalach et al. (2017) found that simultaneous additions of nutrients and water led to larger biomass increases, approximately 69%, than either resource alone. The addition of water along with multiple other important plant growth limiting nutrient (i.e., nitrogen, phosphorus, potassium) through the organic N amendment may explain the observed differences in aboveground biomass response between the two experiments.

While our estimates of standing root biomass were likely conservative due to our sampling method, standing root biomass tended to be higher in the organic N treatment in IP from 0-20 cm relative to the unamended control during peak standing biomass. Ryals and Silver (2013) observed similar increases in root biomass under organic amendment application in annual grasslands, particularly from 0-10 cm. A global meta-analysis of root responses to N additions suggests that in grasslands, root production is not negatively impacted, and root turnover rate may actually increase (Peng et al. 2017). Increases in root turnover have important consequences for soil carbon accumulation. Root C, an important component of soil organic matter (SOM) especially at depth (Rumpel and Kögel-Knabner 2011) or where aboveground plant residues are not readily returned to the soil (Kramer and Gleixner 2008), increases soil C via the proximity of roots to mineral surfaces, physio-chemical protection through soil aggregate formation (Dungait et al. 2012), and root exudation (Rasse et al. 2005).

5.2 No differences in seasonal soil respiration

The application of organic and inorganic N did not increase seasonal cumulative soil respiration in SNP. These findings are contrary to previous findings; a study in California annual grasslands observed 18-19% increases in soil respiration over three-years after organic amendment application (Ryals and Silver 2013) while a global meta-analysis of fifteen publications and 119 observations of soil respiration measured in the lab found that organic amendments increased soil respiration relative to unamended soils. The lack of treatment differences could be a result of low nitrogen application rates in SNP; our application of organic N in SNP was approximately one-tenth of the application rate in Ryals and Silver (2013). Increases in soil respiration after organic amendment application are likely due to increases in root growth and root respiration, but also a result of the decomposition of labile organic inputs, particularly when C:N of the amendment is low, which favors fast growing soil microbial communities (Thangarajan et al. 2013). It is also possible that we did not capture treatment differences in soil CO_2 flux immediately after precipitation events, despite efforts to sample within 24-72 hours of these events. In dryland ecosystems, precipitation pulse events can stimulate large soil CO_2 fluxes, primarily attributable to heterotrophic respiration, which may be caused by the availability of accumulated, easily decomposed substrates (Inglima et al. 2009).

5.3 Soil organic carbon and nitrogen stocks respond to infrequent amendment applications

The infrequent application of organic N in the form of high-quality (low C:N) compost can increase SOC and total N stocks over short and medium durations (two to eight years) in irrigated, semi-arid agroecosystems. In an improved perennial pasture system in the Texas Southern High Plains, Otuya et al. (2020) observed increases in SOC and total N after one and a half years using composted animal manure applied at about half the rate (3.36 Mg ha^{-1}) as this study. In a three-year study in annual grasslands, Ryals et al. (2014) found that a single application of compost resulted in C sequestration of 3 Mg C ha^{-1}. They also found that amendment C and N, and likely new plant C, were incorporated into physically protected SOM pools. And, in their global meta-analysis, Gravuer et al. (2019) observed SOC increases in 233 of 244 observations, and these increases occurred in two years or less in half of these observations. However, in a similar irrigated pasture system in northern Colorado, Mikha et al. (2017) found that composted dairy manure applied at comparable rates as this study did not increase SOC or total N after two years. Similarly, the application of biosolids in nearby semi-arid rangelands increased total soil C and N in the top 8 cm of soil, particularly when applied at equal or greater rates than 21 Mg ha^{-1}, and increases in soil organic matter were still evident 13-14 years after initial application (Ippolito et al. 2010). In this study, there were differences in total C from 0-10 cm eight years after two organic amendment applications in IP. However, these changes were much more pronounced after the second application measured in 2020.

Several mechanisms may explain the observed increases in SOC, total soil C, and soil N in the topsoil in IP. First, increases in topsoil C and N, particularly from 0-10 cm, is likely directly related to the addition of the organic amendment. We did not observe large, visible quantities of compost residue on the soil surface in the second and third experimental years, indicating that the amendment was readily decomposed and incorporated into the topsoil through downward movement by precipitation and irrigation events. Soil microbial analyses undertaken at this site (Chapter 3) indicate that functional diversity in the top 10 cm of the soil shifted over the experimental period where microbial communities in the organic N treatment were better able to process labile C inputs like glucose and xylose. Microbial products from decomposition of labile C inputs are critical for forming stable SOM, particularly mineral associated organic matter (MAOM) (Haddix et al. 2016, Lavallee et al. 2018).

Because most of the aboveground inputs were removed from IP during haying operations, and the organic amendment was not directly incorporated into the soil, root inputs, favorable soil mineralogy, and possibly dissolved organic C were likely the important factors contributing to SOC accumulation in the topsoil. During the 2018 season, the organic amendment had greater root production than the unamended control and greater overall plant productivity under the amendment. The interaction of organic matter with silt and clay particles is an important C stabilization mechanism. Among multiple biotic and abiotic factors that influence SOC stabilization, soil texture, i.e., clay and silt content, is a key factor, but potential SOC storage also depends on C inputs (Wiesmeier et al. 2019). The interactions of C inputs with the soil mineral fraction, in addition to the molecular composition of the C inputs, and the accessibility of this material to the microbial community (Sollins et al. 1996, Sokol et al. 2019), all influence the transformation of plant and litter inputs into soil organic matter. Despite the increases in the topsoil, we did not observe a change over time in SOC or total soil C stocks from 0-50 cm over the experimental period in either the organic N treatment or the unamended control. The high variation in SIC at depth and the resulting impacts on total soil C stocks made it difficult to detect a treatment effect over the entire soil profile.

5.4 Soil inorganic carbon contributions to total carbon estimates

The quantification of net soil C sequestration rates in semi-arid and other regions with significant SIC stocks must consider how practices aimed at atmospheric GHG removal impact this important C pool in the short and long-term. Recommendations for measuring carbon sequestration for carbon dioxide removal focus exclusively on SOC (Billings et al. 2020, Smith et al. 2020), with little to no discussion of SIC. It is possible that management practices implemented to increase SOC sequestration decrease SIC stocks resulting in no net change or even a loss in total soil C.

At our site, SIC contributed considerably to total soil C stocks in these soils, particularly in the subsurface (> 20 cm), and the gains we observed in SOC in the topsoil were not enough to offset this variation leading to no change in total soil C over time below 10 cm. In IP, SIC stocks represented one-third of total soil C from 0-20 cm, but this increased to one-half in the 20-50 cm depth interval. In SNP, the contributions of SIC to total soil C from 0-20 cm was similar to IP, but it represented nearly 60% of total soil C from 0-80 cm. These proportions are similar to other cultivated soils in the Central Great Plains (Denef et al. 2008). Land management, particularly irrigation and fertilization, can influence the SIC cycle with consequences for ecosystem C cycling (Sanderman 2012). Irrigation of high pH soils can lead to soil carbonate formation through the stimulation of plant, root, and soil biotic activity (Denef et al. 2008). In contrast, frequent nitrogen fertilization can decrease soil pH, acidifying the soil, and can lead to substantial decreases in SIC over time (Raza et al. 2020).

We observed similar SIC stocks in the top 20 cm in both IP and SNP suggesting that irrigation of these soils did not lead to an accumulation of SIC in the topsoil, consistent with other studies in the region (Denef et al. 2008). However, the high variability in SIC in IP and SNP below 20 cm highlights the importance of carefully considering both the organic and inorganic C impacts of organic N additions to arid and semi-arid soils as well as the importance of measuring soil C stocks below the top 20 cm. While we did not observe differences between treatments in SIC losses under organic or inorganic N, the organic amendment in IP resulted in a loss of SIC over time since the first application in 2012 from 0-50 cm. The acidification of high pH soils through sustained inorganic nitrogen fertilization can decrease SIC stocks and, thus, total soil C in some regions if gains in organic C do not offset SIC losses (Wu et al. 2009, Raza et al. 2020). While organic amendments that increase SOC should help buffer pH changes, there is potential for acidification and SIC losses in naturally high pH soils common in semi-arid regions.

More broadly, subsoil organic and total carbon stock changes must be quantified under soil management practices considered as NCS. Historically, subsoil carbon stock changes are not quantified in agricultural soils due to difficulty and costs (Harrison et al. 2011). In a long-term study of arable soils, Gregory et al. (2016) observed significant subsoil C losses nearly sixty years after land-use change indicating that deeper soil horizons are susceptible to C losses even when they are not directly impacted by land management decisions. And, in their study investigating the use of organic inputs to increase soil C in row crop systems, Tautges et al. (2019) observed significant losses of subsoil carbon under cover cropping, but important gains under poultry manure additions. In our case, we did not observe a change in total soil C over the entire profile under between treatments or a change over time within treatments. While many studies quantifying soil C stock changes over time assume soils below 20 cm can be considered stable (Harrison et al. 2011), this can only be ascertained through long-term evaluation of soil C pools deeper in the profile.

5.5 Quantifying the climate benefits of organic amendments in semi-arid soils

The increase in organic soil C, total N, and plant productivity in IP indicates that moderate, infrequent applications of organic amendments to perennial pastures increases soil health and pasture production in irrigated semi-arid agroecosystems. High quality organic amendments, by supplying greater amounts of plant limiting nutrients simultaneously, like nitrogen, phosphorus, and potassium, can increase plant growth while providing long-term climate benefits (Gravuer et al. 2019). While we focus here on C and N, organic amendments contain other nutrients like phosphorus, that may also be limiting in this environment. Interestingly, we did not observe consistent treatment differences in IP until after the second application of the organic N treatment in 2018, which was similar in application rate and nutrient content as in 2012. But in the non-irrigated, semi-native pasture (SNP), where we applied organic N at just over half of the application rate in IP, we did not observe any differences between treatments over this same period. These findings suggest that dryland systems are likely to be less responsive to amendments due to water limitations, and that further research is needed to understand the optimal application rate and frequency of organic amendments to irrigated and dryland perennial pasture systems to maximize short and medium-term climate and soil health goals. Further, there is a need to research the economic costs and benefits of these management decisions, and the ability to leverage additional revenue streams from conservation programs and carbon markets is necessary to increase producer adoption (Basche et al. 2020).

The impacts of organic N applications on soil N_2O and a complete life cycle assessment of feedstock diversion for organic amendment production merits further investigation. Agricultural soils are a significant contributor to global N_2O emissions (IPCC 2019), and the application of excess N can stimulate N_2O emissions enough to offset soil C gains and perceived climate benefits (Zhou et al. 2017, Guenet et al. 2021). While amendment application did not increase soil N_2O in lab incubations (Ryals and Silver 2013), the addition of crop residues and organic materials to agricultural soils can stimulate soil N_2O emissions via increased N supply and promoting anaerobic conditions needed for N_2O production (Chen et al. 2013). However, modest N additions that stay within the crop N requirements may not substantially impact N_2O emissions (Shcherbak et al. 2014), and composts along with other stable organic amendments are likely low risk for increased soil N_2O emissions (Charles et al. 2017). High frequency N_2O flux sampling (Parkin 2008) supplemented with modeling (Giltrap et al. 2020) can help determine the soil N_2O impacts of amendment application in semi-arid systems. Finally, life cycle assessments of the alternative uses of amendment feedstocks (e.g., manure, landfill, anaerobic digestion) can help determine the full environmental footprint of organic amendment applications (DeLonge et al. 2013).

6. Conclusion

Moderate, infrequent applications of high-quality compost increased plant production and topsoil total soil C and SOC stocks in an irrigated, improved perennial pasture system in northern Colorado. Aboveground biomass increased by almost 40% over the experimental period, and SOC stocks – after subtracting C additions from the amendment and accounting for the changes over time in the unamended control – increased over time by 0.46 Mg C ha^{-1} yr^{-1} from 0-20 cm, but there were no changes in soil C stocks when measured throughout the entire profile. Total soil N stocks demonstrated consistent and persistent increases under the organic amendment after two applications over eight years.

Application of the same amendment at a lower rate in an adjacent non-irrigated, semi-native rangeland pasture did not increase plant production or soil C or N stocks. However, it is possible that the experimental duration was too short to observe changes in this lower productivity system, and that similar pasture types may require higher or more frequent application rates to stimulate a soil C response, which may not be economically feasible without additional incentives. The increases in plant and SOC in the improved pasture indicates that organic amendment additions to low-input irrigated pastures in semi-arid climates can enhance soil health and pasture production. But from a natural climate solution perspective, more work is needed to understand the total soil carbon impacts of organic amendment applications to semi-arid soils.

CHAPTER 3: INFREQUENT ORGANIC NITROGEN APPLICATIONS TO PERENNIAL PASTURES INFLUENCES PLANT AND SOIL COMMUNITIES AND THEIR INTERACTIONS

1. Summary

Managed grasslands are globally important reservoirs of soil organic carbon. Overgrazing and climate change inhibit the ability of these ecosystems to deliver critical ecosystem services and sustain rural livelihoods. Organic amendment application in the form of compost is one of several improved grassland management strategies recommended to improve soil organic matter, soil nutrient cycling, and forage production providing climate and environmental co-benefits. However, the potential impacts on plant and soil microbial communities and their functions remains understudied in semi-arid perennial pasture systems. During a three-year experiment, we examined plant and soil dynamics after the application of a single addition of organic, addition of inorganic nitrogen or no addition (control) to an improved, irrigated pasture and a non-irrigated, semi-native rangeland, respectively. The improved pasture responded positively to the addition of organic N over the experimental period, while the semi-native pasture was less responsive. In the improved pasture, aboveground plant production was 34-40% higher relative to the unamended control, and root biomass measured to 20 cm also increased immediately following application. Plant diversity and species evenness increased in the organic N treatment, and noxious weed relative abundances showed either no change or small decreases over the period. Belowground, total PLFA microbial biomass and bacteria biomass increased over time in the improved pasture under organic N. The respiration of labile plant substrates, i.e., glucose and xylose, was higher in the organic N treatment; these plant carbon compounds are often

associated with root exudation suggesting that the microbial community may be responding to changes in plant carbon allocation rather than directly responding to the added carbon from the organic amendment. We also observed decreases in soil pH and increases in plant available soil phosphorus and potassium. The stronger initial plant responses and the gradual change in microbial community composition and function suggests a plant-mediated response to organic N in this system, which may impact soil carbon cycling. The increase in provisioning and regulating ecosystem services and the absence of major ecological tradeoffs indicates infrequent application of high-quality organic amendments can benefit low-input irrigated perennial pastures. Water-limited, semi-native pastures appear to be more resistant to change under one-time organic and inorganic N additions.

2. Introduction

Grazed grasslands and pastures represent nearly sixty percent of the global land area occupied by agriculture (Goldewijk et al. 2017), but more than a quarter of the soils in these ecosystems are classified as degraded (Bai et al. 2008). Managed grassland ecosystems are also under threat from overgrazing (Mysterud 2006), land use change (Li et al. 2018), alterations in precipitation patterns and amount (Knapp et al. 2020), changing fire regimes (Brooks et al. 2004), and invasive alien species (Firn et al. 2013) that can reduce biodiversity and the delivery of ecosystem services (Bengtsson et al. 2019). The improved management of grassland soils and ecosystems can build soil organic matter (SOM) and boost soil health, simultaneously enhancing the carbon (C) capture and sequestration potential across vast land areas while increasing the resilience of these ecosystems to anthropogenic drivers of global change (Bossio et al. 2020). Management practices like improved grazing, seeding with legumes, and inorganic and organic nitrogen (N) fertilization (Conant et al. 2001, Conant and Paustian 2002, Conant et al. 2017) increase plant C inputs by alleviating N limitation (LeBauer and Treseder 2008, Fay et al. 2015), and in the case of organic amendments, phosphorus (P) and other nutrients, which can also constrain plant production responses in grasslands (Menge and Field 2007).

The application of organic amendments, which add N, P, and other plant growth limiting nutrients coupled with C, can improve soil C accrual and forage production without increasing non-CO_2 trace gas emissions, resulting in a net climate benefit (Ryals and Silver 2013, Ryals et al. 2014). The use of organic amendments, such as composts and biosolids, presents an opportunity to create a circular nutrient economy by recycling C and nutrients like N and P from municipal and feedlot waste streams back to the land (Butler and Muir 2006, Ippolito et al. 2010, DeLonge et al. 2013). Moreover, improvements in soil structure from surface-applied compost can increase water infiltration and reduce surface runoff, thus increasing the available water for plant production (Bakr et al. 2015), with positive feedbacks on plant C uptake and residue C inputs to sustain higher soil organic C (SOC) stocks. Strategic use of organic nutrient sources in perennial agroecosystems like grazed pastures and hay lands can restore these and other agroecosystem functions (Drinkwater and Snapp 2007), aligning with several of the United Nations Sustainable Development Goals (Keesstra et al. 2016).

In Colorado, unimproved or semi-native grasslands and shrublands that include grazing by domestic livestock represent forty-six percent of the land cover, and when combined with agricultural land, these two land cover types represent almost two-thirds of land cover in the state (Failey and Dilling 2010). In 2019, total agricultural acreage in Colorado was 31.8 million acres with hay and alfalfa production, i.e., improved pastures, representing only 1.4 million acres, or

4.4% of total agricultural production acreage (USDA NASS 2020). While the total acreage dedicated to hay and alfalfa production in Colorado is relatively small, hay production value in 2019 was 934.6 million USD, which was 300 million USD and nearly 600 million USD greater than corn and wheat production values in the state, respectively (USDA NASS 2020).

Enhanced invasibility following nutrient additions of annual grasses such as cheatgrass (*Bromus tectorum* L.) and invasive forbs like field bindweed (*Convolvulus arvensis*), both Class C noxious weeds in Colorado (CDA 2020), is a major concern in these types of low-input grasslands (Gross et al. 2005). The potential for unintended impacts to plant and microbial communities must be better understood before considering widespread applications of organic N to improved and unimproved managed grasslands (Gravuer et al. 2019). Greater N loading in terrestrial ecosystems can alter above and belowground community composition and function (Pardo et al. 2011).

At the local scale, alterations in plant community composition and functional traits can modify net primary production, forage availability, and forage suitability for cattle (Augustine et al. 2017). More broadly, these shifts can impact ecosystem level evapotranspiration diminishing ecosystem resilience to climate change (Knapp et al. 2008). There is concern that fast growing invasive annuals like *B. tectorum* may respond more positively to N enrichment than native short-grass species (Lowe et al. 2003). The invasion of *B. tectorum* in native perennial grasslands has substantially altered fire regimes, C cycling, and higher trophic level diversity (Pimentel et al. 2000). In a composted biosolids application experiment in a Colorado semi-arid grassland, compost actually decreased exotic species in the highest application treatment (Ippolito et al.

2010). Likewise, a one-time application of green-waste compost to California annual grasslands increased productivity but did not increase the prevalence of exotic plant species (Ryals et al. 2016).

Belowground, changes in total microbial biomass and functional groups can enhance or suppress soil nutrient cycling, decomposition, and beneficial plant-microbe interactions. A meta-analysis of five biomes including temperate grasslands found that total microbial biomass, especially fungal biomass, decreased an average by 15% with N enrichment and these decreases were more pronounced when the total N applied was higher and over long periods of time (Treseder 2008). The decreases in total microbial biomass suppressed soil CO_2 emissions and slowed SOM decomposition (Treseder 2008, Riggs and Hobbie 2016). However, the impact of N addition on microbial functional groups varies considerably. Inorganic N addition experiments to semi-arid grasslands have observed a strong negative response of actinomycetes, positive responses by total bacteria and gram-positive bacteria as an entire functional group, and limited response by fungi to N and water additions (Wang et al. 2017b). Actinomycetes contribute to important soil functions like plant nutrient availability and organic matter cycling helping to promote plant growth (Bhatti et al. 2017), and gram-positive bacteria, more broadly, provide these and other functions like biocontrol and bioremediation (Francis et al. 2010).

The objectives of our study were to quantify how a single addition of organic or inorganic N in irrigated and dryland perennial pasture systems in Colorado would impact 1) plant production, 2) plant and microbial community responses, and 3) the relationships between plants, microbial communities, and soil properties. We investigated above and belowground responses during three growing seasons from 2018-2020 at a field site in northern Colorado.

3. Methods

3.1 Study site

3.1.1 Site description

A complete description of the site location and the two experiments is contained in Chapter 2. Briefly, two simultaneous field experiments were conducted in Loveland Colorado, USA (40° 24' 26.7156" N, 105° 11' 16.6272" W). The first experiment was on an irrigated perennial pasture (improved pasture; IP), and the second experiment on an adjacent non-irrigated semi-native rangeland (semi-native pasture; SNP). We collected field data for three growing seasons from 2018 to 2020 in both experiments. The climate is semi-arid; the growing season is from March-April through October. The 30-year mean annual precipitation is 421 mm and the mean annual temperature is 9.5°C (4 km resolution; PRISM Climate Group 2020). Daily precipitation and air temperature data were collected from an onsite weather station installed in August 2018 (Figure 7). The soils in the study region are mesic Aridic Arguistolls, and the parent material is alluvium derived from sandstone and shale. More details on baseline soil characteristics are provided in Table 10.

The first experimental site, IP, was irrigated by a center pivot beginning in late April or early May through mid-September at an average annual rate of 592 mm ha^{-1}. The pasture forage was a mixture of cool-season grasses (e.g., *Bromus inermis*) and legumes (e.g., *Medicago sativa*). The pasture was cut once annually for hay in June or July. There was periodic grazing by beef cattle in the winter and early spring.

Table 10. Baseline mean ± SE soil properties of each field experiment measured in 2012 (IP) and 2018 (SNP). The reported soil carbon (C) and nitrogen (N) stocks were quantified using equivalent soil mass. IP: improved pasture; SNP: semi-native pasture.

Field experiment	Depth (cm)	Clay (%)	Silt (%)	Sand (%)	pH	Bulk Density (g cm^{-3})	Soil organic C (Mg C ha^{-1})	Soil inorganic C (Mg C ha^{-1})	Soil N (kg N ha^{-1})
IP	0-10	34.6	19.9	45.4	7.8	1.01 ± 0.01	18.9 ± 0.8	10.5 ± 1.9	2.3 ± 0.05
	10-20	34.3	19.3	46.5	8.0	1.14 ± 0.01	12.3 ± 0.5	11.1 ± 1.9	1.8 ± 0.04
SNP	0-10	44.7	23.0	32.3	8.1	1.33 ± 0.05	19.5 ± 1.0	7.0 ± 0.8	2.1 ± 0.1
	10-20	45.2	21.2	33.7	8.3	1.40 ± 0.04	13.7 ± 1.0	10.8 ± 0.8	1.7 ± 0.1

3.1.2 General ranch management

The first experimental site was an irrigated, perennial pasture seeded in 2007 that was a mixture of cool-season grasses and legumes (i.e., *Bromus spp.*, *Dactylis glomerata,* and *Medicago sativa*). The pasture was primarily managed for hay production, but it was also grazed in late autumn, winter, and early spring by beef cattle. Depending on spring rainfall, irrigation began in April or May of each year. Irrigation water was delivered by a center pivot and average annual application was 592 mm ha^{-1}. More details pertaining to annual irrigation rates and frequency during the experimental period is described in Table 6.

The second experimental site is a non-irrigated, semi-native perennial pasture that was not seeded and was occasionally harrowed in lower elevation areas to improve soil aeration and pasture productivity. The non-irrigated pasture was comprised of a mixture of cool and warm-season grasses, legumes, forbs, and shrubs (i.e., *Bromus spp.*, *Thinopyrum intermedium*, *M. sativa*, *Lepidium virginicum, A. frigida*). The pasture was managed for beef cattle grazing, which varied in timing and frequency.

3.1.3 Experiment design – Improved pasture (IP)

The field experiment in the irrigated pasture (hereafter improved pasture, IP) was established in 2012 and continued until July 2020. There were two levels of treatment – organic N and control –with eight replicate blocks of 10 x 10 meter plots (Figure 8). The organic N treatment was applied at two time points; the first application occurred in March 2012 and the second in March 2018 just prior to pasture green-up in early April (Table 6). The organic N treatment consisted of commercially available composted livestock manure (Humalfa; Iliff, Colorado, USA) with a C:N ratio 8. The amendment was applied at a rate of 11.2 Mg ha^{-1} by compost spreader, which was equivalent to 123 kg N ha^{-1}, 974 kg organic C ha^{-1}, 171 kg P ha^{-1}, and 213 kg K ha^{-1}. The control plots that did not receive the organic amendment treatment were carefully marked and given a 5-meter buffer to avoid any potential spread of compost by wind. A description of the compost management and nutrient composition is provided inTable 6. All plots were cut for hay in late June or early July each summer. Cattle grazing varied annually, but the entire irrigated pasture area, including research plots, were continuously grazed in the winter.

3.1.4 Experiment design – Semi-native dryland pasture (SNP)

The second experiment was on semi-native dryland pastures (hereafter SNP) adjacent to IP (Figure 8). It was established in March 2018 and continued until October 2020 as a randomized complete block design with three levels of treatment – organic N, inorganic N, and control – and six replicate plots measuring 70 x 30 m. Both the organic and inorganic N treatments were applied once at the start of the experimental period. The organic N treatment was comprised of the same compost applied in the IP experiment. The application rate was lower than in IP due to the lower expected productivity of the site and to be more realistic in terms of the level of potential investment a rancher might make on semi-native range. The organic amendment was applied at a rate of 6.7 Mg ha^{-1} by compost spreader on March 25, 2018, which was equivalent to 74 kg N ha^{-1}, 583 kg organic C ha^{-1}, 103 kg P ha^{-1}, and 127 kg K ha^{-1}. The inorganic N treatment was intended to provide a similar amount of available mineral N as the compost treatment during the first year after application. We assumed that 25% of the N applied in the organic N treatment was bioavailable during the first growing season (Colorado State University Extension 2015). Urea N was applied by hand approximately two weeks after the organic N treatment in April 2018 at a rate of 20 kg N ha^{-1}. Additional descriptions of the organic and inorganic N amendments can be found in Table 6.

Grazing in the SNP by beef cattle occurred during each experimental year but it was managed independently by a different leasee each year. The stocking rate generally ranged from 15.8 – 23.8 head ha^{-1}. In 2018 and 2019, beef cattle were allowed access once in early fall (September) to two blocks and rotated through the experimental blocks every 10-14 days until all blocks were grazed. In these two years, grazing of SNP plots typically ended in mid-December or early January. In 2020, beef stockers were allowed access to only four of the blocks in late spring.. Cattle were rotated through the blocks for up to seven days in late April and early May. A description of grazing management for SNP is available in Table 6.

3.2 Aboveground biomass measurements

Across both experiments, biomass data were collected from 2018 to 2020 at peak biomass and before haying or grazing events. In IP, aboveground biomass samples were collected after the second compost application in 2018 at peak standing biomass and prior to haying in June or July

(Table 6). Biomass was clipped a few centimeters above the soil surface to avoid contamination by manure or soil from two 0.25 m^2 quadrats from each plot. For SNP, aboveground biomass was collected at peak standing biomass, which occurred in late July or early August over the experimental period.

Immediately after collection, aboveground biomass samples were dried at 55°C for a minimum 48 hours to obtain mass on an oven-dried basis. Samples were then finely ground with a ball mill and analyzed for C and N concentration using an LECO Tru-Spec CN analyzer (Leco Corporation; St. Joseph, Minnesota, USA).

3.3 Measurements of plant diversity and abundance

Annual vegetation surveys were made during each experimental year immediately prior to haying (IP) or during peak biomass (SNP). Surveys were conducted using the step-point method described by Woensby (1973). Briefly, a triangular metal frame was lowered perpendicularly to the soil surface at a 30° angle. A single plant species was recorded at every sampling interval nearest to the point frame in a 180° arc.

In IP, plant species data and basal cover were collected along two randomized transects every 0.25 m for a total of 50 sampling points per plot. Similarly, samples were collected along two randomized transects every 0.5 m for a total of 100 sampling points per plot in SNP given the larger area of these plots. These data were used to estimate relative abundance of plant species, species richness and evenness, and α-diversity (i.e., Shannon Index).

3.4 Belowground biomass measurements

Belowground biomass was determined using the soil core method (Milchunas 2009). An analysis of the depth distribution of roots (0-50 cm) prior to the first compost application in IP in 2012 indicated that less than 20% of roots occurred below 20 cm. Thus, annual belowground biomass measurements were made at two intervals: 0-10 cm and 10-20 cm. For IP, four soil cores (4 cm diameter) were collected per plot during each experimental year two weeks after haying to capture the belowground response to this disturbance event. In SNP, five soil cores were collected per plot in 2018 two weeks after the grazing event. Samples were not collected in 2019 due to the delay of grazing into the fall and winter months in SNP when the soil was frozen. In 2020, we were unable to sample after the grazing events in the spring, but soil cores were collected in mid-September 2020 which was consistent with the sampling timeframe in 2018.

All soil cores were bulked in the field and stored in coolers until returned to the lab. After weighing the entirety of the sample and removing a subsample for gravimetric water content (GWC) analysis, soils were stored at 4°C for no more than four months until processing. Roots were extracted by hand at two different sieving stages. First, all large visible roots (i.e., coarse roots) were extracted before passing the entire sample through an 8 mm sieve. In a second stage, remaining roots (i.e., fine roots) were extracted from a 150 g air dried soil subsample before passing through a 2mm sieve. At both stages, soil was carefully removed from the roots before drying at 55°C for a minimum of 48 hours and weighing. On average, roots were extracted for a minimum of 30 minutes per size class stage for a total minimum cumulative time of 60 minutes per sample. We multiplied the dry mass of the root samples by a 45% C concentration factor (Ma et al. 2018) to obtain C content of belowground biomass.

3.5 Soil microbial diversity measurements

To assess the soil microbial community response to management, we analyzed phospholipid fatty acids (PLFA). In both IP and SNP, eight soil cores with a core diameter of approximately 1.3 cm were collected per plot to a depth of 10 cm. These samples were collected twice annually: once in the spring during vegetation "green-up" and two weeks after a haying or grazing event (Table 6). Soil cores were bulked in the field and kept in coolers until returned to the lab. After determining GWC, soils were sieved to 2mm within one week of collection and subsamples stored at -80°C before being freeze-dried. After freeze drying, subsamples were thoroughly picked for roots to avoid confounding with fungal biomarkers. PLFA subsamples were then shipped to Ward Laboratory, Inc. (Kearney, Nebraska, USA) for extraction and separation as described in Rosenzweig et al. (2018) that was adapted from (Hamel et al. 2006). Fatty acid biomarkers were categorized into the following microbial functional groups: 16:1 ω5c (arbuscular mycorrhizal fungi) and 18:2 ω6c (saprophytic and ectomycorrhizal fungi) were grouped as fungal biomass; 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, and 17:0 anteiso, represented gram-positive bacteria; 16:1 ω7c, 17:1ω8c, 18:1 ω7c, and 18:1 ω5c were grouped as gram-negative bacteria; 16:0 10-methyl, 17:0 10-methyl, and 18:0 10-methyl were grouped as actinomycetes (Frostegård and Bååth 1996, Zelles 1999, Frostegård et al. 2011). Total bacterial biomass also included the straight-chain saturated fatty acid biomarkers 15:0 and 17:0. All functional groups were analyzed individually to ascertain treatment effects on specific groups of organisms. The abundances of bacterial and fungal biomarkers up to 20C chains and including no more than two branches were summed to represent total microbial biomass (Quideau et al. 2016, Rosenzweig et al. 2018) for a total of 67 biomarkers in IP and 60 biomarkers in SNP.

Soil samples collected after haying or grazing disturbance were also assessed for microbial community functional diversity using the MicroResp® method of Campbell et al. (2003) and described in Delgado-Baquerizo et al. (2017). MicroResp® is a community-level physiological profile technique (CLPP) that measures basal respiration and substrate-induced respiration. Sieved soils were incubated for 72 hours at 25°C and 60% water-filled pore space (WFPS) before MicroResp® analyses. Two lab replicates per sample were incubated with deionized water (basal respiration) and five additional substrates: glucose, cellulose, xylose, glucosamine, and lignin. After the addition of the substrates, the samples were left uncovered for one hour prior to incubation to remove abiotic CO_2 due to the high pH of these soils. Samples were then covered and incubated for six hours and read at 570 nm on a microplate absorbance reader (Bio-Rad Laboratories; Hercules, California, USA). The substrate-induced respiration is calculated as the respiration in the substrates (e.g., glucose) minus basal respiration.

3.6 Soil bulk density, texture, and nutrient content

In IP and SNP, soil bulk density, texture, and nutrient measurements were made on the same soil samples collected for root biomass. We collected cores (4 cm diameter) with a truck mounted Giddings hydraulic soil probe Giddings Machine Company; Windsor, Colorado, USA). For bulk density, we collected five cores in IP and SNP (0-10, 10-20 cm). The 2012 baseline soil sampling regime in IP is described in Chapter 2. Soil water content was determined gravimetrically by a subsample before and after drying at 105°C. Bulk density estimates were mass corrected for coarse particles > 2mm diameter following the recommended approach for dryland soils in Throop et al. (2012). The total dry mass of the sample was then multiplied by the volume of each core and the number of cores per samples to estimate 2mm mass corrected bulk density.

Soil texture, phosphorus, and potassium analyses were conducted by Ward Laboratories Inc. (Lincoln, Nebraska, USA) on the same cores collected for root biomass. Soil texture was determined by hydrometer (Gee and Bauder 1986) and pH by a 1:1 slurry of soil and deionized water. Available soil P content was extracted via the Mehlich-3 and soil available K was determined by the exchangeable K test.

3.7 Statistical analyses

We analyzed all plant and soil data using linear mixed models in R version 3.6.3 (R Core Team 2013) using the *lme4* package (Bates et al. 2014). The two experiments (IP and SNP) were analyzed separately. We assessed change over time using repeated measure multivariate analysis of variance (MANOVA) where there were multiple years of observations. Treatment and year were fixed effects and block and plot were random effects. Otherwise, we applied one-way analysis of variance (ANOVA) to test for statistically significant treatment effects; block was included as a random effect. Degrees of freedom were corrected according to the method recommended by Kenward and Roger (1997). To meet normality assumptions, MicroResp® data were log-transformed and error estimates were reported as 95% confidence intervals. We removed outliers in rare instances when they exceeded the upper 75% or lower 25% quartile ranges (generally impacting less than 6% of samples per variable). Significant differences between treatments and treatment by year were assessed with pairwise comparisons using the *emmeans* package (Lenth 2020) in R to estimate least-squares means. Data are reported as least squares means followed by standard error unless otherwise noted. We determined statistical significance at $P \leq 0.10$.

The α-diversity of plant communities was assessed using the Shannon Diversity index (Shannon 1948, McCune et al. 2002):

$$H' = \sum_{i=1}^{R} p_i ln p_i \tag{1}$$

where p_i is the proportion of individuals of the *i*th species and R is species richness.

We evaluated the effect of treatments on individual plant species relative abundances by calculating the log response ratio (Hedges et al. 1999, Stein et al. 2010, Ryals et al. 2016) for each plant species as:

$$lnRR_i = ln\left(\frac{RA_{treatment_i}}{RA_{control_i}}\right) \tag{2}$$

where $lnRR_i$ is the log response ratio of relative abundance for $species_i$, $RA_{treatment_i}$ and $RA_{control_i}$ refer to the average species relative abundance in the treatment and control plots, respectively. The lnRR were estimated individually for each block and averaged over all years. When there were zeros in the relative abundance dataset, we treated these values as missing since it is not possible to estimate lnRR. The species included in our lnRR represented 98% (five species) of the relative abundance in IP and 97% (10 species) of the relative abundance in SNP. These species occurred in ≥ 10% of plots across all years. A more positive value indicates the treatment plot had greater relative abundance, and a negative value indicates the treatment plot had lower relative abundance than the control. To test for significant differences, we conducted t-tests for each species to test if lnRR was different from zero (i.e., no difference between treatment and control).

We evaluated PLFA and plant community data separately for each experiment using non-metric multidimensional scaling (NMDS) using the *vegan* package in R (Oksanen et al. 2019). We included the PLFA community data, microbial induced respiration, plant, and soil data collected in the summer of each year from 0-10 cm. The same plant species used in each experiment for our lnRR analysis were used in the multivariate analysis. We removed one outlier plot from both of the IP and SNP data sets prior to analysis. The distance matrix was estimated using the Sorenson (Bray-Curtis) index with 20 random restarts and two axes. Stress values < 20 were considered acceptable for the final NMDS solutions (McCune et al. 2002). Environmental variables were overlaid on the final NMDS solution and we estimated Spearman-rank correlations with the axes using the *envfit* function due to non-normal distributions of the species variables. After NMDS, we evaluated different species groupings by treatment and year by implementing permutational MANOVA (PERMANOVA) using the *adonis* function on the species Sorenson (Bray-Curtis) dissimilarity matrix (Anderson 2001). Species groupings were considered significant at $P \leq 0.10$. To evaluate pairwise comparisons when there were more than two groups, we applied the *pairwise.adonis2* function (Martinez Arbizu 2020). All PERMANOVA models were stratified by block.

4. Results

4.1 Annual weather and irrigation

A description of annual weather and irrigation management can be found in detail in Chapter 2.

4.2 Aboveground biomass

In IP, the organic N treatment increased aboveground biomass production (Figure 9, Figure 13). In 2018 and 2020, aboveground biomass C and N were greater where compost had been applied than the unamended control. Average aboveground biomass between the two treatments was about 29% lower in 2019 than in 2018 and 2020 (Appendix 3 Table 1), likely due to water limitation, and we did not observe any differences between treatments in 2019.

In SNP, the inorganic N treatment resulted in greater aboveground biomass N during the first experimental year relative to the control, but this relationship reversed in the third experimental year (Figure 13; Appendix 3 Table 1). In 2018, aboveground biomass C (Figure 9) was around 35% higher in the inorganic N treatment, but in 2020 the inorganic N treatment was 23% lower than the control (*P* = 0.06). There were no differences in aboveground biomass C between any other treatment comparisons. Aboveground biomass N was also about 14 kg N ha^{-1} higher in the inorganic N treatment relative to the control in 2018 and 12 kg N ha^{-1} higher in the organic N treatment relative to the control (Figure 13), but there were no other treatment differences during the experimental period.

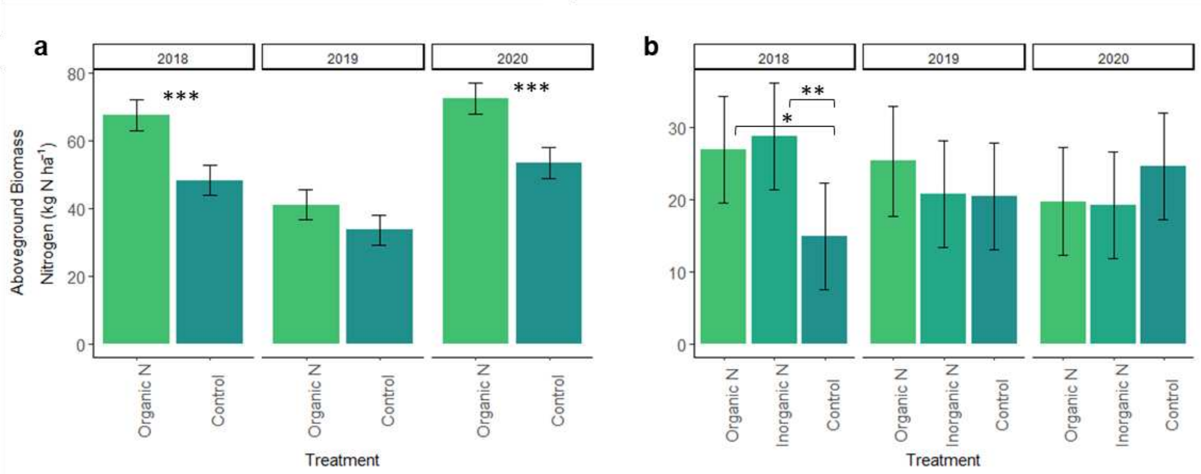


Figure 13. Least squares means of aboveground biomass nitrogen (kg N ha^{-1}) responses for a) improved pasture (IP) and b) semi-native pasture (SNP) during a three-year experiment near

Loveland, CO. Significant p-values for treatments within year; $P > 0.1$ (non-significant; NS), $P \leq 0.1$ (*) , $P \leq 0.05$ (**), and $P \leq 0.01$ (***). Error bars represent standard error; IP (n=8) and SNP (n=6).

4.3 Plant diversity and species composition

In IP, addition of organic N to these pastures changed plant community composition during the experimental period, but it did not increase the prevalence of noxious weeds. The organic N treatment led to decreases in the relative abundances of *Bromus* spp., which includes both *B. commutatus* and *B. inermis,* relative to the control (Figure 14a). The relative abundance of *D. glomerata* increased in the organic N treatment relative to the control. On average, the organic N treatment led to a decrease of approximately 13% in *Bromus* spp. and more than a 250% increase in *D. glomerata* relative abundances. We identified the presence of one Class C noxious weed (CDA 2020), *C. arvensis*. The relative abundance of this species did not change (Figure 14a) with the addition of the organic N.

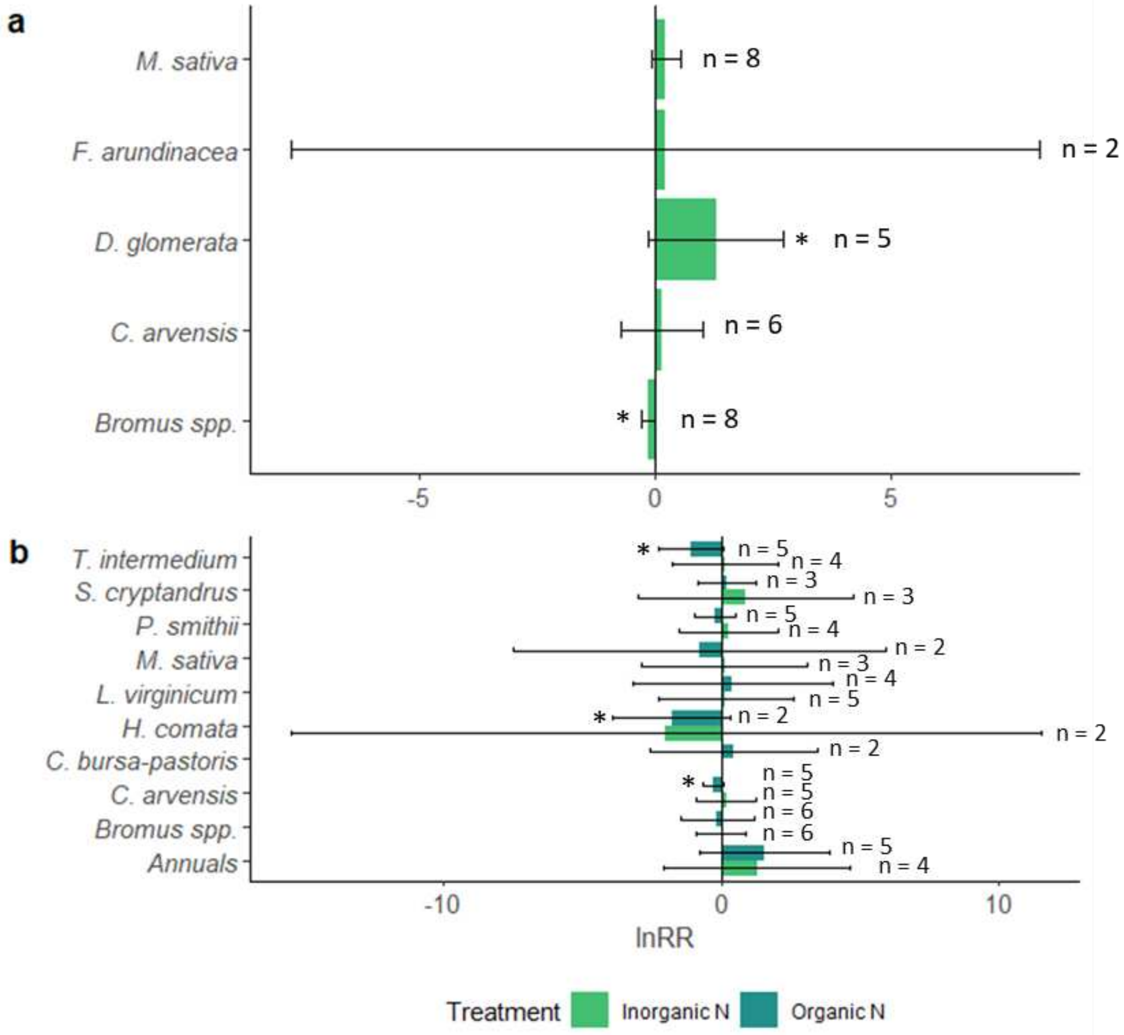


Figure 14. The impact of organic and inorganic N treatments on the relative abundances of plant species with ≥ 10 percent of plot occurrences across a) improved pasture (IP) and b) semi-native pasture (SNP) during a three-year experiment near Loveland, CO. The bars are paired treatment means of the log response ratios (lnRR) averaged over three-years. Positive values indicate an increase in relative abundance compared to the unamended control, and negative values indicate a decrease in relative abundance compared to the unamended control. Significant p-values for treatments relative to control; $P > 0.1$ (non-significant; NS), $P \leq 0.1$ (*) , $P \leq 0.05$ (**), and $P \leq 0.01$ (***). Error bars represent 95% confidence intervals. Plant species abbreviations: Annuals (*Bromus secalinus*, *B. tectorum*), *Bromus* spp. (*B. commutatus*, *B. inermis*), *C. arvensis* (*Convolvulus arvensis*), *C. bursa-pastoris* (*Capsella bursa-pastoris*), *D. glomerata* (*Dactylis glomerata*), *F. arundinacea* (*Festuca arundinacea*), *H. comata* (*Hesperostipa comata*), *L. virginicum* (*Lepidium virginicum*), M. sativa (*Medicago sativa*), *P. smithii* (*Pascopyrum smithii*), *S. cryptandrus* (*Sporobolus cryptandrus*), *T. intermedium* (*Thinopyrum intermedium*).

In SNP, the organic N treatment had lower relative abundances of *Hesperostipa comata* and *Thinopyrum intermedium* than the control (Figure 14b). There were no differences in species relative abundances between the inorganic N treatment and the control. The widespread invasive species *C. arvensis,* and *B. tectorum* and *Bromus secalinus* (grouped together as "invasive annuals") were present in SNP prior to the start of the experiment. We did not find meaningful increases in these species with addition of inorganic N, but the organic N treatment led to a 31% significant decrease in *C. arvensis* relative to the control (Figure 14b).

Aboveground plant diversity, species richness, and species evenness were higher during the experimental period in the organic N treatment in IP (Figure 15a). Across all years, the average Shannon H for the organic N treatment was about twice that of the control, 0.61 ± 0.09 versus 0.37 ± 0.09, respectively. There were smaller differences in species evenness and richness between the two treatments (Appendix 3 Table 1). In 2019, which was the driest year, we observed the greatest differences between the two treatments across all diversity measures. There were no differences between treatments or years in SNP for plant diversity, species richness, or species evenness (Figure 15b).

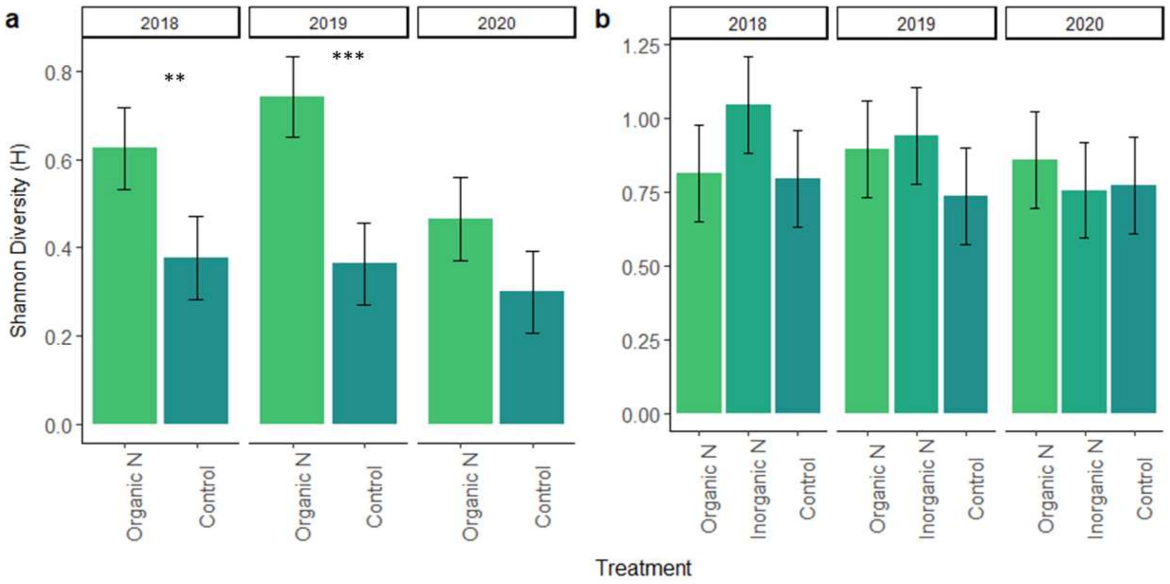


Figure 15. Least squares means of Shannon diversity (H) for a) improved pasture (IP) and b) semi-native pasture (SNP) during a three-year experiment near Loveland, CO. Significant p-

values for treatments within year; $P > 0.1$ (non-significant; NS), $P \leq 0.1$ (*) , $P \leq 0.05$ (**), and $P \leq 0.01$ (***). Error bars represent standard error; IP (n=8) and SNP (n=6).

4.4 Standing root biomass

The application of organic N led to changes in standing root biomass allocation. Coarse root biomass and total root biomass from 0-10 cm was greater in the organic N treatment (Figure 9a, Appendix 3 Table 2). There were no differences in the fine root fractions at any depth or total belowground biomass from 0-20 cm, which included both coarse and fine root fractions (Appendix 3 Table 2).

In SNP, there were no differences in total belowground biomass or the coarse root fraction by depth between treatments (Figure 9b; Appendix 3 Table 2). Like the aboveground biomass C in SNP, we found that fine roots in the inorganic N and organic N treatments were higher in 2018 but lower in 2020 than the control from 10-20 cm (Appendix 3 Table 2). In 2018, fine roots at this depth interval were an average of 42% higher in N treatments, but 28% lower in 2020 relative to the unamended control.

4.5 Microbial PLFA

There were no differences in microbial biomass or PLFA functional groups during the first two experimental years in IP. In 2020, total microbial biomass and bacterial biomass were higher in the organic N treatment. Total microbial biomass was 24% greater in the organic N treatment compared to the control after the hay harvest in 2020. We observed 19% greater bacterial biomass in the organic N treatment relative to the unamended control prior to the start of the growing season in 2020 (Appendix 3 Table 3), and the same pattern after hay harvest in 2020 where total bacterial biomass was 22% greater (Table 11). The differences in bacterial biomass in 2020 were likely driven by an increase in actinomycetes biomass. We observed 27% greater actinomycetes biomass in the organic N treatment in spring 2020 (Appendix 3 Table 3), and a similar pattern after hay harvest although these differences were not significant (Table 11).

We did not observe any differences in microbial biomass or functional groups in SNP except for gram-positive to gram-negative bacteria (Table 11). The organic N treatment and unamended control exhibited 66% and 52% lower gram-positive to gram-negative bacteria ratios, respectively, after grazing in 2020 relative to the inorganic N treatments. In general, across both experiments, total microbial biomass and biomass by functional groups were higher in the spring and lower after hay or grazing events (Table 11; Appendix 3 Table 3).

Table 11. Annual least-squares means ± standard error (SE) of PLFA biomarker responses (nmol g^{-1}) to control and treatments after post- harvest events from two field experiments. Samples collected from 0 – 10 cm. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6)[1]. Significant p-values ($P \le 0.1$) indicated by different letters; $P > 0.1$ (non-significant; NS), $P \le 0.1$ (*) , $P \le 0.05$ (**), and $P \le 0.01$ (***).

		IP			SNP[1]			
Variable	Year	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Functional group								
Total bacteria (nmol g^{-1})	2018	214 ± 33a	200 ± 33a	NS	161 ± 41a	173 ± 41a	163 ± 41a	NS
	2019	178 ± 33a	171 ± 33a	NS	__	__	__	
	2020	555 ± 36a	454 ± 39b	*	95 ± 50a	46 ± 50a	105 ± 50a	NS
Total fungi (nmol g^{-1})	2018	30.3 ± 8.3a	35.3 ± 8.3a	NS	17.8 ± 4.6a	18.3 ± 4.6a	22.2 ± 4.6a	NS
	2019	17.1 ± 9.0a	20.0 ± 9.0a	NS	__	__	__	
	2020	49.2 ± 9.0a	42.2 ± 8.3a	NS	0.0 ± 5.7a	0.0 ± 5.7a	0.0 ± 5.7a	NS
Total actinomycetes (nmol g^{-1})	2018	39.1 ± 9.8a	38.8 ± 9.8a	NS	30.4 ± 9.5a	30.6 ± 9.5a	30.9 ± 9.5a	NS
	2019	34.3 ± 9.8a	30.2 ± 10.6a	NS	__	__	__	
	2020	102.4 ± 9.8a	83.2 ± 10.6a	NS	12.8 ± 11.7a	8.8 ± 11.7a	15.1 ± 11.7a	NS
Gram positive bacteria (nmol g^{-1})	2018	80.3 ± 16.8a	77.0 ± 16.8a	NS	57.4 ± 19.0a	58.2 ± 19.0a	54.7 ± 19.0a	NS
	2019	67.5 ± 16.8a	70.3 ± 16.8a	NS	__	__	__	
	2020	223.0 ± 18.0a	228.0 ± 18.0a	NS	33.6 ± 23.5a	15.5 ± 23.5a	39.6 ± 23.5a	NS
Gram negative bacteria (nmol g^{-1})	2018	89.5 ± 15.5a	79.8 ± 15.5a	NS	68.4 ± 16.2a	79.4 ± 16.2a	71.1 ± 16.2a	NS
	2019	70.6 ± 15.5a	63.4 ± 16.7a	NS	__	__	__	
	2020	131.2 ± 15.5a	116.0 ± 15.5a	NS	31.0 ± 20.0a	19.1 ± 20.0a	34.5 ± 20.0a	NS
Ratios								
Fungi:Bacteria	2018	0.14 ± 0.02a	0.17 ± 0.02a	NS	0.11 ± 0.01a	0.11 ± 0.01a	0.13 ± 0.01a	NS
	2019	0.11 ± 0.02a	0.14 ± 0.02a	NS	__	__	__	
	2020	0.10 ± 0.02a	0.07 ± 0.02a	NS	0.0 ± 0.02a	0.0 ± 0.02a	0.0 ± 0.02a	NS
Gram Positive:Gram Negative	2018	0.91 ± 0.14a	0.98 ± 0.14a	NS	0.87 ± 0.45a	0.74 ± 0.45a	0.81 ± 0.45a	NS
	2019	1.07 ± 0.14a	1.12 ± 0.14a	NS	__	__	__	
	2020	1.59 ± 0.15a	1.45 ± 0.17a	NS	1.17 ± 0.66b	3.45 ± 0.56a	1.39 ± 0.45a	*,**[2]
Biomass								
Total microbial biomass (nmol g^{-1})	2018	415 ± 70a	387 ± 70a	NS	308 ± 63a	320 ± 63a	339 ± 63a	NS
	2019	381 ± 75a	330 ± 75a	NS	__	__	__	
	2020	923 ± 70a	744 ± 70b	*	331 ± 78a	214 ± 78a	372 ± 78a	NS

[1] For 2020 SNP, n = 4

[2] * = Organic N; ** = Control

4.6 Soil microbial functional diversity

Total microbial substrate utilization and functional diversity – defined here as differences in substrate utilization – were higher in the organic N treatment in IP relative to the unamended control in 2018 and in 2020. The differences in substrate utilization between treatments were greater for more labile plant materials like glucose and xylose than for more recalcitrant substrates like lignin (Figure 16a). During the first experimental year after organic N application in IP, total microbial-induced substrate respiration was 14% higher in the organic N treatment (Appendix 3 Table 4). Total microbial-induced substrate respiration was also lower in 2019 across both treatments following the patterns observed in aboveground biomass. We found that microbial-induced substrate respiration was 13% higher in the organic N treatment compared to the control for xylose across all years ($P = 0.01$). In 2018, microbial-induced substrate respiration was 21% higher in the organic N treatment for glucose (Figure 16a; Appendix 3 Table 4). There were no differences between treatments for microbial functional evenness.

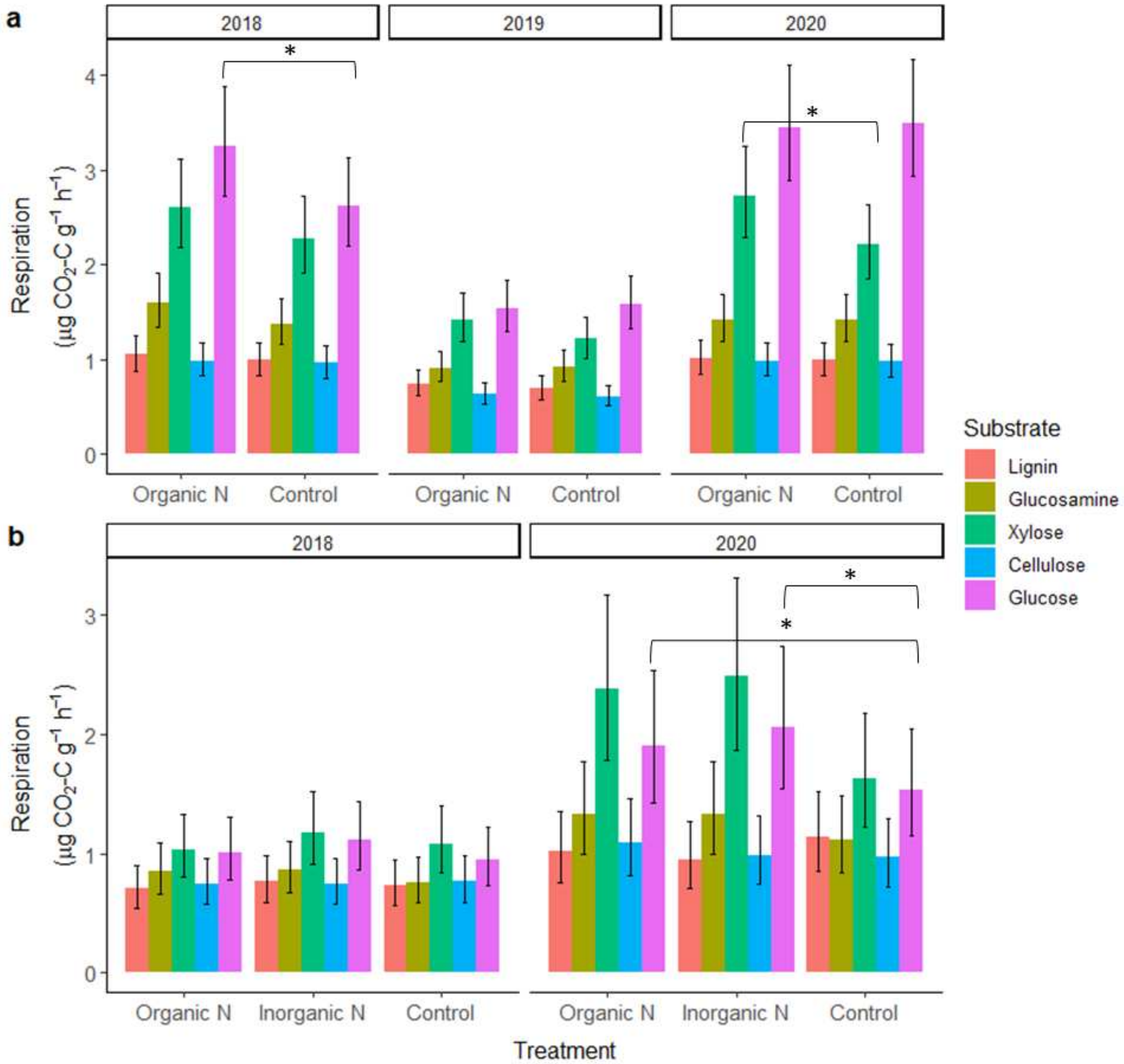


Figure 16. Back-transformed least-squares means of microbial functional diversity responses from MicroResp® incubations for improved pasture (IP; top) and semi-native pasture (SNP; bottom) averaged across a three-year experiment near Loveland, CO. Significant p-values for treatments within year; $P > 0.1$ (non-significant; NS), $P \leq 0.1$ (*) , $P \leq 0.05$ (**), and $P \leq 0.01$ (***). Error bars represent 95% confidence intervals; IP (n=8) and SNP (2018, n=6; 2020, n=4).

In SNP, we did not observe treatment differences in microbial substrate utilization in 2018, but in 2020 the N treatments had 82% higher microbial induced-substrate respiration for glucose than the unamended control (Appendix 3 Table 4). Total microbial induced-substrate respiration across treatments was higher in 2020 than 2018 ($P < 0.001$), but this was likely attributable to differences in seasonal sampling (spring vs. late fall). There were no differences between treatments for microbial functional evenness.

4.7 Soil characteristics

Compost additions impacted soil pH and nutrient availability in both IP and SNP. In IP, the organic N treatment lowered soil pH from 0-10 cm after the second amendment application in March 2018 (Table 12). From 10-20 cm, the soil pH was initially lower in the organic N treatment, but we did not observe any differences at this depth in the third experimental year. Available soil P was higher in the organic N treatment than the control across both depth intervals (Table 12), and there was a treatment by year interaction (P <0.001). Prior to the second compost application, there were no differences between treatments in available soil P from 0-10 cm. After the compost application in March 2018, soil available P was higher in the organic N treatment at both depth intervals for each sampling time point. Soil available K was similarly elevated in the organic N treatment compared to the control for all soil depths and sampling time points (Table 12).

Table 12. Annual least-squares means ± standard error (SE) of soil physical and chemical responses to control and treatments after post- harvest events from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values ($P \leq 0.1$) indicated by different letters; $P > 0.1$ (non-significant; NS), $P \leq 0.1$ (*) , $P \leq 0.05$ (**), and $P \leq 0.01$ (***).

			IP			SNP			
Variable	Year	Depth (cm)	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
pH	Baseline[1]	0-10	7.9 ± 0.04a	8.0 ± 0.03a	NS	__	__	__	
	2018		7.9 ± 0.03b	8.0 ± 0.03a	***	8.0 ± 0.04b	8.1 ± 0.04a	8.0 ± 0.04ab	*
	2019		7.8 ± 0.03b	7.9 ± 0.03a	**	__	__	__	
	2020		7.8 ± 0.03b	7.9 ± 0.03a	***	7.9 ± 0.04a	8.0 ± 0.04a	7.9 ± 0.04a	NS
	2018	10-20	8.0 ± 0.03b	8.1 ± 0.04a	*	__	__	__	
	2020		8.0 ± 0.03a	8.1 ± 0.04a	NS	8.1 ± 0.05a	8.1 ± 0.05a	8.1 ± 0.05a	NS
Bulk density[1] (g cm^{-3})	2012	0-10	1.01 ± 0.01	1.01 ± 0.01		__	__	__	
	2018		1.40 ± 0.02a	1.43 ± 0.02a	NS	1.33 ± 0.05	1.33 ± 0.05	1.33 ± 0.05	
	2020		1.22 ± 0.03a	1.22 ± 0.03a	NS	1.38 ± 0.03a	1.34 ± 0.03a	1.32 ± 0.04a	NS
	2012	10-20	1.14 ± 0.01	1.14 ± 0.01		__	__	__	
	2018		1.52 ± 0.03a	1.56 ± 0.03a	NS	1.40 ± 0.04	1.40 ± 0.04	1.40 ± 0.04	
	2020		1.41 ± 0.03a	1.39 ± 0.03a	NS	1.43 ± 0.03a	1.40 ± 0.03a	1.41 ± 0.03a	NS
Phosphorus (g kg^{-1})	Baseline[1]	0-10	0.01 ± 0.004a	0.009 ± 0.004a	NS	__	__	__	
	2018		0.04 ± 0.004a	0.02 ± 0.004b	***	0.03 ± 0.003a	0.02 ± 0.003b	0.01 ± 0.003b	**
	2019		0.03 ± 0.004a	0.01 ± 0.004b	***	__	__	__	
	2020		0.04 ± 0.004a	0.02 ± 0.004b	***	0.04 ± 0.003a	0.02 ± 0.003b	0.02 ± 0.003b	***
	2018	10-20	0.007 ± 0.0008a	0.007 ± 0.0008a	NS	__	__	__	
	2020		0.009 ± 0.0008b	0.005 ± 0.0008b	***	0.01 ± 0.002a	0.007 ± 0.002a	0.007 ± 0.002a	NS
Potassium (g kg^{-1})	Baseline[1]	0-10	0.22 ± 0.02a	0.18 ± 0.02b	**	__	__	__	
	2018		0.21 ± 0.02a	0.18 ± 0.02b	*	0.45 ± 0.03a	0.43 ± 0.03a	0.38 ± 0.03a	NS
	2019		0.22 ± 0.02a	0.18 ± 0.02b	*	__	__	__	
	2020		0.24 ± 0.02a	0.18 ± 0.02b	***	0.43 ± 0.03a	0.39 ± 0.03a	0.38 ± 0.03a	NS
	2018	10-20	0.13 ± 0.01a	0.11 ± 0.01a	NS	__	__	__	
	2020		0.14 ± 0.01a	0.11 ± 0.01b	**	0.27 ± 0.02a	0.23 ± 0.02ab	0.17 ± 0.02b	**

[1] Collected in March 2018

The organic N treatment lowered soil pH from 0-10 cm relative to the unamended control during the first experimental year in SNP. However, there were no differences between treatments in 2020 (Table 12). We did not observe any treatment differences from 10-20 cm in soil pH. Soil available P from 0-10 cm was 50% higher (*P* = 0.002) in the organic N treatment compared to both the inorganic N treatment and the unamended control. There were no differences in soil available P after three years in the 10-20 cm depth. For soil available K, there were no treatment differences from 0-10 cm, but from 10-20 cm the organic N treatment was around 60% higher than the control. There were no differences between the organic and inorganic N treatments at this depth. Bulk density values did not differ between treatments over the experimental period in IP or SNP.

4.8 Relationships between above and belowground communities

The NMDS exhibited differences in plant and microbial communities between treatments in IP (Figure 17a), but the spatial separation by PLFA functional groups and plant relative abundances were not statistically robust based on the results of the PERMANOVA ($P = 0.29$). However, we observed a shift over time in plant and soil community groupings from 2018 to 2020 ($P \leq 0.001$). Microbial functional groups demonstrated a stronger association with axis one, while plant communities were more associated with axis two (Figure 17a). Results from the environmental variable loadings (Appendix 3 Table 5) and correlation analyses (Appendix 3 Figure 1) indicated stronger positive associations between microbial substrate respiration ($P \leq 0.01$) – like glucose, xylose, and glucosamine – and aboveground biomass C, i.e., shoot C ($P = 0.005$) with axis one while total root biomass carbon demonstrated a strong negative association ($P \leq 0.001$).

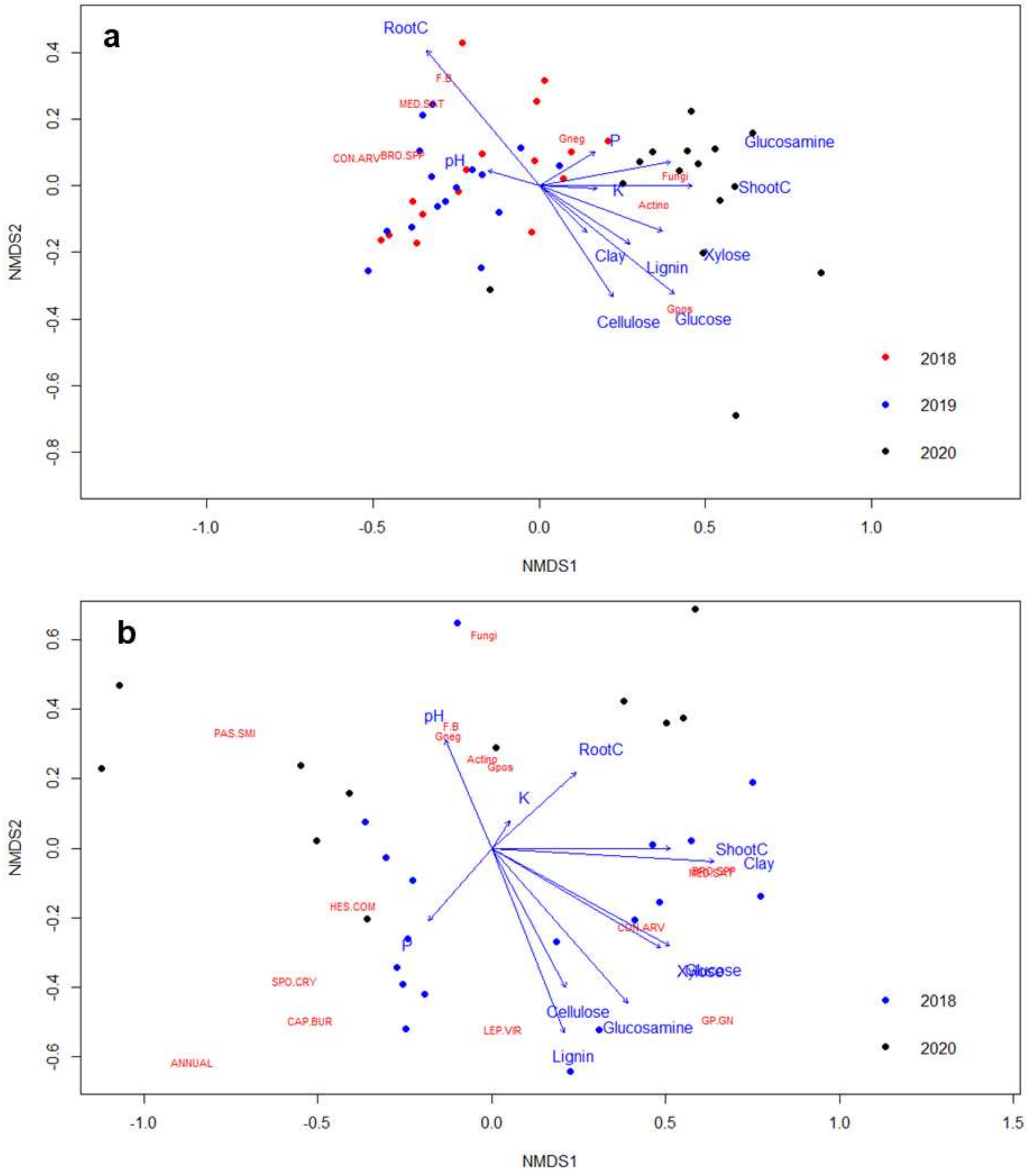


Figure 17. Non-metric multidimensional scaling (NMDS) ordination of microbial community composition as measured by PLFA and plant community composition of relative species abundances for a) improved pasture (IP) and b) semi-native pasture (SNP) averaged across a three-year experiment near Loveland, CO. Plant species: ANNUAL (*Bromus secalinus*, *B. tectorum*), BRO.SPP (*B. commutatus*, *B. inermis*), CON.ARV (*Convolvulus arvensis*), CAP.BUR (*Capsella bursa-pastoris*), DAC.GLO (*Dactylis glomerata*), FES.ARU (*Festuca arundinacea*), HES.COM (*Hesperostipa comata*), LEP.VIR (*Lepidium virginicum*), MED.SAT

(*Medicago sativa*), PAS.SMI (*Pascopyrum smithii*), SPO.CRY (*Sporobolus cryptandrus*), THI.INT (*Thinopyrum intermedium*).

In SNP, we did not observe any spatial separation of communities by treatment, but there was a shift over time from 2018 to 2020 based on the results of the PERMANOVA ($P = 0.002$). Plant relative abundances exhibited more negative associations with axis one, while belowground PLFA functional groups demonstrated more negative associations with axis two. All microbial-induced substrate respiration variables (Figure 17b, Appendix 3 Table 5) were negatively associated with axis one and positively associated with axis two ($P \leq 0.02$), while only aboveground biomass C was negatively associated with axis one ($P \leq 0.01$).

5. Discussion

Plant and soil microbial composition and function shifted in response to a single application of organic N in IP but not SNP over the three-year experimental period. Organic N application also increased plant productivity, plant N uptake, and plant available soil nutrients. Nitrogen additions did not facilitate increased abundances of invasive annual grasses like *B. tectorum* or *B. secalinus* and led to a decrease in the Class C perennial noxious weed *C. arvensis* in SNP. These findings support the strategic use of infrequent organic amendment applications to enhance agroecosystem services in semi-arid irrigated perennial pastures. But the marginal responses in SNP indicates that abiotic constraints may limit the viability of an infrequent or limited application strategy, suggesting that the application of organic amendments to semi-arid dryland pastures may require a different management approach than non-water limited systems.

5.1 Plant productivity responses to organic and inorganic nitrogen

Plant and root biomass responded to nitrogen additions in both IP and SNP immediately after application, but only IP demonstrated sustained increases in aboveground plant biomass over time. The differences in responses between the two experiments may be a result of both the application rate and form of N as well as initial differences in plant community and cover. In a meta-analysis of 92 studies applying organic amendments to rangelands, aboveground plant production responded positively at relatively low rates of amendment application (~10 Mg ha^{-1}), but dryland climates were more responsive than Mediterranean climates to higher application rates (Gravuer et al. 2019). The same study also found that increases in aboveground biomass were sustained over time in Mediterranean climates, but that biomass increases in dryland climates decreased over time. In a semi-native rangeland in norther Colorado, Ippolito et al. (2010) found that composted biosolids increased perennial plant and litter cover, but this effect was most notable on plots that received higher amendment rates, i.e., > 10 Mg ha^{-1}, and plots where amendment application was repeated. In this study, we applied about 33% less organic amendment during a single application in SNP.

The inorganic N treatment in SNP showed initial responses in plant productivity and N uptake relative to the organic N treatment and the unamended control. The application of N in a preferential form for plant uptake likely facilitated this difference in response between treatments (Schimel and Bennett 2004). However, these differences in plant productivity were not sustained in the second or third experimental years, indicating that this N was quickly assimilated by plants or microbes, or possibly lost from this system via leaching, gaseous N losses, or through direct residue removal by grazers. The initial plant productivity responses suggest that this semi-native rangeland pasture is N-limited, but the additions of N in both inorganic and organic N forms were not sufficient to alleviate this limitation. Other grassland N experiments in dryland ecosystems observed similar, muted responses in aboveground biomass even when inorganic N was applied at up to 8 kg ha^{-1} annually over seven years (Phillips et al. 2020). The lack of plant responses to both organic and inorganic N in this and other dryland systems merits closer study of critical N loads and the relationship between N, water availability, and other plant limiting nutrients (Hooper and Johnson 1999, Phillips et al. 2020).

Water availability was a key difference between IP and SNP management. While the amount of N applied was higher in IP and there was a longer history of organic amendment application in this system, we observed no differences between treatments in aboveground biomass in 2019 when irrigation rate was lowest. However, in the following wetter year of 2020, the organic N treatment effect was still evident and resulted in 34% greater biomass production than the unamended control, and this response was 9% higher than aboveground biomass immediately after application in 2018. This observation supports both the capacity of organic amendments to provide a slow release of N over time to facilitate sustained increased in plant productivity. However, it also highlights the potentially stronger controls of other co-limiting resources, like water, on aboveground responses.

5.2 Plant community responses to nitrogen amendments

The response of plant communities to N addition varies in the literature with several studies finding decreases (Fornara and Tilman 2012) no change (Ryals et al. 2016, McHugh et al. 2017, Phillips et al. 2020), or increases in plant diversity (Ippolito et al. 2010). We observed an increase in plant diversity and species evenness in IP relative to the unamended control, while species richness was only higher in 2019. The increases in plant diversity were most likely driven by the suppression of *B. commutatus* and *B. inermis* – the dominant plant species at the site – and increases in *D. glomerata* and small, but non-significant increases in *M. sativa* in the organic N treatment. These plant species are responsive to concomitant increases in N and P additions, particularly *D. glomerata* (Ryser et al. 1997), while P is a critical factor for N-fixing plants (Vance et al. 2000).

In SNP, the application of N did not lead to changes in plant diversity, species evenness, or richness over the experimental period, which is largely consistent with other dryland N addition experiments. We did observe decreases in the relative abundances of two C_3 grasses, one native (*H. comata*) and one exotic (*T. intermedium*), in the organic N treatment relative to the control. This finding contradicts previous observations in long-term N fertilization treatments, where N addition led to increases in species composition of exotic C_3 grasses (Fornara and Tilman 2012). Similarly, Ippolito et al. (2010) found that organic N additions increased native perennial grasses, like *H. comata*, relative to non-amended plots.

The results of our study demonstrate that organic amendments do not increase plant invasion by exotic annual grasses or perennial noxious weeds over the short-term. In the intermountain west of North America, *B. tectorum* is a major ecological concern because of its ability to increase fire frequency and size (Link et al. 2006), alter ecosystem biogeochemical cycles (Rimer and Evans 2006, Blank 2008), and change above and belowground community dynamics (Belnap and Phillips 2001). The noxious weed, *C. arvensis*, is of major management concern in agroecosystems because of its perennial lifecycle, competitive ability with agronomic crops, and difficulty to control (Davis et al. 2018). While longer-term evaluation of plant community dynamics under amendment application is needed (Gravuer et al. 2019), our study aligns with previous findings that infrequent organic amendment applications do not increase the prevalence of invasive species (Ippolito et al. 2010, Ryals et al. 2016) demonstrating that the form of N may be critical for the invasion of exotic plant species in grasslands (Borden and Black 2011).

5.3 Changes in soil properties and plant available nutrients

The organic N amendment reduced soil pH and increased plant available soil P and K in both IP and SNP in the topsoil. Soil pH is an important edaphic factor in regulating biogeochemical cycles (Brady et al. 2008), and soil pH decreases in alkaline soils can impact phosphorus mineralization processes (Neina 2019). Under soil pH > 7, phosphorus is generally fixed by Ca and unavailable for plant uptake (Penn and Camberato 2019). Applications of inorganic N to alkaline soils in semi-arid grassland ecosystems in the Colorado Plateau did not alter soil pH (McHugh et al. 2017), but the application of organic materials to alkaline soils in other semi-arid regions of the world decreased soil pH likely due to the production of organic acids during mineralization of organic matter (Sarwar et al. 2008). The direct application of an organic material containing P, and the reduction in soil buffering capacity are two possible mechanisms for the increased response in plant available soil phosphorus under the organic amendment.

5.4 Microbial community and functional diversity responses

Total microbial biomass, bacteria biomass, and functional groups like actinomycetes increased under organic amendment application in IP. In SNP, microbial functional groups primarily responded to season and grazing events, but the ratio of gram-positive to gram-negative bacteria was lower in both N treatments after grazing in 2020. Total fungi and fungi:bacteria ratios did not differ between treatments in either IP or SNP. Prior grassland fertilization experiments showed a negative response of fungal communities to inorganic N (Denef et al. 2009), while gram-positive bacteria and actinomycetes responded more favorably to N fertilization (Denef et al. 2009, Ramirez et al. 2012) although other semiarid grasslands demonstrate negative responses in this functional group (Wang et al. 2017b). Under organic amendment applications, several studies report increases in total microbial biomass and shifts in the ratios of gram-positive to gram-negative bacteria and mycorrhizal to total fungi, while fungal to bacterial ratios did not change (Lynch et al. 2005, Bastida et al. 2008, Hurisso et al. 2013). While both gram-positive and gram-negative utilize plant C substrates, gram-positive bacteria may prefer older SOM-derived C while gram-negative tend to utilize more recent plant-derived C inputs (Fanin et al. 2019), and these tendencies are more pronounced at depth (Kramer and Gleixner 2008).In our study, the low C:N ratio of the organic amendment (8:1) likely favored faster growing bacterial communities relative to fungi given their lower C to nutrient biomass requirements.

Microbially-induced respiration of common plant substrates increased under organic N in both experiments. The respiration of labile plant C compounds like glucose and xylose were elevated immediately and two years following amendment application in IP. In SNP, both N treatments had higher glucose-induced respiration than the unamended control after three growing seasons. Simple sugars like glucose and xylose are associated with root C exudation (Campbell et al. 1997), and the increased respiration of these substrates by the microbial communities under organic N suggests that microbes may be responding to changes in plant C allocation rather than directly responding to the added C from the organic amendment.

The observed shifts over time in microbial community composition and function measured by substrate-induced respiration likely impacted soil C and N cycling (Chapter 2). Labile plant constituents are the dominant source of microbial products, which form the main precursors to stable soil organic matter formation (Cotrufo et al. 2013). Root exudates in addition to dissolved organic C and other labile plant litter inputs may lead to rapid formation of mineral-associated organic matter (Haddix et al. 2016, Lavallee et al. 2018), which is a more stable form of soil organic carbon. Given the increased respiration of these substrates by the microbial community in the organic N treatment, it is possible that these C inputs were preferentially processed by the microbial community leading to increases in soil organic C stock (McClelland et al., *in prep*). Like other grassland fertilization studies, we observed increases in total bacteria biomass, and the abundances of fast growing bacterial like actinomycetes (Ramirez et al. 2012). While we did not explicitly examine the respiration of plant vs. soil organic matter derived C inputs, the increase in fast-growing microbial functional groups and greater labile substrate respiration under N addition may suppress microbial decomposition of the resident soil organic carbon pool leading to greater soil C sequestration over time (Liu and Greaver 2010, Ramirez et al. 2012).

5.5 Interactions between plants, soil properties, and microbial communities

We assessed relationships between plant and microbial communities, plant and root production, microbial respiration, and soil properties under different N management strategies in two distinct perennial grasslands. Previous investigations of above and belowground responses to N addition in similar ecosystems suggests that N applications directly impact plant C allocation (Leff et al. 2015), which, in turn, indirectly impacts soil microbial communities (Ramirez et al. 2010, Leff et al. 2015, McHugh et al. 2017). The stronger initial plant productivity and community responses to the organic amendment application and the gradual change in microbial community response over the experimental period, particularly in IP, corroborates these previous findings (Zak et al. 2003, Lamb et al. 2011, Chaparro et al. 2012). Our multivariate analyses indicated a strong association between aboveground plant C and microbial functional groups and substrate-induced respiration. The close alignment of changes in plant C and microbial function observed in the univariate and multivariate analyses suggests that soil microbial activity is changing in response to plant shifts in production (Clegg 2006), but that the composition of the microbial community is slower to change. Interestingly, we found a negative association between PLFA microbial functional groups and root biomass C, indicating that plants are effective competitors for nutrients in this system probably due to both arbuscular mycorrhizal fungi associations and microsite availability (Schimel and Bennett 2004).

We also observed a less robust, positive association of plant relative abundances, i.e., *D. glomerata*, and aboveground biomass C with soil available P in IP. While soil P also increased under the organic N treatment in SNP, the lack of response across plant taxa to greater soil nutrient availability was likely related to co-limitation with water in this non-irrigated system (Bloom et al. 1985). Soil P was also positively associated with bacteria functional groups in the NMDS. Compared to fungi, bacteria support greater P solubilization in soils (Sharma et al. 2013), by lowering soil pH through the secretion of organic acids (Bhattacharyya et al. 2016). The plant productivity and individual taxa response to plant available P under non-water limiting conditions suggests that in addition to addressing soil N limitation, organic amendment applications also supply other limiting nutrients to support plant growth with potentially positive impacts on plant nutritional quality (Miner et al. 2020).

5.6 Implications for semi-arid perennial pasture management

Infrequent applications of high-quality organic amendments to irrigated perennial pastures can increase positive ecosystem outcomes while minimizing adverse impacts. From a land management perspective, organic N application can increase forage production and plant N uptake, while increasing soil health via increased plant nutrient availability, soil microbial biomass and activity, and soil C sequestration (McClelland et al. *in prep*) without increasing noxious weed species. However, it remains uncertain how long these benefits last between amendment applications, and how plant and microbial communities may respond in the mid- to long-term, i.e., 5-10 years (Gravuer et al. 2019). And, in the case of dryland pastures, higher and/or more frequent application rates may not necessarily supply the same benefits as applications to irrigated pastures. Economic costs and barriers to adoption must also be considered. While the residual benefits of organic amendment application may last several years lowering costs relative to inorganic N fertilization, large-scale adoption faces several challenges including, but not limited to, access to organic materials, infrastructure and labor, and distance between production facilities and application sites (Weindorf et al. 2011).

6. Conclusions

A single application of an organic N amendment to a semi-arid irrigated perennial pasture increased plant production, plant diversity, soil microbial diversity and function, and soil quality over three growing seasons. In an adjacent semi-native dryland pasture, inorganic, but not organic N application increased plant production during the first growing season, and there were some shifts in microbial community composition and function over time under inorganic and organic N. The relative abundance of noxious annual and perennial weeds did not increase in either experiment. The increase in provisioning and regulating ecosystem services and the absence of major ecological tradeoffs indicates infrequent application of high-quality organic amendments can benefit low-input irrigated perennial pastures. The marginal responses in the dryland pasture suggest that co-limitation with water or greater resilience to N deposition may inhibit a similar, low-frequency N amendment strategy as in non-water limited pastures and grasslands.

CHAPTER 4: QUANTIFYING BIODIVERSITY IMPACTS IN LIVESTOCK LIFE CYCLE ASSESSMENTS

1. Summary

Livestock production is among the main anthropogenic activities contributing to biodiversity loss due to land-use and land-use change. The Food and Agriculture Organization of the United Nations recently published guidelines for quantitative assessment of biodiversity impacts from livestock within a life cycle assessment (LCA) framework, but these approaches have not yet been widely adopted. We applied and compared the two recommended approaches – potential species loss and pressure-state-response (PSR) – for two different pasture land-use intensities at a cattle ranch in central Florida, and we demonstrate a novel method for adapting the PSR framework for use in LCA. Our results illustrate how indicator selection and functional unit may result in discrepancies between the two methods. We provide a decision-making framework to guide ecologists and LCA practitioners in selecting between the two approaches. Given the projected global demand for livestock products, it is critical that biodiversity impacts are incorporated into holistic evaluations of livestock system sustainability to meet the United Nations Sustainable Development Goals.

2. Introduction

Globally, biodiversity is under threat. Anthropogenic activities over the past 50,000 years have transformed up to one-half of Earth's land surface area leading to irreversible biodiversity loss (Vitousek et al. 1997, Tilman et al. 2017). The leading threats to species extinctions include land use and land use change, habitat fragmentation, overhunting, invasive species, and pollution

(Newbold et al. 2015, Maxwell et al. 2016). Coupled with the climate crisis, these drivers continue to accelerate the sixth mass extinction event (Bellard et al. 2012, Ceballos et al. 2020).

Among anthropogenic activities, livestock production exerts considerable influence on biodiversity and the environment. Agriculture, including livestock, directly impacts biodiversity through habitat conversion, resource demand, changes to water quality and ecotoxicity, spread of invasive alien species, and over-exploitation (Millennium Ecosystem Assessment 2005). However, livestock play a crucial role in human nutrition and livelihoods, particularly in developing countries (Randolph et al. 2007), and they can enhance provisioning ecosystem services supported by a landscape (Steinfeld and Gerber 2010). The livestock sector has an important role in achieving the United Nations Sustainable Development Goals, but environmental and socio-economic tradeoffs and synergies must be evaluated and addressed (FAO 2018).

Life cycle assessment (LCA) is a technique for evaluating the environmental impacts associated with the production and consumption of goods, processes, or services. They are frequently applied in livestock supply chains (de Vries and de Boer 2010, Thoma et al. 2013, McClelland et al. 2018, Rotz et al. 2019) to evaluate numerous environmental impacts (e.g., climate change, ozone depletion, eutrophication potential) during the life cycle impact assessment (LCIA) phase of the analysis. Historically, livestock LCIA of biodiversity are either lacking (McClelland et al. 2018) or extrapolate impacts from land-use (Teillard et al. 2016) due to the complexity in accounting for the different ways livestock systems affect biodiversity and the scale at which these effects occur.

The Food and Agriculture Organization (FAO) of the United Nations Livestock Environmental Assessment and Performance Partnership (LEAP) recently released guidelines to quantitatively assess biodiversity impacts from livestock (FAO 2020). The guidelines describe two quantitative approaches for estimating biodiversity impacts from livestock (Appendix 4 Figure 1). The first approach is an LCA method for regional and global scales, and the second method pertains to local-level assessments using pressure-state-response (PSR) indicators. The selection of an approach is contingent on the stated goal, scale, and constraints (e.g., data availability) of the assessment (FAO 2020). However, few examples exist to guide ecologists and LCA practitioners in selecting between and applying the two methods. Furthermore, comparative approaches of biodiversity impact assessments are understudied and such work is necessary to continue building consensus around an acceptable set of methodologies (Curran et al. 2016).

The objective of this study was to demonstrate the incorporation of local biodiversity data into livestock LCA based on the recently published FAO LEAP guidelines by: **i)** applying a land use-based method for regional assessments (i.e., Chaudhary and Brooks, 2018) and **ii)** developing a site-specific biotic integrity index (BII) based on a local PSR assessment that is easily integrated into LCA methodology. We then evaluated the differences and similarities of these two approaches in the context of their ability to assess biodiversity impacts for a ranch in central Florida. We also developed a decision-making framework with the goal of supporting practitioners that wish to incorporate site-specific biodiversity data into their LCA following the FAO LEAP guidelines.

3. Methods

3.1 Buck Island Ranch at the MacArthur Agroecology Research Center (MAERC)

We undertook a biodiversity assessment of the Buck Island Ranch located at the MacArthur Agroecology Research Center (MAERC) (27° 9' 9.6078" N, 81° 11' 40.7142" W). The MAERC is part of the Archbold Biological Station (https://www.archbold-station.org/) and is one of four sites under the Archbold-University of Florida Long-term Agroecosystem Research (LTAR) network. Buck Island Ranch is a commercial cow-calf operation that manages more than 3,000 head of beef cattle on 4,249 hectares making it among the top 20 beef cattle producers in Florida (Swain et al. 2013). The primary output at the farm-gate is weaned calves, which are sent for finishing in northern Florida. Other by-products produced from this system include sod, hunting leases, and conservation easements.

Buck Island Ranch is in the headwaters of the Florida Everglades, an ecologically-sensitive region that covers more than 1 million hectares and includes the globally threatened Florida scrub ecosystem (USDA ARS 2020). A detailed land use history of the ranch prior to acquisition by Archbold Biological Station is described in Swain et al. (2013), which includes a description of the draining and modification of seasonal wetlands and the conversion of the dry prairie ecosystem to warm-season forage grass pastures from the mid-1940s to mid-1960s. Habitat restoration efforts began after acquisition in 1988 and there has been no further habitat loss.

Beef cattle at Buck Island Ranch graze pastures under two types of management of approximately equal land area: improved pastures (IMP) and semi-native pastures (SN). The improved pastures receives synthetic fertilizer (Medley et al. 2015) and the average stocking rate is 1.2 head acre^{-1}. The dominant vegetation in the improved pastures is bahia grass (*Paspalum notatum* Flueggé), an introduced warm season forage species. In the semi-native pastures, there is no history of fertilization (Medley et al. 2015) and the average stocking rate is 0.6 head acre^{-1}. The dominant vegetation in the semi-native pastures is a mixture of *P. notatum* and native grasses (Boughton et al. 2011). More than 600 isolated and seasonally flooded, freshwater wetlands are contained within the two pasture types. From 2014 to 2019, Buck Island Ranch maintained an average of 2,912 cows and 2,088 calves annually, including replacement heifers, plus breeding bulls. The dominant breed is a Brahman-Angus cross. A detailed description of animal use days per acre and yield is included in Appendix 4 Table 1.

3.2 Goal and scope of analysis

The goal of this study was to pilot two approaches to assessing biodiversity impacts by comparing the biodiversity impacts of producing cattle under two pasture management regimes – IMP and SN – at the Buck Island Ranch. The functional unit for this study was 1 kg of live weight (LW) yr^{-1} leaving the ranch, and the system boundary extended from cradle-to-farm gate. Allocation between co- or byproducts, and downstream impacts are beyond the scope of this study.

3.3 Biodiversity assessments

3.3.1 Land-use intensity potential species loss approach

The life cycle inventory (LCI) was informed by data from 2014 – 2018. We compiled data pertaining to the land occupation and total yield (kg LW yr^{-1}) by pasture type. For this analysis, we used weaned calf LW yield rather than total LW yield because these data were available at the pasture level (Appendix 4 Table 1).

We used the WWF Terrestrial Ecoregions of the World database (https://arcg.is/0HvWDv) to identify the appropriate ecoregion classification for Buck Island Ranch. The ranch is classified as Florida sand pine scrub (NA0513). We followed the steps outlined in Chaudhary and Brooks (2018) to classify the broad land use and management type for IMP and SN at Buck Island

Ranch. We designated IMP as “pasture – intense use” and SN as “pasture – minimal use” because of the differences in fertilizer use and stocking density.

To estimate the potential biodiversity damage related to land-use (i.e., land occupation) caused by 1 kg of LW yr^{-1} beef sourced from the different pasture types at Buck Island Ranch, we identified the disaggregated taxa and mean species potential species loss (PSL) by management regime and ecoregion reported in Chaudhary and Brooks (2018). We reported aggregated and disaggregated PSL given that different taxa may show different sensitivities to land use intensity (Appendix 4 Table 2). The estimates of potential biodiversity damage ($\text{BD}_{ecoregion,g}$ in PSL) and the upper and lower 95% confidence intervals for each taxon *g* were calculated as:

$$BD_{\text{ecoregion,g}} = \frac{CF_{ecoregion,g,i,c}}{\frac{1}{Y_{i,c}}} \tag{1}$$

where $\text{CF}_{\text{ecoregion}}$ is the CF for the designated ecoregion, *i* is the land use class, *c* is the average value, and Y is the yield for the product of interest.

3.3.2 Biotic integrity index

As an alternative to the land use intensity-based approach, we developed a biotic integrity index (BII) in keeping with the FAO LEAP recommendations for local biodiversity assessments. An index of biotic integrity indicates how well an ecosystem functions relative to an original or restored ecosystem prior to human intervention (Lackey 2009). To develop this index, we selected seven pressure and state indicators from the PSR framework. One indicator per thematic category (Figure 18; Appendix 4 Table 3) was selected in consultation with Archbold Biological Station. The selected indicators were reflective of key biodiversity issues and represented both positive and negative impacts on biodiversity (FAO 2020). The use of pressure and state indicators, rather than qualitative response indicators, enabled us to develop one quantitative value for each pasture type to integrate into a LCIA. We chose a nearby nature preserve, Kissimmee Prairie Preserve State Park (https://www.floridastateparks.org/parks-and-trails/kissimmee-prairie-preserve-state-park), as the reference system to compare the biodiversity integrity (Milà i Canals et al. 2007) of IMP and SN.

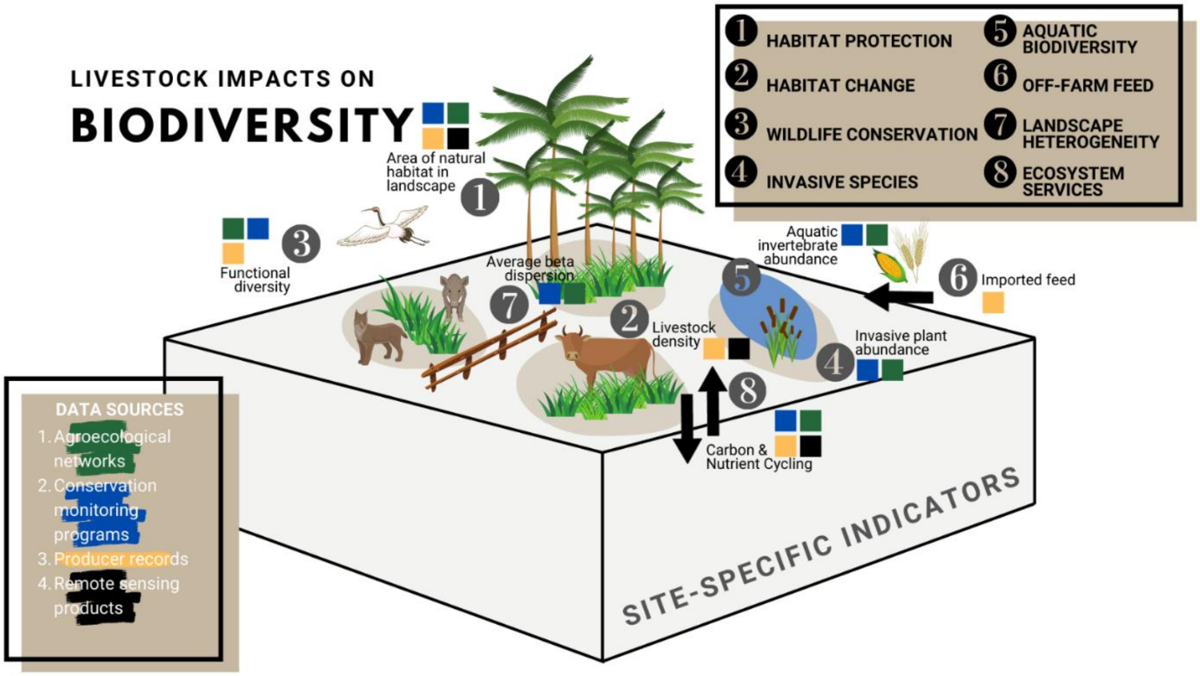


Figure 18. Primary categories for livestock impact on local to landscape-scale biodiversity. The upper right corner contains the eight thematic areas identified by FAO (2020). Examples of site-specific indicators to inform these categories are marked in gray next to the thematic category number on the landscape. The colored square in the lower left corner indicates potential data sources for these common indicators: 1) agroecological networks, 2) conservation monitoring programs, 3) producer records, and 4) remote sensing products.

The data used to inform these indicators are described in Appendix 4 Table 3. Most of the datasets represent multiple sampling years, except for the indicators selected to inform the habitat protection and pollution and aquatic biodiversity categories. The supplemental information (Appendix 4 Table 4) contains a detailed description of how data were collected to inform each indicator.

To integrate the values into the BII, we used average annual values to derive a single estimate for each indicator. Because the index was developed on a scale ranging from zero to one with one representing maximum biodiversity integrity, we took the inverse value of indicators that represented negative biodiversity impacts. These values were then normalized due to the difference in units and magnitude of each indicator. Results of the normalization procedure can be found in Appendix 4 Table 5.

The final step in the development of the BII was to assign a weighting factor to each thematic area. Weighting factors reflect the relative importance or "weight" of each indicator in the final index, and assignment is a critical step to integrating multiple indicators into a single value. After consulting with Archbold Biological Station, we decided to apply an equal weighting factor to all categories. A final estimate of biodiversity impact for each system was estimated as:

$$BII_{systemi} = \sum_{c=1}^{n} WF_c \times NV_{systemi,c} \qquad (2)$$

where BII is the biotic integrity index, WF is the weighting factor, $NV_{system\,i}$ is the normalized value for each $system_i$, and *c* is the biodiversity category. A value closer to one indicates higher biodiversity integrity (i.e., positive biodiversity impact) and a value closer to zero indicates low biodiversity integrity (i.e., negative biodiversity impact).

4. Results

4.1 Land-use intensity potential species loss approach

Overall, biodiversity damage was similar between the two pasture systems (Figure 19), but there were appreciable differences for disaggregated taxa. The estimated mean aggregated taxa response due to land occupation per 1 kg of LW was about three times greater for IMP than SN; mean biodiversity damage was 4.97 x 10^{-11} PSL m^{-3} (95% CI; 3.68 x 10^{-11} – 6.35 x 10^{-11}) and 1.61 x 10^{-11} PSL m^{-3} (95% CI; 1.18 x 10^{-11} – 2.12 x 10^{-11}) for IMP and SN, respectively. However, there were notable differences in PSL by taxa between systems. Across all taxa, biodiversity damage to plants was approximately ten times greater than any other taxa. The mean biodiversity damage to plants was 4.1 times greater for IMP than for SN (Figure 19). Biodiversity impacts from pasture management for amphibians, birds, mammals, and reptiles were on the same order of magnitude, but the impacts for IMP were two to four times greater than SN.

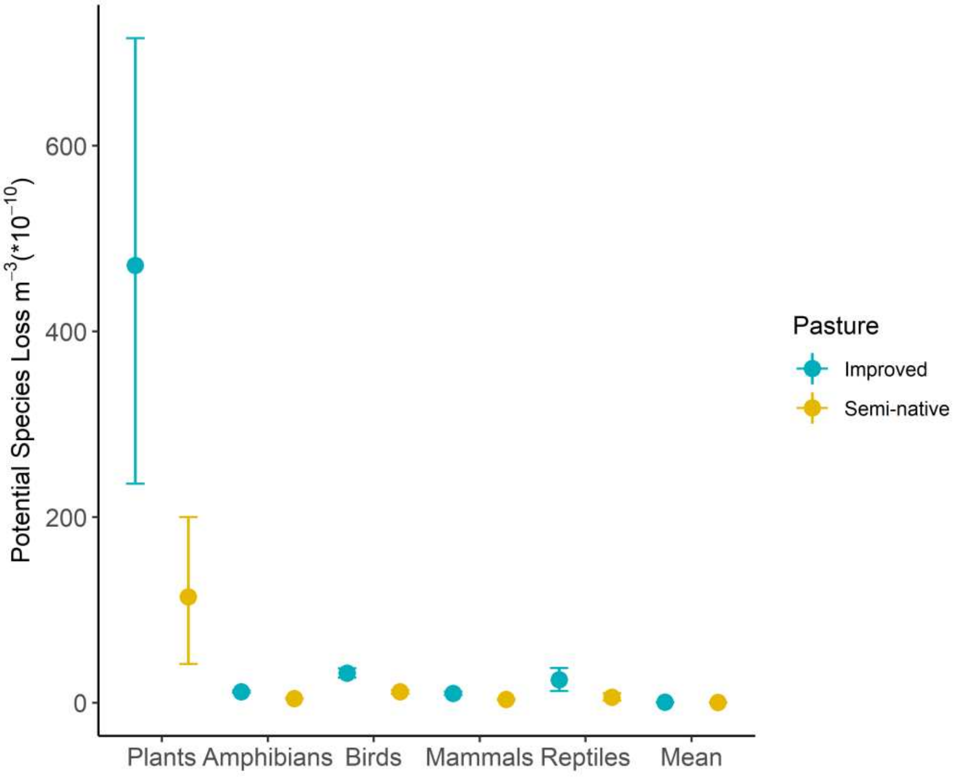


Figure 19. Biodiversity impacts for improved (IMP) and semi-native pasture (SN) at Buck Island Ranch using the Chaudhary and Brooks (2018) method. Biodiversity damage is measured as potential species loss caused by 1 kg LW of beef. Error bars represent upper and lower 95% confidence intervals.

4.2 Biotic integrity index

The reference system, Kissimmee Prairie Preserve State Park, maintained the most biotic integrity (i.e. had the smallest biodiversity impact), followed by SN and IMP (Figure 20). The final index values for the reference, SN, and IMP were 0.9, 0.5, and 0.1, respectively. Thus, on a cumulative basis, SN provided five times the biotic integrity of IMP. However, on a functional unit basis (per kg LW), IMP had 33% greater biotic integrity than SN (3×10^{-6} and 4×10^{-6} per kg of LW, respectively: data not shown).

There were a few differences in the cumulative rankings between systems for the categories used to inform the index. The reference system provided the greatest biotic integrity in each category of the index apart from aquatic diversity (Figure 20; Appendix 4 Table 5. The mean, inverse, and normalized values for each indicator included in the biotic integrity index for improved pasture (IMP), semi-native pasture (SN), and the reference system (REF) located at the Kissimmee Prairie Preserve State Park.), for which SN had the greatest aquatic species abundance closely followed by IMP. However, IMP was ranked lowest in all other categories used in the BII. While the observed values for each indicator were relatively similar for SN and IMP, particularly for off-farm feed and aquatic biodiversity, there were noticeable differences in a few other categories. For example, IMP had about twice the amount of associated habitat change relative to SN, and about three times the prevalence of invasive alien species. IMP also provided fewer benefits compared to SN in the habitat protection, wildlife conservation, and landscape-scale conservation categories.

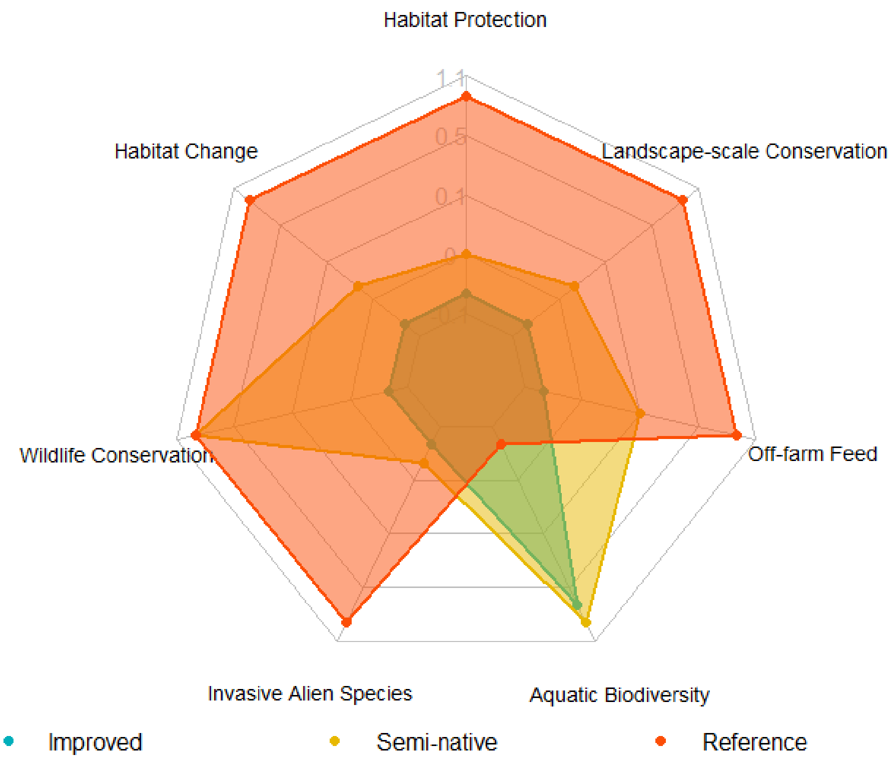


Figure 20. Biodiversity impacts for improved pasture (IMP), semi-native pasture (SN), and a reference system (REF) for Buck Island Ranch using a biotic integrity index with the normalized and weighted values demonstrating the overall biotic integrity of each system. Values closer to one indicate higher biotic integrity.

5. Discussion

The SN pasture outperformed the IMP pasture at Buck Island Ranch according to the regional scale approach, i.e., Chaudhary and Brooks (2018), and on a cumulative basis using our BII. However, when we relativized impact per unit of beef produced using the BII, IMP performed slightly better because of the higher yield under this management system. This finding contradicted our initial hypothesis, but it was not unexpected given the greater efficiency in beef production from IMP.

There was significant uncertainty in the plant taxa response using the biodiversity damage approach of Chaudhary and Brooks (2018). This is likely a result of the taxon affinity calculations and subsequent calculation of characterization factors. Habitat classifications for some plant and reptile species were not available through the IUCN Habitat Classification Scheme (IUCN 2015) that was used to classify taxon affinity to broad land-use categories (Chaudhary and Brooks 2018). For the BII approach, the greatest source of uncertainty is attributed to the input data used to inform our indicators for the reference system. We relied on publicly available data or expert opinion to inform these indicators. While we did not undertake a formal sensitivity analysis, it is likely that the final normalized values were impacted by these assumptions.

The attribution of weaned calf LW to each pasture type also impacted our results. This attribution was necessary to compare different management systems for our scenario, but it is not reflective of the ranch-level management of Buck Island Ranch. Cow-calf pairs rotate between all pasture types and we had to make assumptions about the productivity of each pasture type based on annual weaned-calf LW and animal use days per hectare. These assumptions influenced the final estimates of biodiversity impact on a functional unit basis. If we had used the average annual yield at the ranch-level, the biodiversity impact would have been smaller in SN. While this decision is not reflective of the ranch-level management of Buck Island Ranch, it was suitable for our demonstration of how to apply the two approaches.

Our findings suggest four important considerations for integrating biodiversity impacts into livestock LCA. First, they highlight the importance of disaggregating biodiversity impacts.

While a single, aggregated value is required for integration into the LCIA, from a management perspective, information at the taxon or indicator level is critical for developing responses to improve specific biodiversity outcomes. We encourage practitioners to report biodiversity impacts at both an aggregated and disaggregated level irrespective of the approach they use for impact assessment. From our study, there were clear differences in the impacts to different taxa, particularly plants, using the Chaudhary and Brooks (2018) method. Under the BII, we were able to distinguish similarities and differences between the two management systems across broad categories, such as for aquatic biodiversity and invasive alien species, respectively. This lends the disaggregated Chaudhary and Brooks (2018) data to management decisions relevant to specific taxa or species, while the BII lends itself to tracking general biodiversity progress of the operation over time at the macroscale and across biodiversity categories.

Second, they support the need for greater transparency in the reporting of indicators to inform livestock biodiversity impacts. Transparency is essential for reproducibility, but also for understanding subjective judgements made throughout the evaluation process (Curran et al. 2016). Our final set of indicators included subjective measures relevant to the management and biodiversity contexts within the greater landscape of Buck Island Ranch, but they were also based on the availability of data to inform them. These choices undoubtedly had an impact on the results of our system comparisons. Thus, the indicators provided in our study can be used as a guide, but we caution that practitioners carefully select indicators relevant to their ecoregion and production system.

Our results also show that practitioners should carefully consider the tradeoffs that may exist when evaluating the biodiversity impact of a production system on a cumulative or a footprint basis. Productivity is a significant driver of footprint-based results (Capper and Bauman 2013).

This was evident in our evaluation of the BII from both a cumulative and a footprint perspective. The usefulness of each unit in decision making depends on the goal of livestock impact assessment; for example, if the goal is to understand the absolute biodiversity impact, we encourage practitioners to evaluate these impacts on a cumulative basis, but if the goal is maximize livestock production while minimizing environmental harm, a footprint basis is more appropriate. Similar to our recommendation to report both cumulative and disaggregated results for the biodiversity damage indicators, we also recommend practitioners to report impacts on both a cumulative and footprint basis.

Finally, including a relevant reference system is essential for benchmarking progress towards a biodiversity optimum. In our case, Kissimmee Prairie Preserve State Park is a realistic point of comparison because it includes modest livestock production while supporting high levels of biodiversity and natural habitat. The BII demonstrates where biodiversity impacts are similar to the ‘best-practice’ system (e.g., habitat change, invasive alien species), and even cases where Buck Island Ranch or another commercial operation might supply additional biodiversity benefits (i.e., aquatic biodiversity). Given these considerations, we provide a decision-making framework (Figure 21) to help practitioners and stakeholders determine the best approach to use in their biodiversity impact assessment.

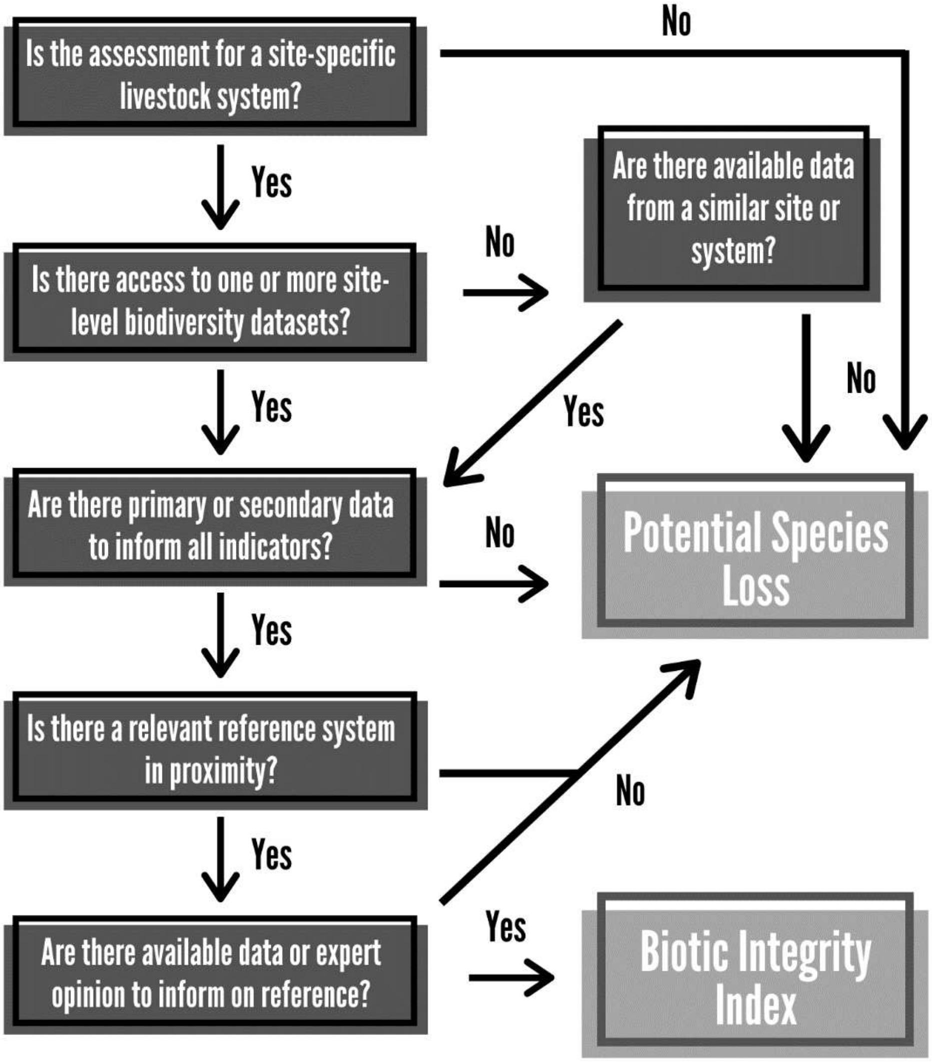


Figure 21. Decision-making framework for practitioners deciding between biodiversity impact assessment approaches to integrate into life cycle assessment. Potential species loss refers to the approach undertaken by Chaudhary and Brooks (2018) and the biotic integrity index is the adapted pressure-state-response approach applied in this study.

6. Conclusion

We demonstrated two different approaches for integrating biodiversity impact assessments into LCA based on the FAO (2020) guidelines for livestock using a ranch in central Florida as a pilot study. Our work presents a novel approach for conducting site-specific biodiversity LCIA for livestock through the application of a BII following the PSR framework, which can be applied in other livestock systems at similar scales. Overall, we found differences in the estimated biodiversity impact of two different beef grazing management systems when we applied the two methods. These differences can be attributed to differences in quantifying biodiversity impact – potential species loss (land occupation) vs. biotic integrity (integrated measure of land and non-land related impacts) – and whether biodiversity impacts are evaluated on a cumulative or footprint basis. The subjectivity introduced through these choices can be addressed through careful consultation with stakeholders and increased transparency in the methodology and reporting of results. To meet the United Nations 2030 Agenda for Sustainable Development, it is critical that biodiversity impacts are incorporated into holistic evaluations of livestock system sustainability.

DISCUSSION AND CONCLUSION

Agricultural soil management is at the forefront of the conservation on climate change like never before. The findings of this dissertation contribute to a growing body of scientific evidence in support of agricultural soil management to facilitate CDR. The adoption of cover crops on croplands, and strategic applications of organic amendments to improved grasslands can deliver climate benefits without exacerbating environmental harms. And, new guidance for integrating biodiversity impact assessments into LCA of agricultural productions systems, increases the capacity of the scientific community to quantify and evaluate ecosystem services tradeoffs that might arise from wider adoption.

Another key theme to emerge from this work is the capacity of land managers to directly influence soil C responses to BMPs. Under cover cropping, the magnitude of soil C response is influenced by decisions pertaining to cover crop planting and termination, cover crop yield, cropping rotation, and tillage management. The quality of the organic amendment (i.e., C:N ratio), amount, and frequency applied may also be important management drivers to increase soil C response in grassland agroecosystems.

Decision-support tools like COMET-Farm (Paustian et al. 2009) and the Cool Farm Tool (Hillier et al. 2011), and environmental footprint assessments, will benefit from the results of this dissertation, particularly Chapters 1 and 4. The cover crop dataset developed in the first chapter can inform model parameterization and validation efforts (McClelland et al. in review) and improve confidence in model estimates of soil C sequestration resulting from cover crops. The pilot study in the final chapter demonstrates two ways biodiversity impacts can be integrated into more holistic assessments of livestock sustainability. Improved, data-driven decision support tools that can evaluate the benefits and impacts of various agricultural management practices are essential to meeting consumer demands for climate-friendly agricultural systems and for the development of more sustainable supply chains. In addition, quantitatively driven tools will continue to play an important role in the growth of C markets and C offset projects.

Unresolved scientific questions remain on the net climate – and net environmental – benefits of wider adoption of cover crops and organic amendments. More research is needed to determine whether these same management drivers also influence soil C responses to cover crops in tropical climates (Poeplau and Don 2015, Abdalla et al. 2019). And, while previous research suggests there is not a tradeoff in soil N_2O emission with cover crop adoption (Basche et al. 2014), the number of publications on this topic is small relative to soil C responses, and merits further investigation, especially to improve biogeochemical models (McClelland et al. in review). The quantification of albedo under cover crops remains understudied at the field (Kaye and Quemada 2017) and global scale (Quemada et al. 2020). Alterations to soil N2O emissions and albedo could offset the soil C gains observed with cover crops (Levy 2019, Guenet et al. 2021). Many of these same questions persist for organic amendment applications, but also include uncertainties in the response of other dryland and perennial grassland systems to application. Furthermore, there are few LCA of organic amendment application to soil (DeLonge et al. 2013) presenting another technical barrier to wider adoption. The best use of organic amendment feedstocks, e.g., biochar, compost, or bioenergy, is unclear from both a climate and environmental perspective.

Removing socio-economic barriers are also critical. At the federal level, increased financial incentives and technical support can accelerate the adoption of NCS on natural and working lands (Sanchez et al. 2018). Many current conservation and subsidy programs are underutilized for this purpose. A recent review of annual Environmental Quality Incentives Program (EQIP) funds found that between 2009 and 2018, agricultural practices known to increase soil health, environmental co-benefits, and agroecosystem resilience represented less than a third of total funding (Basche et al. 2020). In the private sector, supply chain sustainability commitments (e.g., Walmart, General Mills, Patagonia), the formation of C markets (e.g., Nori, IndigoAg, Ecosystem Services Market Consortium), and investment in research and development (e.g., Stripe, Microsoft, Amazon) will continue to accelerate adoption (Amelung et al. 2020). The USDA under the Biden administration should continue to leverage public and private support to rapidly expand cropland and grazing land acres under cover cropping, organic amendments, and other agricultural NCS.

Agriculture is undeniably part of the solution to the climate crisis. But the timeframe to act to avert the worst impacts of climate change grows short. The time is now for scientists and decision-makers in the public and private sectors to come together to advance the adoption of NCS on natural and working lands.

REFERENCES

Abdalla, M., A. Hastings, K. Cheng, Q. Yue, D. Chadwick, M. Espenberg, J. Truu, R. M. Rees, and P. Smith. 2019. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. Global Change Biology **25**:2530-2543.

Acharya, P., R. Ghimire, and Y. Cho. 2019. Linking Soil Health to Sustainable Crop Production: Dairy Compost Effects on Soil Properties and Sorghum Biomass. Sustainability **11**:3552.

Acuna, J. C. M., and M. B. Villamil. 2014. Short-Term Effects of Cover Crops and Compaction on Soil Properties and Soybean Production in Illinois. Agronomy Journal **106**:860-870.

Allen, B. L., J. L. Pikul, J. T. Waddell, and V. L. Cochran. 2011. Long-Term Lentil Green-Manure Replacement for Fallow in the Semiarid Northern Great Plains. Agronomy Journal **103**:1292-1298.

Amelung, W., D. Bossio, W. de Vries, I. Kögel-Knabner, J. Lehmann, R. Amundson, R. Bol, C. Collins, R. Lal, and J. Leifeld. 2020. Towards a global-scale soil climate mitigation strategy. Nature communications **11**:1-10.

Anderson, M. J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences **58**:626-639.

Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. Ecology Letters **9**:683-693.

Assink, M., and C. J. Wibbelink. 2016. Fitting three-level meta-analytic models in R: A step-by-step tutorial. The Quantitative Methods for Psychology **12**:154-174.

Augustine, D. J., J. D. Derner, D. Milchunas, D. Blumenthal, and L. M. Porensky. 2017. Grazing moderates increases in C3 grass abundance over seven decades across a soil texture gradient in shortgrass steppe. Journal of Vegetation Science **28**:562-572.

Austin, E. E., K. Wickings, M. D. McDaniel, G. P. Robertson, and A. S. Grandy. 2017. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. Global Change Biology Bioenergy **9**:1252-1263.

Autret, B., B. Mary, C. Chenu, M. Balabane, C. Girardin, M. Bertrand, G. Grandeau, and N. Beaudoin. 2016. Alternative arable cropping systems: A key to increase soil organic carbon storage? Results from a 16 year field experiment. Agriculture Ecosystems & Environment **232**:150-164.

Bai, Z. G., D. L. Dent, L. Olsson, and M. E. Schaepman. 2008. Proxy global assessment of land degradation. Soil Use and Management **24**:223-234.

Baker, J. S., B. A. McCarl, B. C. Murray, S. K. Rose, R. J. Alig, D. Adams, G. Latta, R. Beach, and A. Daigneault. 2010. Net farm income and land use under a US greenhouse gas cap and trade. Agricultural and Applied Economics Association. Policy Issues 7: April 2010. 5 p.

Bakr, N., T. A. Elbana, A. E. Arceneaux, Y. Zhu, D. C. Weindorf, and H. M. Selim. 2015. Runoff and water quality from highway hillsides: Influence compost/mulch. Soil and Tillage Research **150**:158-170.

Basche, A., K. Tully, N. L. Álvarez-Berríos, J. Reyes, L. Lengnick, T. Brown, J. M. Moore, R. E. Schattman, L. K. Johnson, and G. Roesch-McNally. 2020. Evaluating the untapped potential of US conservation investments to improve soil and environmental health. Frontiers in Sustainable Food Systems **4**:236.

Basche, A. D., S. V. Archontoulis, T. C. Kaspar, D. B. Jaynes, T. B. Parkin, and F. E. Miguez. 2016. Simulating long-term impacts of cover crops and climate change on crop production and environmental outcomes in the Midwestern United States. Agriculture Ecosystems & Environment **218**:95-106.

Basche, A. D., and M. S. DeLonge. 2019. Comparing infiltration rates in soils managed with conventional and alternative farming methods: a meta-analysis. BioRxiv:603696.

Basche, A. D., F. E. Miguez, T. C. Kaspar, and M. J. Castellano. 2014. Do cover crops increase or decrease nitrous oxide emissions? A meta-analysis. Journal of Soil and Water Conservation **69**:471-482.

Bastida, F., E. Kandeler, J. Moreno, M. Ros, C. García, and T. Hernández. 2008. Application of fresh and composted organic wastes modifies structure, size and activity of soil microbial community under semiarid climate. Applied Soil Ecology **40**:318-329.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.

Batjes, N. H. 1996. Total carbon and nitrogen in the soils of the world. European Journal of Soil Science **47**:151-163.

Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller, and F. Courchamp. 2012. Impacts of climate change on the future of biodiversity. Ecology Letters **15**:365-377.

Belnap, J., and S. L. Phillips. 2001. Soil biota in an ungrazed grassland: response to annual grass (Bromus tectorum) invasion. Ecological Applications **11**:1261-1275.

Bengtsson, J., J. Bullock, B. Egoh, C. Everson, T. Everson, T. O'Connor, P. O'Farrell, H. Smith, and R. Lindborg. 2019. Grasslands—more important for ecosystem services than you might think. Ecosphere **10**:e02582.

Bernal, B., D. C. McKinley, B. A. Hungate, P. M. White, T. J. Mozdzer, and J. P. Megonigal. 2016. Limits to soil carbon stability; Deep, ancient soil carbon decomposition stimulated by new labile organic inputs. Soil Biology and Biochemistry **98**:85-94.

Bhattacharyya, P., M. Goswami, and L. Bhattacharyya. 2016. Perspective of beneficial microbes in agriculture under changing climatic scenario: A review. Journal of Phytology **8**:26-41.

Bhatti, A. A., S. Haq, and R. A. Bhat. 2017. Actinomycetes benefaction role in soil and plant health. Microbial pathogenesis **111**:458-467.

Billings, S., K. Lajtha, A. Malhotra, A. Berhe, M. A. de Graaff, S. Earl, J. Fraterrigo, K. Georgiou, S. Grandy, and S. Hobbie. 2020. Soil organic carbon is not just for soil scientists: measurement recommendations for diverse practitioners. Ecological Applications:e2290.

Bishop, T. B., S. Munson, R. A. Gill, J. Belnap, S. L. Petersen, and S. B. S. Clair. 2019. Spatiotemporal patterns of cheatgrass invasion in Colorado Plateau National Parks. Landscape Ecology **34**:925-941.

Blank, R. R. 2008. Biogeochemistry of plant invasion: a case study with downy brome (Bromus tectorum). Invasive Plant Science and Management **1**:226-238.

Bloom, A. J., F. S. Chapin III, and H. A. Mooney. 1985. Resource limitation in plants-an economic analogy. Annual Review of Ecology and Systematics **16**:363-392.

Borden, R. K., and R. Black. 2011. Biosolids Application and Long-Term Noxious Weed Dominance in the Western United States. Restoration Ecology **19**:639-647.

Bossio, D., S. Cook-Patton, P. Ellis, J. Fargione, J. Sanderman, P. Smith, S. Wood, R. Zomer, M. von Unger, and I. Emmer. 2020. The role of soil carbon in natural climate solutions. Nature Sustainability:1-8.

Boughton, E. H., P. F. Quintana-Ascencio, D. Nickerson, and P. J. Bohlen. 2011. Management intensity affects the relationship between non-native and native species in subtropical wetlands. Applied Vegetation Science **14**:210-220.

Bowman, M., S. Wallander, and L. Lynch. 2016. An economic perspective on soil health.

Brady, N. C., R. R. Weil, and R. R. Weil. 2008. The nature and properties of soils. Prentice Hall Upper Saddle River, NJ.

Bremer, D. J., J. M. Ham, C. E. Owensby, and A. K. Knapp. 1998. Responses of soil respiration to clipping and grazing in a tallgrass prairie. Journal of Environmental Quality **27**:1539-1548.

Brennan, E. B., and N. S. Boyd. 2012. Winter cover crop seeding rate and variety affects during eight years of organic vegetables: I. Cover crop biomass production. Agronomy Journal **104**:684-698.

Broadbent, F. 1953. The soil organic fraction. Pages 153-183 Advances in Agronomy. Elsevier.

Brooks, M. L., C. M. D'antonio, D. M. Richardson, J. B. Grace, J. E. Keeley, J. M. DiTomaso, R. J. Hobbs, M. Pellant, and D. Pyke. 2004. Effects of invasive alien plants on fire regimes. Bioscience **54**:677-688.

Brummer, J. 2021. Personal Communication. Colorado State University, Fort Collins, CO, USA.

Buckeridge, C., S. Duvvuri, and W. S. Denney. 2015. Simple, automatic noncompartmental analysis: the PKNCA R package. J Pharmacokinet Pharmacodyn **42**:11-107.

Butler, T. J., and J. P. Muir. 2006. Dairy manure compost improves soil and increases tall wheatgrass yield. Agronomy Journal **98**:1090-1096.

Campbell, C., S. Grayston, and D. Hirst. 1997. Use of rhizosphere carbon sources in sole carbon source tests to discriminate soil microbial communities. Journal of Microbiological Methods **30**:33-41.

Campbell, C. D., S. J. Chapman, C. M. Cameron, M. S. Davidson, and J. M. Potts. 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology **69**:3593-3599.

Capper, J. L., and D. E. Bauman. 2013. The Role of Productivity in Improving the Environmental Sustainability of Ruminant Production Systems. Pages 469-489 *in* H. A. Lewin and R. M. Roberts, editors. Annual Review of Animal Biosciences, Vol 1.

Cardinael, R., T. Chevallier, A. Cambou, C. Beral, B. G. Barthès, C. Dupraz, C. Durand, E. Kouakoua, and C. Chenu. 2017. Increased soil organic carbon stocks under agroforestry: A survey of six different sites in France. Agriculture, Ecosystems & Environment **236**:243-255.

Castellano, M. J., K. E. Mueller, D. C. Olk, J. E. Sawyer, and J. Six. 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. Global Change Biology **21**:3200-3209.

CDA. 2020. Noxious Weed Species ID. Colorado Department of Agriculture, Colorado.

Ceballos, G., P. R. Ehrlich, and P. H. Raven. 2020. Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. Proceedings of the National Academy of Sciences.

Chaparro, J. M., A. M. Sheflin, D. K. Manter, and J. M. Vivanco. 2012. Manipulating the soil microbiome to increase soil health and plant fertility. Biology and Fertility of Soils **48**:489-499.

Chapin, F. S., A. J. Bloom, C. B. Field, and R. H. Waring. 1987. Plant responses to multiple environmental factors. Bioscience **37**:49-57.

Charles, A., P. Rochette, J. K. Whalen, D. A. Angers, M. H. Chantigny, and N. Bertrand. 2017. Global nitrous oxide emission factors from agricultural soils after addition of organic amendments: A meta-analysis. Agriculture, Ecosystems & Environment **236**:88-98.

Chaudhary, A., and T. M. Brooks. 2018. Land use intensity-specific global characterization factors to assess product biodiversity footprints. Environmental science & technology **52**:5094-5104.

Chen, H., X. Li, F. Hu, and W. Shi. 2013. Soil nitrous oxide emissions following crop residue addition: a meta-analysis. Global Change Biology **19**:2956-2964.

Chirinda, N., M. S. Carter, K. R. Albert, P. Ambus, J. E. Olesen, J. R. Porter, and S. O. Petersen. 2010. Emissions of nitrous oxide from arable organic and conventional cropping systems on two soil types. Agriculture Ecosystems & Environment **136**:199-208.

Clark, A. J., A. M. Decker, and J. J. Meisinger. 1994. SEEDING RATE AND KILL DATE EFFECTS ON HAIRY VETCH CEREAL RYE COVER CROP MIXTURES FOR CORN PRODUCTION. Agronomy Journal **86**:1065-1070.

Clark, K. M., D. L. Boardman, J. S. Staples, S. Easterby, T. M. Reinbott, R. J. Kremer, N. R. Kitchen, and K. S. Veum. 2017. Crop Yield and Soil Organic Carbon in Conventional and No-till Organic Systems on a Claypan Soil. Agronomy Journal **109**:588-599.

Clark, M. S., W. R. Horwath, C. Shennan, and K. M. Scow. 1998. Changes in soil chemical properties resulting from organic and low-input farming practices. Agronomy Journal **90**:662-671.

Clegg, C. D. 2006. Impact of cattle grazing and inorganic fertiliser additions to managed grasslands on the microbial community composition of soils. Applied Soil Ecology **31**:73-82.

Colorado State University Extension. 2015. Using manure in the home garden. Colorado State University, Fort Collins, CO.

Conant, R. T., C. E. Cerri, B. B. Osborne, and K. Paustian. 2017. Grassland management impacts on soil carbon stocks: a new synthesis. Ecological Applications **27**:662-668.

Conant, R. T., and K. Paustian. 2002. Potential soil carbon sequestration in overgrazed grassland ecosystems. Global Biogeochemical Cycles **16**:90-91-90-99.

Conant, R. T., K. Paustian, and E. T. Elliott. 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecological Applications **11**:343-355.

Cotrufo, M. F., J. L. Soong, A. J. Horton, E. E. Campbell, M. L. Haddix, D. H. Wall, and W. J. Parton. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience **8**:776.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology **19**:988-995.

Curran, M., D. Maia de Souza, A. Antón, R. F. Teixeira, O. Michelsen, B. Vidal-Legaz, S. Sala, and L. Mila i Canals. 2016. How Well Does LCA Model Land Use Impacts on

Biodiversity?・ A Comparison with Approaches from Ecology and Conservation. Environmental science & technology **50**:2782-2795.

Davis, S., J. Mangold, F. Menalled, N. Orloff, Z. Miller, and E. Lehnhoff. 2018. A meta-analysis of field bindweed (Convolvulus arvensis) management in annual and perennial systems. Weed Science **66**:540-547.

de Vries, M., and I. J. M. de Boer. 2010. Review: Comparing environmental impacts for livestock products: A review of life cycle assessments. Livestock Science **128**:1-11.

Del Re, A. 2015. A practical tutorial on conducting meta-analysis in R. The Quantitative Methods for Psychology **11**:37-50.

Delgado-Baquerizo, M., P. Trivedi, C. Trivedi, D. J. Eldridge, P. B. Reich, T. C. Jeffries, and B. K. Singh. 2017. Microbial richness and composition independently drive soil multifunctionality. Functional Ecology **31**:2330-2343.

DeLonge, M. S., R. Ryals, and W. L. Silver. 2013. A lifecycle model to evaluate carbon sequestration potential and greenhouse gas dynamics of managed grasslands. Ecosystems **16**:962-979.

DeMalach, N., E. Zaady, and R. Kadmon. 2017. Contrasting effects of water and nutrient additions on grassland communities: A global meta-analysis. Global Ecology and Biogeography **26**:983-992.

Denef, K., D. Roobroeck, M. C. M. Wadu, P. Lootens, and P. Boeckx. 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology and Biochemistry **41**:144-153.

Denef, K., C. Stewart, J. Brenner, and K. Paustian. 2008. Does long-term center-pivot irrigation increase soil carbon stocks in semi-arid agro-ecosystems? Geoderma **145**:121-129.

Drinkwater, L. E., and S. S. Snapp. 2007. Nutrients in agroecosystems: Rethinking the management paradigm. Pages 163-+ *in* D. L. Sparks, editor. Advances in Agronomy, Vol 92.

Dungait, J. A., D. W. Hopkins, A. S. Gregory, and A. P. Whitmore. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology **18**:1781-1796.

EPA. 2019. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2018. Environmental Protection Agency, Washington, D.C.

Failey, E. L., and L. Dilling. 2010. Carbon stewardship: land management decisions and the potential for carbon sequestration in Colorado, USA. Environmental Research Letters **5**:024005.

Fanin, N., P. Kardol, M. Farrell, M.-C. Nilsson, M. J. Gundale, and D. A. Wardle. 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biology and Biochemistry **128**:111-114.

FAO. 2018. World Livestock: Transforming the livestock sector through the Sustainable Development Goals. FAO, Rome.

FAO. 2020. Biodiversity and the livestock sector – Guidelines for quantitative assessment – Version 1. Rome.

Fargione, J. E., S. Bassett, T. Boucher, S. D. Bridgham, R. T. Conant, S. C. Cook-Patton, P. W. Ellis, A. Falcucci, J. W. Fourqurean, and T. Gopalakrishna. 2018. Natural climate solutions for the United States. Science Advances **4**:eaat1869.

Fay, P. A., S. M. Prober, W. S. Harpole, J. M. Knops, J. D. Bakker, E. T. Borer, E. M. Lind, A. S. MacDougall, E. W. Seabloom, and P. D. Wragg. 2015. Grassland productivity limited by multiple nutrients. Nature Plants **1**:1-5.

Ferchaud, F. 2020. SimpleESM. A simple R script to calculate soil organic carbon nitrogen stocks at equivalent soil mass (ESM). INRAE, France.

Ferris, H., S. Sanchez-Moreno, and E. B. Brennan. 2012. Structure, functions and interguild relationships of the soil nematode assemblage in organic vegetable production. Applied Soil Ecology **61**:16-25.

Fick, S. E., and R. J. Hijmans. 2017. Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology **37**:4302-4315.

Finney, D. M., C. M. White, and J. P. Kaye. 2016. Biomass Production and Carbon/Nitrogen Ratio Influence Ecosystem Services from Cover Crop Mixtures. Agronomy Journal **108**:39-52.

Firn, J., J. N. Price, and R. D. Whalley. 2013. Using strategically applied grazing to manage invasive alien plants in novel grasslands. Ecological Processes **2**:1-11.

Fischer, G., H. T. van Velthuizen, and F. O. Nachtergaele. 2000. Global agro-ecological zones assessment: methodology and results.

Fornara, D. A., and D. Tilman. 2012. Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. Ecology **93**:2030-2036.

Francis, I., M. Holsters, and D. Vereecke. 2010. The Gram-positive side of plant–microbe interactions. Environmental microbiology **12**:1-12.

Franzluebbers, A., and M. Arshad. 1996. Water-stable aggregation and organic matter in four soils under conventional and zero tillage. Canadian Journal of Soil Science **76**:387-393.

Fronning, B. E., K. D. Thelen, and D. H. Min. 2008. Use of Manure, Compost, and Cover Crops to Supplant Crop Residue Carbon in Corn Stover Removed Cropping Systems. Agronomy Journal **100**:1703-1710.

Frostegård, A., and E. Bååth. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils **22**:59-65.

Frostegård, Å., A. Tunlid, and E. Bååth. 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry **43**:1621-1625.

Fuss, S., J. G. Canadell, P. Ciais, R. B. Jackson, C. D. Jones, A. Lyngfelt, G. P. Peters, and D. P. Van Vuuren. 2020. Moving toward Net-Zero Emissions Requires New Alliances for Carbon Dioxide Removal. One Earth **3**:145-149.

Garcia-Franco, N., E. Hobley, R. Hübner, and M. Wiesmeier. 2018. Climate-smart soil management in semiarid regions. Pages 349-368 Soil management and climate change. Elsevier.

Gee, G., and J. Bauder. 1986. Texture, hydrometer method. Soil Science Scociety of America: Madison, WI, USA.

Gentry, L. E., S. S. Snapp, R. F. Price, and L. F. Gentry. 2013. Apparent Red Clover Nitrogen Credit to Corn: Evaluating Cover Crop Introduction. Agronomy Journal **105**:1658-1664.

Giltrap, D., J. Yeluripati, P. Smith, N. Fitton, W. Smith, B. Grant, C. D. Dorich, J. Deng, C. F. Topp, and M. Abdalla. 2020. Global Research Alliance N2O chamber methodology guidelines: Summary of modeling approaches. Journal of Environmental Quality **49**:1168-1185.

Goldewijk, K. K., A. Beusen, J. Doelman, and E. Stehfest. 2017. Anthropogenic land use estimates for the Holocene–HYDE 3.2. Earth System Science Data **9**:927-953.

Grandy, A. S., and G. P. Robertson. 2007. Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. Ecosystems **10**:59-74.

Gravuer, K., S. Gennet, and H. L. Throop. 2019. Organic amendment additions to rangelands: A meta-analysis of multiple ecosystem outcomes. Global Change Biology **25**:1152-1170.

Gregory, A., J. Dungait, C. Watts, R. Bol, E. Dixon, R. White, and A. Whitmore. 2016. Long-term management changes topsoil and subsoil organic carbon and nitrogen dynamics in a temperate agricultural system. European Journal of Soil Science **67**:421-430.

Griscom, B. W., J. Adams, P. W. Ellis, R. A. Houghton, G. Lomax, D. A. Miteva, W. H. Schlesinger, D. Shoch, J. V. Siikamäki, and P. Smith. 2017. Natural climate solutions. Proceedings of the National Academy of Sciences **114**:11645-11650.

Gross, K. L., G. G. Mittelbach, and H. L. Reynolds. 2005. Grassland invasibility and diversity: responses to nutrients, seed input, and disturbance. Ecology **86**:476-486.

Guenet, B., B. Gabrielle, C. Chenu, D. Arrouays, J. Balesdent, M. Bernoux, E. Bruni, J. P. Caliman, R. Cardinael, and S. Chen. 2021. Can N2O emissions offset the benefits from soil organic carbon storage? Global Change Biology **27**:237-256.

Guo, L. B., and R. Gifford. 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology **8**:345-360.

Gurevitch, J., P. S. Curtis, and M. H. Jones. 2001. Meta-analysis in ecology. Advances in Ecological Research **32**:199-247.

Haddix, M. L., E. A. Paul, and M. F. Cotrufo. 2016. Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. Global Change Biology **22**:2301-2312.

Hamel, C., K. Hanson, F. Selles, A. F. Cruz, R. Lemke, B. McConkey, and R. Zentner. 2006. Seasonal and long-term resource-related variations in soil microbial communities in wheat-based rotations of the Canadian prairie. Soil Biology and Biochemistry **38**:2104-2116.

Hamza, M. A., and W. K. Anderson. 2010. Potential and limitations of soil organic matter build-up in dry areas. African Journal of Agricultural Research **5**:2850-2861.

Hansen, E. M., L. J. Munkholm, J. E. Olesen, and B. Melander. 2015. Nitrate Leaching, Yields and Carbon Sequestration after Noninversion Tillage, Catch Crops, and Straw Retention. Journal of Environmental Quality **44**:868-881.

Harpole, W. S., L. L. Sullivan, E. M. Lind, J. Firn, P. B. Adler, E. T. Borer, J. Chase, P. A. Fay, Y. Hautier, and H. Hillebrand. 2016. Addition of multiple limiting resources reduces grassland diversity. Nature **537**:93-96.

Harris, D., W. R. Horwáth, and C. Van Kessel. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Science Society of America Journal **65**:1853-1856.

Harrison, F. 2011. Getting started with meta-analysis. Methods in Ecology and Evolution **2**:1-10.

Harrison, R. B., P. W. Footen, and B. D. Strahm. 2011. Deep soil horizons: contribution and importance to soil carbon pools and in assessing whole-ecosystem response to management and global change. Forest Science **57**:67-76.

Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. Ecology **80**:1150-1156.

Hedges, L. V., and I. Olkin. 2014. Statistical methods for meta-analysis. Academic press.

Hersom, M. 2005. Pasture stocking density and the relationship to animal performance. Gainsville, Florida.

Hillier, J., C. Walter, D. Malin, T. Garcia-Suarez, L. Mila-i-Canals, and P. Smith. 2011. A farm-focused calculator for emissions from crop and livestock production. Environmental Modelling & Software **26**:1070-1078.

Hobbs, P. R., K. Sayre, and R. Gupta. 2008. The role of conservation agriculture in sustainable agriculture. Philosophical Transactions of the Royal Society B-Biological Sciences **363**:543-555.

Holz, C., L. S. Siegel, E. Johnston, A. P. Jones, and J. Sterman. 2018. Ratcheting ambition to limit warming to 1.5 C–trade-offs between emission reductions and carbon dioxide removal. Environmental Research Letters **13**:064028.

Honegger, M., A. Michaelowa, and J. Roy. 2020. Potential implications of carbon dioxide removal for the sustainable development goals. Climate policy:1-21.

Hooper, D. U., and L. Johnson. 1999. Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. Biogeochemistry **46**:247-293.

Hothorn, T., F. Bretz, P. Westfall, R. M. Heiberger, A. Schuetzenmeister, S. Scheibe, and M. T. Hothorn. 2016. Package ‘multcomp’. Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria.

Howard, P. 1965. The carbon-organic matter factor in various soil types. Oikos:229-236.

Hox, J. 2010. Multivariate multilevel regression models. Multilevel Analysis: Techniques and Applications. 2nd ed. New York, NY: Routledge.

Hubbard, R. K., T. C. Strickland, and S. Phatak. 2013. Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. Soil & Tillage Research **126**:276-283.

Huizenga, H. M., I. Visser, and C. V. Dolan. 2011. Testing overall and moderator effects in random effects meta-regression. British Journal of Mathematical and Statistical Psychology **64**:1-19.

Hurisso, T. T., J. G. Davis, J. E. Brummer, M. E. Stromberger, M. M. Mikha, M. L. Haddix, M. R. Booher, and E. A. Paul. 2013. Rapid changes in microbial biomass and aggregate size distribution in response to changes in organic matter management in grass pasture. Geoderma **193**:68-75.

Inglima, I., G. Alberti, T. Bertolini, F. Vaccari, B. Gioli, F. Miglietta, M. Cotrufo, and A. Peressotti. 2009. Precipitation pulses enhance respiration of Mediterranean ecosystems: the balance between organic and inorganic components of increased soil CO2 efflux. Global Change Biology **15**:1289-1301.

IPCC. 2014. Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY.

IPCC. 2019. Climate Change and Land: An IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse Gas Fluxes In Terrestrial Ecosystems.

Ippolito, J., K. Barbarick, M. Paschke, and R. Brobst. 2010. Infrequent composted biosolids applications affect semi-arid grassland soils and vegetation. Journal of Environmental Management **91**:1123-1130.

IUCN. 2015. IUCN Habitat Classification Scheme.*in* International Union for Conservation of Nature, editor., Cambridge, UK.

Izaurralde, R., W. B. McGill, J. Robertson, N. Juma, and J. Thurston. 2001. Carbon balance of the Breton classical plots over half a century. Soil Science Society of America Journal **65**:431-441.

Jackson, R. B., and J. S. Baker. 2010. Opportunities and constraints for forest climate mitigation. Bioscience **60**:698-707.

James, J., R. Tiller, and J. Richards. 2005. Multiple resources limit plant growth and function in a saline-alkaline desert community. Journal of Ecology **93**:113-126.

Jobbágy, E. G., and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications **10**:423-436.

Kallenbach, C. M., M. D. Wallenstein, M. E. Schipanksi, and A. S. Grandy. 2019. Managing Agroecosystems for Soil Microbial Carbon Use Efficiency: Ecological Unknowns, Potential Outcomes, and a Path Forward. Frontiers in Microbiology **10**.

Kaspar, T. C., T. B. Parkin, D. B. Jaynes, C. A. Cambardella, D. W. Meek, and Y. S. Jung. 2006. Examining changes in soil organic carbon with oat and rye cover crops using terrain covariates. Soil Science Society of America Journal **70**:1168-1177.

Kaye, J. P., and M. Quemada. 2017. Using cover crops to mitigate and adapt to climate change. A review. Agronomy for Sustainable Development **37**.

Keesstra, S. D., J. Bouma, J. Wallinga, P. Tittonell, P. Smith, A. Cerdà, L. Montanarella, J. N. Quinton, Y. Pachepsky, and W. H. Van Der Putten. 2016. The significance of soils and soil science towards realization of the United Nations Sustainable Development Goals. Soil **2**:111-128.

Kenward, M. G., and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics:983-997.

King, A. E., and J. Blesh. 2018. Crop rotations for increased soil carbon: perenniality as a guiding principle. Ecological Applications **28**:249-261.

Knapp, A. K., J. M. Briggs, S. L. Collins, S. R. Archer, M. S. BRET-HARTE, B. E. Ewers, D. P. Peters, D. R. Young, G. R. Shaver, and E. Pendall. 2008. Shrub encroachment in North American grasslands: shifts in growth form dominance rapidly alters control of ecosystem carbon inputs. Global Change Biology **14**:615-623.

Knapp, A. K., A. Chen, R. J. Griffin-Nolan, L. E. Baur, C. J. Carroll, J. E. Gray, A. M. Hoffman, X. Li, A. K. Post, and I. J. Slette. 2020. Resolving the Dust Bowl paradox of grassland responses to extreme drought. Proceedings of the National Academy of Sciences **117**:22249-22255.

Knapp, G., and J. Hartung. 2003. Improved tests for a random effects meta-regression with a single covariate. Statistics in medicine **22**:2693-2710.

Koebel, J. W., S. G. Bousquin, D. H. Anderson, M. D. Cheek, C. Carroll, H. Chen, B. C. Anderson, T. Beck, and A. Brunell. 2020. Kissimmee River Restoration and Other Kissimmee Basin Initiatives.

Kong, A. Y. Y., S. J. Fonte, C. van Kessel, and J. Six. 2009. Transitioning from standard to minimum tillage: Trade-offs between soil organic matter stabilization, nitrous oxide emissions, and N availability in irrigated cropping systems. Soil & Tillage Research **104**:256-262.

Kong, A. Y. Y., and J. Six. 2010. Tracing Root vs. Residue Carbon into Soils from Conventional and Alternative Cropping Systems. Soil Science Society of America Journal **74**:1201-1210.

Kramberger, B., A. Gselman, M. Podvrsnik, J. Kristl, and M. Lesnik. 2013. Environmental advantages of binary mixtures of Trifolium incarnatum and Lolium multiflorum over individual pure stands. Plant Soil and Environment **59**:22-28.

Kramer, C., and G. Gleixner. 2008. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. Soil Biology and Biochemistry **40**:425-433.

Kuo, S., U. M. Sainju, and E. J. Jellum. 1997a. Winter cover crop effects on soil organic carbon and carbohydrate in soil. Soil Science Society of America Journal **61**:145-152.

Kuo, S., U. M. Sainju, and E. J. Jellum. 1997b. Winter cover cropping influence on nitrogen in soil. Soil Science Society of America Journal **61**:1392-1399.

Kuzyakov, Y., J. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry **32**:1485-1498.

Lackey, R. T. 2009. Is Science Biased Toward Natural? Northwest Science **83**:291-293.

Ladoni, M., A. Basir, P. G. Robertson, and A. N. Kravchenko. 2016. Scaling-up: cover crops differentially influence soil carbon in agricultural fields with diverse topography. Agriculture Ecosystems & Environment **225**:93-103.

Lamb, E. G., N. Kennedy, and S. D. Siciliano. 2011. Effects of plant species richness and evenness on soil microbial community diversity and function. Plant and Soil **338**:483-495.

Lauren, J. G., G. S. Pettygrove, and J. M. Duxbury. 1994. Methane emissions associated with a green manure amendment to flooded rice in California. Biogeochemistry **24**:53-65.

Lavallee, J., R. Conant, E. Paul, and M. Cotrufo. 2018. Incorporation of shoot versus root-derived 13 C and 15 N into mineral-associated organic matter fractions: results of a soil slurry incubation with dual-labelled plant material. Biogeochemistry **137**:379-393.

LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology **89**:371-379.

Lee, J., J. W. Hopmans, D. E. Rolston, S. G. Baer, and J. Six. 2009. Determining soil carbon stock changes: simple bulk density corrections fail. Agriculture, Ecosystems & Environment **134**:251-256.

Leff, J. W., S. E. Jones, S. M. Prober, A. Barberán, E. T. Borer, J. L. Firn, W. S. Harpole, S. E. Hobbie, K. S. Hofmockel, and J. M. Knops. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences **112**:10967-10972.

Lenth, R. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.4.6.

Levy, C. R. 2019. Climate Impact of Land-Use Change in the Northeastern United States: Measuring Albedo and Carbon Sequestration in Willow and Forest Bioenergy Systems. Cornell University.

Li, W., P. Ciais, B. Guenet, S. Peng, J. Chang, V. Chaplot, S. Khudyaev, A. Peregon, S. Piao, and Y. Wang. 2018. Temporal response of soil organic carbon after grassland-related land-use change. Global Change Biology **24**:4731-4746.

Liang, C., J. P. Schimel, and J. D. Jastrow. 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology **2**:17105.

Linares, R., M. de la Fuente, P. Junquera, J. R. Lissarrague, and P. Baeza. 2014. Effects of soil management in vineyard on soil physical and chemical characteristics.*in* J. M. Aurand, editor. 37th World Congress of Vine and Wine and 12th General Assembly of the Oiv.

Link, S. O., C. W. Keeler, R. W. Hill, and E. Hagen. 2006. Bromus tectorum cover mapping and fire risk. International Journal of Wildland Fire **15**:113-119.

Liu, L., and T. L. Greaver. 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecology Letters **13**:819-828.

Lowe, P. N., W. K. Lauenroth, and I. C. Burke. 2003. Effects of nitrogen availability on competition between Bromus tectorum and Bouteloua gracilis. Plant Ecology **167**:247-254.

Lü, X.-T., F. A. Dijkstra, D.-L. Kong, Z.-W. Wang, and X.-G. Han. 2014. Plant nitrogen uptake drives responses of productivity to nitrogen and water addition in a grassland. Scientific Reports **4**:4817.

Lunt, H. A. 1931. The carbon-organic matter factor in forest soil humus. Soil Sci **32**:e33.

Lynch, D., R. Voroney, and P. Warman. 2005. Soil physical properties and organic matter fractions under forages receiving composts, manure or fertilizer. Compost Science & Utilization **13**:252-261.

Ma, S., F. He, D. Tian, D. Zou, Z. Yan, Y. Yang, T. Zhou, K. Huang, H. Shen, and J. Fang. 2018. Variations and determinants of carbon content in plants: a global synthesis. Biogeosciences **15**:693-702.

Mancinelli, R., S. Marinari, P. Brunetti, E. Radicetti, and E. Campiglia. 2015. Organic mulching, irrigation and fertilization affect soil CO2 emission and C storage in tomato crop in the Mediterranean environment. Soil & Tillage Research **152**:39-51.

Mancinelli, R., S. Marinari, V. Di Felice, M. C. Savin, and E. Campiglia. 2013. Soil property, CO2 emission and aridity index as agroecological indicators to assess the mineralization of cover crop green manure in a Mediterranean environment. Ecological Indicators **34**:31-40.

Marcillo, G., and F. Miguez. 2017. Corn yield response to winter cover crops: An updated meta-analysis. Journal of Soil and Water Conservation **72**:226-239.

Martinez Arbizu, P. 2020. pairwiseAdonis: Pairwise multilevel comparison using adonis.

Maxwell, S. L., R. A. Fuller, T. M. Brooks, and J. E. Watson. 2016. Biodiversity: The ravages of guns, nets and bulldozers. Nature News **536**:143.

Mazzoncini, M., T. B. Sapkota, P. Barberi, D. Antichi, and R. Risaliti. 2011. Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content. Soil & Tillage Research **114**:165-174.

McClelland, S., C. Arndt, D. Gordon, and G. Thoma. 2018. Type and number of environmental impact categories used in livestock life cycle assessment: A systematic review. Livestock Science **209**:39-45.

McClelland, S. C., K. Paustian, S. Williams, and M. E. Schipanski. in review. Modeling cover crop biomass production and related emissions to improve farm-scale decision-support tools Agricultural Systems.

McCune, B., J. B. Grace, and D. L. Urban. 2002. Analysis of ecological communities. MjM software design Gleneden Beach, OR.

McDaniel, M. D., L. K. Tiemann, and A. S. Grandy. 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications **24**:560-570.

McHugh, T. A., E. M. Morrissey, R. C. Mueller, L. V. Gallegos-Graves, C. R. Kuske, and S. C. Reed. 2017. Bacterial, fungal, and plant communities exhibit no biomass or compositional response to two years of simulated nitrogen deposition in a semiarid grassland. Environmental microbiology **19**:1600-1611.

McNaughton, S., D. Milchunas, and D. Frank. 1996. How can net primary productivity be measured in grazing ecosystems? Ecology **77**:974-977.

Medley, K. A., E. H. Boughton, D. G. Jenkins, J. E. Fauth, P. J. Bohlen, and P. F. Quintana-Ascencio. 2015. Intense ranchland management tips the balance of regional and local factors affecting wetland community structure. Agriculture, Ecosystems & Environment **212**:207-244.

Menge, D. N., and C. B. Field. 2007. Simulated global changes alter phosphorus demand in annual grassland. Global Change Biology **13**:2582-2591.

Mikha, M. M., D. P. Widiastuti, T. T. Hurisso, J. E. Brummer, and J. G. Davis. 2017. Influence of Composted Dairy Manure and Perennial Forage on Soil Carbon and Nitrogen Fractions during Transition into Organic Management. Agriculture **7**:37.

Milà i Canals, L., C. Bauer, J. Depestele, A. Dubreuil, R. F. Knuchel, G. Gaillard, O. Michelsen, R. Müller-Wenk, and B. Rydgren. 2007. Key elements in a framework for land use impact assessment within LCA The International Journal of Life Cycle Assessment **12**:5-15.

Milchunas, D. G. 2009. Estimating root production: comparison of 11 methods in shortgrass steppe and review of biases. Ecosystems **12**:1381-1402.

Millar, R. J., J. S. Fuglestvedt, P. Friedlingstein, J. Rogelj, M. J. Grubb, H. D. Matthews, R. B. Skeie, P. M. Forster, D. J. Frame, and M. R. Allen. 2017. Emission budgets and pathways consistent with limiting warming to 1.5 C. Nature Geoscience **10**:741-747.

Millennium Ecosystem Assessment. 2005. Ecosystems and human well-being.

Miner, G., J. Delgado, J. Ippolito, C. Stewart, D. Manter, S. Del Grosso, B. Floyd, and R. D'Adamo. 2020. Assessing manure and inorganic nitrogen fertilization impacts on soil health, crop productivity, and crop quality in a continuous maize agroecosystem. Journal of Soil and Water Conservation **75**:481-498.

Minoshima, H., L. E. Jackson, T. R. Cavagnaro, S. Sanchez-Moreno, H. Ferris, S. R. Temple, S. Goyal, and J. P. Mitchell. 2007. Soil food webs and carbon dynamics in response to conservation tillage in California. Soil Science Society of America Journal **71**:952-963.

Mitchell, J. P., A. Shrestha, W. R. Horwath, R. J. Southard, N. Madden, J. Veenstra, and D. S. Munk. 2015. Tillage and Cover Cropping Affect Crop Yields and Soil Carbon in the San Joaquin Valley, California. Agronomy Journal **107**:588-596.

Moher, D., A. Liberati, J. Tetzlaff, D. G. Altman, and P. Group. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS med **6**:e1000097.

Morra, L., D. Cerrato, M. Bilotto, and S. Baiano. 2017. Introduction of sorghum Sorghum bicolor (L.) Moench green manure in rotations of head salads and baby leaf crops under greenhouse. Italian Journal of Agronomy **12**:40-46.

Murrell, E. G., M. E. Schipanski, D. M. Finney, M. C. Hunter, M. Burgess, J. C. LaChance, B. Baraibar, C. M. White, D. A. Mortensen, and J. P. Kaye. 2017. Achieving diverse cover crop mixtures: Effects of planting date and seeding rate. Agronomy Journal **109**:259-271.

Mysterud, A. 2006. The concept of overgrazing and its role in management of large herbivores. Wildlife Biology **12**:129-141.

National Academies of Sciences, E., and Medicine. 2018. Negative Emissions Technologies and Reliable Sequestration: A Research Agenda. The National Academies Press, Washington, DC.

Neina, D. 2019. The role of soil pH in plant nutrition and soil remediation. Applied and Environmental Soil Science **2019**.

Newbold, T., L. N. Hudson, S. L. Hill, S. Contu, I. Lysenko, R. A. Senior, L. Börger, D. J. Bennett, A. Choimes, and B. Collen. 2015. Global effects of land use on local terrestrial biodiversity. Nature **520**:45-50.

Nyakatawa, E. Z., K. C. Reddy, and K. R. Sistani. 2001. Tillage, cover cropping, and poultry litter effects on selected soil chemical properties. Soil & Tillage Research **58**:69-79.

O. Hoegh-Guldberg, D. J., M. Taylor, M. Bindi, S. Brown, I. Camilloni, A. Diedhiou, R. Djalante, K. Ebi, F. Engelbrecht, J. Guiot, Y. Hijioka, S. Mehrotra, A. Payne, S. I. Seneviratne, A. Thomas, R. Warren, G. Zhou. 2018. Impacts of 1.5ºC global warming on natural and human systems.*in* P. Z. V. Masson-Delmotte, H. O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterfield (eds.), editor. Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty.

Oksanen, J., F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter, R. B. O. H. R. Minchin, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs and Helene, and C. Wagner-Riddle. 2019. vegan: Community Ecology Package.

Otuya, R., L. C. Slaughter, C. P. West, S. K. Deb, and V. Acosta-Martinez. 2020. Compost and legume management differently alter soil microbial abundance and soil carbon in semi-arid pastures. Soil Science Society of America Journal.

Pardo, L. H., M. E. Fenn, C. L. Goodale, L. H. Geiser, C. T. Driscoll, E. B. Allen, J. S. Baron, R. Bobbink, W. D. Bowman, and C. M. Clark. 2011. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. Ecological Applications **21**:3049-3082.

Parkin, T. B. 2008. Effect of sampling frequency on estimates of cumulative nitrous oxide emissions. Journal of Environmental Quality **37**:1390-1395.

Paustian, K., J. Brenner, M. Easter, K. Killian, S. Ogle, C. Olson, J. Schuler, R. Vining, and S. Williams. 2009. Counting carbon on the farm: Reaping the benefits of carbon offset programs. Journal of Soil and Water Conservation **64**:36A-40A.

Paustian, K., M. Easter, K. Brown, A. Chambers, M. Eve, A. Huber, E. Marx, M. Layer, M. Stermer, and B. Sutton. 2018. Field-and farm-scale assessment of soil greenhouse gas mitigation using COMET-Farm. Precision Conservation: Geospatial Techniques for Agricultural and Natural Resources Conservation:341-359.

Paustian, K., E. Larson, J. Kent, E. Marx, and A. Swan. 2019. Soil C sequestration as a biological negative emission strategy. Frontiers in Climate **1**:8.

Paustian, K., J. Lehmann, S. Ogle, D. Reay, G. P. Robertson, and P. Smith. 2016. Climate-smart soils. Nature **532**:49-57.

Paustian, K., J. Six, E. Elliott, and H. Hunt. 2000. Management options for reducing CO2 emissions from agricultural soils. Biogeochemistry **48**:147-163.

Peng, Y., D. Guo, and Y. Yang. 2017. Global patterns of root dynamics under nitrogen enrichment. Global Ecology and Biogeography **26**:102-114.

Penn, C. J., and J. J. Camberato. 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. Agriculture **9**:120.

Peregrina, F., E. P. Perez-Alvarez, and E. Garcia-Escudero. 2014a. The short term influence of aboveground biomass cover crops on C sequestration and beta-glucosidase in a vineyard ground under semiarid conditions. Spanish Journal of Agricultural Research **12**:1000-1007.

Peregrina, F., E. P. Pérez-Álvarez, and E. García-Escudero. 2014b. The short term influence of aboveground biomass cover crops on C sequestration and β-glucosidase in a vineyard ground under semiarid conditions. Spanish Journal of Agricultural Research **12**:1000-1007.

Philibert, A., C. Loyce, and D. Makowski. 2012. Assessment of the quality of meta-analysis in agronomy. Agriculture, Ecosystems & Environment **148**:72-82.

Phillips, M. L., D. E. Winkler, R. H. Reibold, B. B. Osborne, and S. C. Reed. 2020. Muted responses to chronic experimental nitrogen deposition on the Colorado Plateau. Oecologia:1-12.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. Bioscience **50**:53-65.

Poeplau, C., and A. Don. 2015. Carbon sequestration in agricultural soils via cultivation of cover crops–A meta-analysis. Agriculture, Ecosystems & Environment **200**:33-41.

PRISM Climate Group. 2020. Time Series Values for Individual Locations. Oregon State University http://prism.oregonstate.edu.

Puget, P., and L. Drinkwater. 2001. Short-term dynamics of root-and shoot-derived carbon from a leguminous green manure. Soil Science Society of America Journal **65**:771-779.

Quemada, M., L. Lassaletta, A. Leip, A. Jones, and E. Lugato. 2020. Integrated management for sustainable cropping systems: Looking beyond the greenhouse balance at the field scale. Global Change Biology **26**:2584-2598.

Quideau, S. A., A. C. McIntosh, C. E. Norris, E. Lloret, M. J. Swallow, and K. Hannam. 2016. Extraction and analysis of microbial phospholipid fatty acids in soils. Journal of visualized experiments: JoVE.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramirez, K. S., J. M. Craine, and N. Fierer. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology **18**:1918-1927.

Ramirez, K. S., C. L. Lauber, R. Knight, M. A. Bradford, and N. Fierer. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology **91**:3463-3470.

Randolph, T. F., E. Schelling, D. Grace, C. F. Nicholson, J. Leroy, D. Cole, M. Demment, A. Omore, J. Zinsstag, and M. Ruel. 2007. Invited review: Role of livestock in human nutrition and health for poverty reduction in developing countries. Journal of Animal Science **85**:2788-2800.

Rasse, D. P., C. Rumpel, and M.-F. Dignac. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil **269**:341-356.

Raudenbush, S. W., and A. S. Bryk. 2002. Hierarchical linear models: Applications and data analysis methods. Sage.

Raza, S., N. Miao, P. Wang, X. Ju, Z. Chen, J. Zhou, and Y. Kuzyakov. 2020. Dramatic loss of inorganic carbon by nitrogen-induced soil acidification in Chinese croplands. Global Change Biology **26**:3738-3751.

Reeder, J., and G. Schuman. 2001. Influence of livestock grazing on C sequestration in semi-arid mixed-grass and short-grass rangelands. Environmental pollution **116**:457-463.

Riggs, C. E., and S. E. Hobbie. 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biology and Biochemistry **99**:54-65.

Rimer, R., and R. Evans. 2006. Invasion of downy brome (Bromus tectorum L.) causes rapid changes in the nitrogen cycle. The American midland naturalist **156**:252-258.

Rogelj, J., M. Schaeffer, M. Meinshausen, R. Knutti, J. Alcamo, K. Riahi, and W. Hare. 2015. Zero emission targets as long-term global goals for climate protection. Environmental Research Letters **10**:105007.

Rosenthal, R. 1984. Meta-analytic procedures for social research. Beverly Hills, CA: Sage.

Rosenzweig, S. T., S. J. Fonte, and M. E. Schipanski. 2018. Intensifying rotations increases soil carbon, fungi, and aggregation in semi-arid agroecosystems. Agriculture, Ecosystems & Environment **258**:14-22.

Rotz, C. A., S. Asem-Hiablie, S. Place, and G. Thoma. 2019. Environmental footprints of beef cattle production in the United States. Agricultural Systems **169**:1-13.

Rumpel, C., and I. Kögel-Knabner. 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. Plant and Soil **338**:143-158.

Ryals, R., V. T. Eviner, C. Stein, K. N. Suding, and W. L. Silver. 2016. Grassland compost amendments increase plant production without changing plant communities. Ecosphere **7**.

Ryals, R., M. Kaiser, M. S. Torn, A. A. Berhe, and W. L. Silver. 2014. Impacts of organic matter amendments on carbon and nitrogen dynamics in grassland soils. Soil Biology and Biochemistry **68**:52-61.

Ryals, R., and W. L. Silver. 2013. Effects of organic matter amendments on net primary productivity and greenhouse gas emissions in annual grasslands. Ecological Applications **23**:46-59.

Ryser, P., B. Verduyn, and H. Lambers. 1997. Phosphorus allocation and utilization in three grass species with contrasting response to N and P supply. New Phytologist **137**:293-302.

Sainju, U. M., and B. P. Singh. 1997. Winter cover crops for sustainable agricultural systems: Influence on soil properties, water quality, and crop yields. Hortscience **32**:21-28.

Sainju, U. M., B. P. Singh, and W. F. Whitehead. 2002. Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentrations in sandy loam soils in Georgia, USA. Soil & Tillage Research **63**:167-179.

Sainju, U. M., H. P. Singh, and B. P. Singh. 2017. Soil Carbon and Nitrogen in Response to Perennial Bioenergy Grass, Cover Crop and Nitrogen Fertilization. Pedosphere **27**:223-235.

Sainju, U. M., W. F. Whitehead, and B. P. Singh. 2005. Carbon accumulation in cotton, sorghum, and underlying soil as influenced by tillage, cover crops, and nitrogen fertilization. Plant and Soil **273**:219-234.

Sanchez, D. L., G. Amador, J. Funk, and K. J. Mach. 2018. Federal research, development, and demonstration priorities for carbon dioxide removal in the United States. Environmental Research Letters **13**:015005.

Sanderman, J. 2012. Can management induced changes in the carbonate system drive soil carbon sequestration? A review with particular focus on Australia. Agriculture, Ecosystems & Environment **155**:70-77.

Sanderman, J., T. Hengl, and G. J. Fiske. 2017. Soil carbon debt of 12,000 years of human land use. Proceedings of the National Academy of Sciences **114**:9575-9580.

Sanz-Cobena, A., S. Garcia-Marco, M. Quemada, J. L. Gabriel, P. Almendros, and A. Vallejo. 2014. Do cover crops enhance N2O, CO2 or CH4 emissions from soil in Mediterranean arable systems? Science of the Total Environment **466**:164-174.

Sarwar, G., H. Schmeisky, N. Hussain, S. Muhammad, M. Ibrahim, and E. Safdar. 2008. Improvement of soil physical and chemical properties with compost application in rice-wheat cropping system. Pakistan Journal of Botany **40**:275-282.

Schimel, J. P., and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology **85**:591-602.

Schipanski, M. E., M. Barbercheck, M. R. Douglas, D. M. Finney, K. Haider, J. P. Kaye, A. R. Kemanian, D. A. Mortensen, M. R. Ryan, J. Tooker, and C. White. 2014. A framework for evaluating ecosystem services provided by cover crops in agroecosystems. Agricultural Systems **125**:12-22.

Seifert, C. A., G. Azzari, and D. B. Lobell. 2019. Corrigendum: Satellite detection of cover crops and their effects on crop yield in the Midwestern United States (2018 Environ. Res. Let. 13 064033). Environmental Research Letters **14**:039501.

Senthilkumar, S., A. N. Kravchenko, and G. P. Robertson. 2009. Topography Influences Management System Effects on Total Soil Carbon and Nitrogen. Soil Science Society of America Journal **73**:2059-2067.

Shannon, C. E. 1948. A mathematical theory of communication. The Bell system technical journal **27**:379-423.

Sharma, S. B., R. Z. Sayyed, M. H. Trivedi, and T. A. Gobi. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus **2**:1-14.

Shcherbak, I., N. Millar, and G. P. Robertson. 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. Proceedings of the National Academy of Sciences **111**:9199-9204.

Sherrod, L., G. Dunn, G. Peterson, and R. Kolberg. 2002. Inorganic carbon analysis by modified pressure-calcimeter method. Soil Science Society of America Journal **66**:299-305.

Six, J., R. T. Conant, E. A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil **241**:155-176.

Six, J., E. Elliott, and K. Paustian. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology and Biochemistry **32**:2099-2103.

Smith, P., J. F. Soussana, D. Angers, L. Schipper, C. Chenu, D. P. Rasse, N. H. Batjes, F. van Egmond, S. McNeill, and M. Kuhnert. 2020. How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. Global Change Biology **26**:219-241.

Smith, R. G., L. W. Atwood, and N. D. Warren. 2014. Increased productivity of a cover crop mixture is not associated with enhanced agroecosystem services. PLoS One **9**:e97351.

Snapp, S., S. Swinton, R. Labarta, D. Mutch, J. Black, R. Leep, J. Nyiraneza, and K. O'neil. 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. Agronomy Journal **97**:322-332.

Snapp, S. S., L. E. Gentry, and R. Harwood. 2010. Management intensity - not biodiversity - the driver of ecosystem services in a long-term row crop experiment. Agriculture Ecosystems & Environment **138**:242-248.

Sobecki, T., D. Moffitt, J. Stone, C. Franks, and A. Mendenhall. 2001. A broad-scale perspective on the extent, distribution, and characteristics of US grazing lands. The potential of US grazing lands to sequester carbon and mitigate the greenhouse effect:21-63.

Soil Survey Staff, N. R. C. S., United States Department of Agriculture,. 2017. Web Soil Survey.

Sokol, N. W., J. Sanderman, and M. A. Bradford. 2019. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Global Change Biology **25**:12-24.

Sollins, P., P. Homann, and B. A. Caldwell. 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma **74**:65-105.

Steenwerth, K., and K. M. Belina. 2008. Cover crops enhance soil organic matter, carbon dynamics and microbiological function in a vineyard agroecosystem. Applied Soil Ecology **40**:359-369.

Stein, C., S. B. Unsicker, A. Kahmen, M. Wagner, V. Audorff, H. Auge, D. Prati, and W. W. Weisser. 2010. Impact of invertebrate herbivory in grasslands depends on plant species diversity. Ecology **91**:1639-1650.

Steinfeld, H., and P. Gerber. 2010. Livestock production and the global environment: Consume less or produce better? Proceedings of the National Academy of Sciences **107**:18237-18238.

Stott, D. E., and B. N. Moebius-Clune. 2017. Soil health: challenges and opportunities. Pages 109-121 Global Soil Security. Springer.

Strock, J. S., P. M. Porter, and M. Russelle. 2004. Cover cropping to reduce nitrate loss through subsurface drainage in the northern US Corn Belt. Journal of Environmental Quality **33**:1010-1016.

Swain, H. M., E. H. Boughton, P. J. Bohlen, and L. O. G. Lollis. 2013. Trade-offs among ecosystem services and disservices on a Florida ranch. Rangelands **35**:75-87.

Syswerda, S. P., and G. P. Robertson. 2014. Ecosystem services along a management gradient in Michigan (USA) cropping systems. Agriculture, Ecosystems & Environment **189**:28-35.

Tabak, M. A., M. S. Norouzzadeh, D. W. Wolfson, S. J. Sweeney, K. C. VerCauteren, N. P. Snow, J. M. Halseth, P. A. Di Salvo, J. S. Lewis, and M. D. White. 2019. Machine learning to classify animal species in camera trap images: Applications in ecology. Methods in Ecology and Evolution **10**:585-590.

Tautges, N. E., J. L. Chiartas, A. C. Gaudin, A. T. O'Geen, I. Herrera, and K. M. Scow. 2019. Deep soil inventories reveal that impacts of cover crops and compost on soil carbon sequestration differ in surface and subsurface soils. Global Change Biology **25**:3753-3766.

Teillard, F., D. M. de Souza, G. Thoma, P. J. Gerber, and J. A. Finn. 2016. What does Life-Cycle Assessment of agricultural products need for more meaningful inclusion of biodiversity? Journal of Applied Ecology **53**:1422-1429.

Terra, J. A., D. W. Reeves, J. N. Shaw, and R. L. Raper. 2005. Impacts of landscape attributes on carbon sequestration during the transition from conventional to conservation management practices on a Coastal Plain field. Journal of Soil and Water Conservation **60**:438-446.

Thangarajan, R., N. S. Bolan, G. Tian, R. Naidu, and A. Kunhikrishnan. 2013. Role of organic amendment application on greenhouse gas emission from soil. Science of the Total Environment **465**:72-96.

Thoma, G., J. Popp, D. Nutter, D. Shonnard, R. Ulrich, M. Matlock, D. S. Kim, Z. Neiderman, N. Kemper, and C. East. 2013. Greenhouse gas emissions from milk production and consumption in the United States: A cradle-to-grave life cycle assessment circa 2008. International Dairy Journal **31**:S3-S14.

Thompson, S. G., and S. J. Sharp. 1999. Explaining heterogeneity in meta-analysis: a comparison of methods. Statistics in medicine **18**:2693-2708.

Thomsen, I. K., and B. T. Christensen. 2004. Yields of wheat and soil carbon and nitrogen contents following long-term incorporation of barley straw and ryegrass catch crops. Soil Use and Management **20**:432-438.

Throop, H., S. Archer, H. Monger, and S. Waltman. 2012. When bulk density methods matter: Implications for estimating soil organic carbon pools in rocky soils. Journal of Arid Environments **77**:66-71.

Tilman, D., M. Clark, D. R. Williams, K. Kimmel, S. Polasky, and C. Packer. 2017. Future threats to biodiversity and pathways to their prevention. Nature **546**:73-81.

Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science **294**:843-845.

Treseder, K. K. 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. Ecology Letters **11**:1111-1120.

Tummers, B. 2006. DataThief III.

USDA ARS. 2020. LTAR Sites: Archbold-University of Florida, South Central Florida. USDA ARS,.

USDA NASS. 2019. 2017 Census of Agriculture.

USDA NASS. 2020. 2019 State Agriculture Overview Colorado. USDA National Agricultural Statistics Service, Washington, D.C.

Vance, C. P., P. H. Graham, and D. L. Allan. 2000. Biological nitrogen fixation: phosphorus-a critical future need? Pages 509-514 Nitrogen fixation: From molecules to crop productivity. Springer.

Vasquez, E., R. Sheley, and T. Svejcar. 2008. Nitrogen enhances the competitive ability of cheatgrass (Bromus tectorum) relative to native grasses. Invasive Plant Science and Management **1**:287-295.

Viechtbauer, W. 2010. Conducting meta-analyses in R with the metafor package. J Stat Softw **36**:1-48.

Vincent-Caboud, L., J. Peigné, M. Casagrande, and E. M. Silva. 2017. Overview of organic cover crop-based no-tillage technique in Europe: Farmers' practices and research challenges. Agriculture **7**:42.

Vitousek, P. M., H. A. Mooney, J. Lubchenco, and J. M. Melillo. 1997. Human domination of Earth's ecosystems. Science **277**:494-499.

Wang, F., R. R. Weil, and X. Nan. 2017a. Total and permanganate-oxidizable organic carbon in the corn rooting zone of US Coastal Plain soils as affected by forage radish cover crops and N fertilizer. Soil and Tillage Research **165**:247-257.

Wang, Q. G., W. Klassen, Y. C. Li, and M. Codallo. 2009. Cover Crops and Organic Mulch to Improve Tomato Yields and Soil Fertility. Agronomy Journal **101**:345-351.

Wang, Q. R., Y. C. Li, and A. Alva. 2012. Cover Crops in Mono- and Biculture for Accumulation of Biomass and Soil Organic Carbon. Journal of Sustainable Agriculture **36**:423-439.

Wang, R., M. Dorodnikov, F. A. Dijkstra, S. Yang, Z. Xu, H. Li, and Y. Jiang. 2017b. Sensitivities to nitrogen and water addition vary among microbial groups within soil aggregates in a semiarid grassland. Biology and Fertility of Soils **53**:129-140.

Weindorf, D. C., J. P. Muir, and C. Landeros-Sánchez. 2011. Organic compost and manufactured fertilizers: economics and ecology. Integrating agriculture, conservation and ecotourism: Examples from the field:27-53.

Welch, R. Y., G. D. Behnke, A. S. Davis, J. Masiunas, and M. B. Villamil. 2016. Using cover crops in headlands of organic grain farms: effects on soil properties, weeds and crop yields. Agriculture, Ecosystems & Environment **216**:322-332.

Wendling, M., R. Charles, J. Herrera, C. Amossé, B. Jeangros, A. Walter, and L. Büchi. 2019. Effect of species identity and diversity on biomass production and its stability in cover crop mixtures. Agriculture, Ecosystems & Environment **281**:81-91.

White, C. M., S. T. DuPont, M. Hautau, D. Hartman, D. M. Finney, B. Bradley, J. C. LaChance, and J. P. Kaye. 2017. Managing the trade off between nitrogen supply and retention with cover crop mixtures. Agriculture Ecosystems & Environment **237**:121-133.

Wiebe, N., B. Vandermeer, R. W. Platt, T. P. Klassen, D. Moher, and N. J. Barrowman. 2006. A systematic review identifies a lack of standardization in methods for handling missing variance data. Journal of clinical epidemiology **59**:342-353.

Wiesmeier, M., L. Urbanski, E. Hobley, B. Lang, M. von Luetzow, E. Marin-Spiotta, B. van Wesemael, E. Rabot, M. Liess, and N. Garcia-Franco. 2019. Soil organic carbon storage as a key function of soils-a review of drivers and indicators at various scales. Geoderma **333**:149-162.

Woensby, C. 1973. Modified step-point system for botanical conposition and basal cover estimates. Rangeland Ecology & Management/Journal of Range Management Archives **26**:302-303.

Wu, H., Z. Guo, Q. Gao, and C. Peng. 2009. Distribution of soil inorganic carbon storage and its changes due to agricultural land use activity in China. Agriculture, Ecosystems & Environment **129**:413-421.

Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock, and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology **84**:2042-2050.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils **29**:111-129.

Zhou, M., B. Zhu, S. Wang, X. Zhu, H. Vereecken, and N. Brüggemann. 2017. Stimulation of N2O emission by manure application to agricultural soils may largely offset carbon benefits: A global meta-analysis. Global Change Biology **23**:4068-4083.

Zhou, X., H. Wu, Z. Xu, and C. Chen. 2010. Winter cover crops increase soil carbon and nitrogen cycling processes and microbial functional diversity. Pages 47-50. International Union of Soil Sciences (IUSS), c/o Institut für Bodenforschung, Universität für Bodenkultur, Wien.

APPENDICES

APPENDIX 1 CHAPTER 1

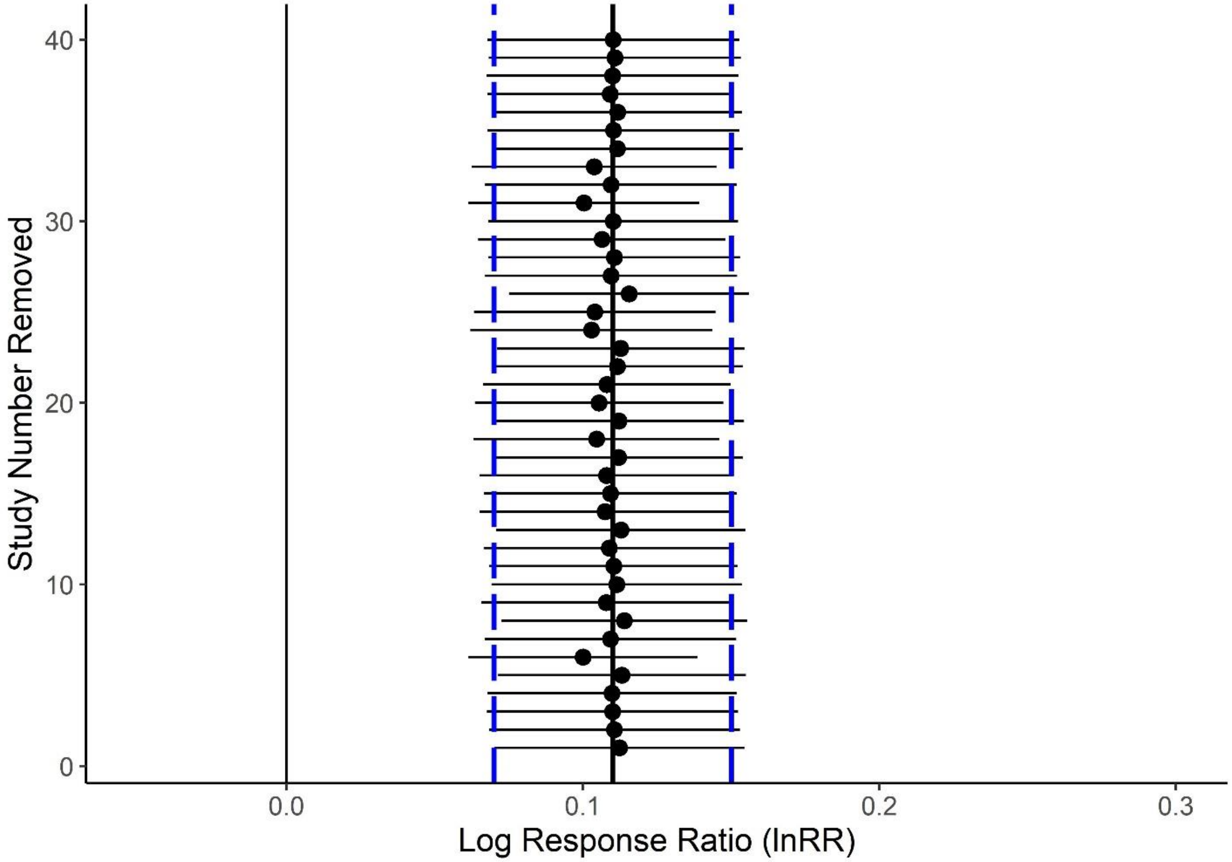
Appendix 1 Table 1. Summary of publications included in the meta-analysis.

Reference	Location	Time since introduction	Cropping system	Cover crop	Functional type	Soil sampling depth (cm)[1]	No. in sensitivity analysis
Campbell et al. (1991)	Saskatchewan, Canada	30	Wheat-Fallow	Legume	Legume	0-15	36
Kuo et al. (1997)	Washington, USA	4	Maize	Legume and non-legume	Legume, grass, and brassica	0-30	19
Clark et al. (1998)	California, USA	5	Tomato-Safflower-Maize-Bean	Legume and non-legume	Mixture	0-30	2
Nyakatawa et al. (2001)	Alabama, USA	3	Cotton	Non-legume	Grass	0-30	29
Sainju et al. (2002)	Georgia, USA	5, 6	Tomato, Tomato-Eggplant	Legume and non-legume	Legume, grass	0-20	27
Thomsen and Christensen (2004)	Jutland, Denmark	10	Spring Barley	Non-legume	Grass	0-20	40
Sainju et al. (2005)	Georgia, USA	6	Cotton-Sorghum	Legume and non-legume	Legume, grass, mixture	0-30	28
Terra et al. (2005)	Alabama, USA	3	Maize-Cotton	Legume and non-legume	Mixture	0-30	15
Kaspar et al. (2006)	Iowa, USA	7	Maize-Soybean	Non-legume	Grass	0-5	13
Minoshima et al. (2007)	California, USA	2	Tomato-Chickpea	Legume and non-legume	Legume, grass	0-30	34

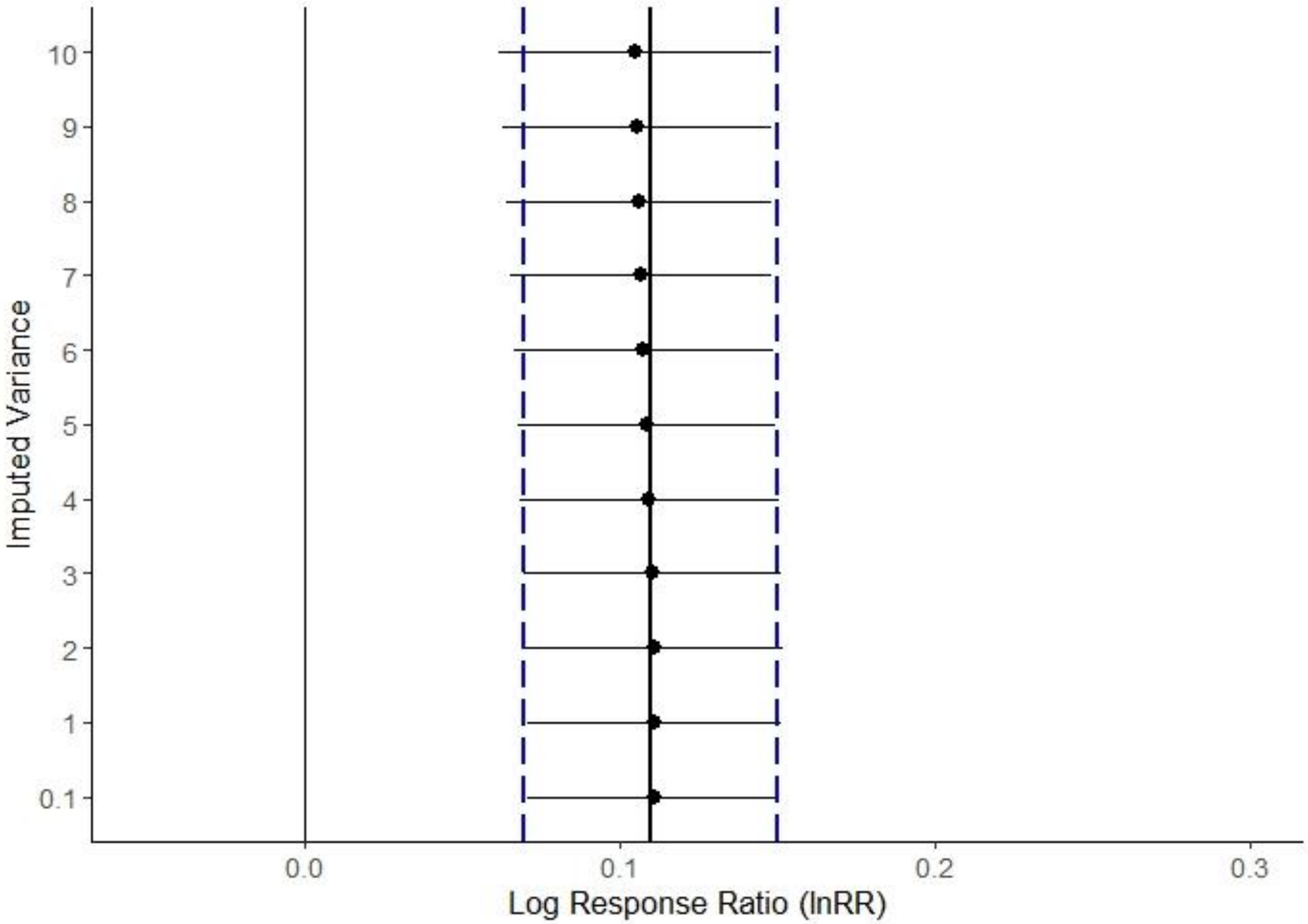
Steenwerth and Belina (2008)	California, USA	6	Vineyard	Non-legume	Grass	0-15	31
Fronning et al. (2008)	Michigan, USA	3	Maize-Soybean	Non-legume	Grass	0-25	17
Senthilkumar et al. (2009)	Michigan, USA	18	Maize-Soybean-Wheat	Legume	Legume	0-40	9
Kong et al. (2009)	California, USA	1	Maize-Tomato	Legume	Mixture	0-15	7
Wang et al. (2009)	Florida, USA	2	Tomato	Legume and non-legume	Legume, grass	0-10	33
Zhou et al. (2010)	New South Wales, Australia	1	Wheat	Legume and non-legume	Legume, grass, brassica	0-10	20
Snapp et al. (2010)	Michigan, USA	16	Maize	Legume and non-legume	Legume, mixture	0-25	10
Chirinda et al. (2010)	Jutland and Sjælland, Denmark	11	Winter wheat	Legume and non-legume	Mixture	0-25	26
Hamza and Anderson (2010)	Western Australia, Australia	4	Cereal-Legume	Legume and non-legume	Mixture	0-10	24
Allen et al. (2011)	Montana, USA	12	Wheat	Legume	Legume	0-30	12
Mazzoncini et al. (2011)	Central, Italy	15	Maize-Wheat	Legume and non-legume	Legume, mixture	0-30	35
Ferris et al. (2012)	California, USA	15	Vegetable	Legume and non-legume	Mixture, grass, brassica	0-30	3
Wang et al. (2012)	Florida, USA	1	___	Legume and non-legume	Legume, grass, brassica, mixture	0-10	16
Gentry et al. (2013)	Michigan, USA	16	Maize-Soybean-Wheat	Legume and non-legume	Mixture	0-25	6
Hubbard et al. (2013)	Georgia, USA	3	Sweet corn	Legume	Legume	0-2.5	18
Mancinelli et al. (2013)	Viterbo, Italy	2	Vegetable	Legume and non-legume	Legume, grass	0-20	23

Acuna and Villamil (2014)	Illinois, USA	2	Soybean	Legume and non-legume	Mixture, brassica	0-50	11
Linares et al. (2014)	Central, Spain	8	Vineyard	Non-legume	Grass	0-30	37
Peregrina et al. (2014)	La Rioja, Spain	4	Vineyard	Legume and non-legume	Legume, grass	0-5	25
Syswerda and Robertson (2014)	Michigan, USA	13	Maize-Soybean-Wheat	Legume	Legume	0-100	4
Hansen et al. (2015)	Jutland, Denmark	8	Cereal-Legume	Non-legume	Mixture	0-25	22
Mancinelli et al. (2015)	Venice, Italy	2	Tomato	Legume and non-legume	Legume, grass, brassica, other	0-25	38
Mitchell et al. (2015)	California, USA	10	Tomato-Cotton	Legume and non-legume	Mixture	0-30	14
Welch et al. (2016)	Illinois, USA	2	Maize-Soybean	Legume and non-legume	Mixture, brassica	0-50	5
Ladoni et al. (2016)	Michigan, USA	5	Maize-Soybean	Legume and non-legume	Mixture	0-15	8
Autret et al. (2016)	Ile de France, France	16	Cereal-Legume	Legume and non-legume	Mixture	0-30	21
Clark et al. (2017)	Missouri, USA	3	Wheat-Maize-Soybean	Legume and non-legume	Mixture, grass	0-15	1
Sainju et al. (2017)	Georgia, USA	3	Energy cane, Elephant grass	Legume	Legume	0-30	30
Wang et al. (2017)	Maryland, USA	5	Corn silage-Soybean	Non-legume	Brassica	0-37.5	32
Morra et al. (2017)	Salerno, Italy	1	Vegetable	Non-legume	Grass	0-30	39

[1] Original sampling depth. All studies were converted to a standard depth of 0-30 cm.



Appendix 1 Figure 1. The results of the sensitivity analysis, which was completed using a Jacknife technique. The solid black line represents the overall lnRR and the blue dashed lines the lower and upper 95% confidence interval of the overall lnRR (prior to publication removal). The solid black line represents a null effect. Recomputed lnRR and 95% confidence intervals that do not cross zero were considered significant.



Appendix 1 Figure 2. The results of the imputation sensitivity analysis. The solid black line represents the overall lnRR and the blue dashed lines the lower and upper 95% confidence interval of the overall lnRR (at each level of imputed variance). The solid black line represents a null effect. Recomputed lnRR and 95% confidence intervals that do not cross zero were considered significant.

APPENDIX 2 CHAPTER 2

Appendix 2 Table 1. Annual least-squares means ± standard error (SE) of aboveground and standing root biomass responses to control and treatments from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values for treatments within year indicated by different letters; $P \leq 0.05$ (*), and $P \leq 0.01$ (**).

Variable	Depth (cm)	Year	IP			SNP			
			Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Aboveground biomass									
Biomass C^1 (Mg C ha^{-1})	__	2018	1.84 ± 0.12a	1.31 ± 0.12b	**	0.73 ± 0.17ab	0.77 ± 0.17a	0.49 ± 0.17b	NS
		2019	1.25 ± 0.12a	1.07 ± 0.12a	NS	0.98 ± 0.18a	0.85 ± 0.17a	0.86 ± 0.17a	NS
		2020	2.00 ± 0.12a	1.49 ± 0.12b	**	0.69 ± 0.17a	0.62 ± 0.17a	0.79 ± 0.17a	NS
Biomass N^1 (kg N ha^{-1})	__	2018	67.60 ± 4.81a	48.30 ± 4.81b	**	26.90 ± 7.41ab	28.80 ± 7.41b	14.90 ± 7.41a	*
		2019	41.20 ± 4.81a	33.70 ± 4.81a	NS	25.40 ± 7.63a	20.80 ± 7.41a	20.40 ± 7.41a	NS
		2020	77.20 ± 4.81a	53.50 ± 4.81b	**	19.80 ± 7.41a	19.30 ± 7.41a	24.60 ± 7.41a	NS
Standing root biomass									
Biomass C (Mg C ha^{-1})	0-10	2018	2.62 ± 0.22a	2.04 ± 0.22b	*	1.15 ± 0.25a	1.39 ± 0.25a	1.56 ± 0.25a	NS
		2019	2.05 ± 0.22a	1.64 ± 0.22a	NS	__	__	__	
		2020	0.90 ± 0.22a	0.85 ± 0.22a	NS	0.53 ± 0.25a	0.65 ± 0.25a	0.64 ± 0.25a	NS
	10-20	2018	0.57 ± 0.06a	0.54 ± 0.06a	NS	0.37 ± 0.14a	0.47 ± 0.14a	0.71 ± 0.14a	NS
		2019	0.41 ± 0.06b	0.54 ± 0.06a	NS	__	__	__	
		2020	0.38 ± 0.06a	0.43 ± 0.06a	NS	0.42 ± 0.14a	0.32 ± 0.14a	0.40 ± 0.14a	NS
	0-20	2018	3.18 ± 0.23a	2.58 ± 0.23b	*	1.51 ± 0.15a	1.71 ± 0.17a	1.53 ± 0.17a	NS
		2019	2.46 ± 0.23a	2.18 ± 0.23a	NS	__	__	__	
		2020	1.28 ± 0.23a	1.27 ± 0.23a	NS	0.95 ± 0.15a	0.97 ± 0.15a	1.04 ± 0.15a	NS

Appendix 2 Table 2. Annual least-squares means ± standard error (SE) of equivalent soil mass (ESM) carbon and nitrogen stock responses to control and treatments from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values for treatments within year indicated by different letters; $P \geq 0.05$ (non-significant; NS), $P \leq 0.05$ (*), and $P \leq 0.01$ (**).

			IP[1]			SNP[2]			
Variable	Year	Depth (cm)	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Soil organic carbon (Mg C ha^{-1})	2018	0-10	22.4 ± 0.8a	20.7 ± 0.8b	*	19.5 ± 1.0	19.5 ± 1.0	19.5 ± 1.0	
	2020		26.3 ± 0.8a	22.6 ± 0.8b	**	21.1 ± 1.1a	21.1 ± 1.1a	20.9 ± 1.0a	NS
	2018	10-20	16.9 ± 0.5a	17.1 ± 0.5a	NS	13.7 ± 1.0	13.7 ± 1.0	13.7 ± 1.0	
	2020		16.6 ± 0.5a	15.8 ± 0.5a	NS	14.9 ± 1.4a	13.9 ± 1.4a	14.0 ± 1.4a	NS
	2018	20-50	29.6 ± 1.3a	28.8 ± 1.1a	NS	25.6 ± 2.3	25.6 ± 2.3	25.6 ± 2.3	
	2020		30.4 ± 1.3a	27.2 ± 1.1a	NS	24.4 ± 2.6a	21.5 ± 2.4a	21.9 ± 2.4a	NS
	2018	50-80	__	__		19.0 ± 1.2	19.0 ± 1.2	19.0 ± 1.2	
	2020		__	__		19.0 ± 1.9a	15.3 ± 1.7a	16.5 ± 1.7a	NS
Soil inorganic carbon (Mg C ha^{-1})	2018	0-10	8.1 ± 0.9b	11.5 ± 0.9a	*	7.0 ± 0.8	7.0 ± 0.8	7.0 ± 0.8	
	2020		9.0 ± 0.9a	10.0 ± 0.9a	NS	4.4 ± 1.8a	8.4 ± 1.8a	8.0 ± 1.8a	NS
	2018	10-20	10.1 ± 0.9a	12.3 ± 0.9a	NS	10.8 ± 0.8	10.8 ± 0.8	10.8 ± 0.8	
	2020		9.9 ± 0.9a	11.5 ± 0.9a	NS	5.6 ± 2.1a	12.5 ± 2.1ab	12.6 ± 2.1b	NS
	2018	20-50	40.5 ± 3.5a	42.6 ± 3.0a	NS	40.8 ± 4.0	40.8 ± 4.0	40.8 ± 4.0	
	2020		35.4 ± 3.5a	42.0 ± 3.0a	NS	32.9 ± 8.8a	51.3 ± 7.8a	51.7 ± 7.8a	NS
	2018	50-80	__	__		53.0 ± 2.9	53.0 ± 2.9	53.0 ± 2.9	
	2020		__	__		49.0 ± 8.0a	64.5 ± 7.3a	55.7 ± 7.3a	NS
Soil total carbon (Mg C ha^{-1})	2018	0-10	29.8 ± 1.0a	31.3 ± 0.9a	NS	26.4 ± 1.6	26.4 ± 1.6	26.4 ± 1.6	
	2020		35.0 ± 1.0a	31.7 ± 0.9b	*	26.6 ± 2.2a	31.0 ± 2.2a	28.9 ± 2.2a	NS
	2018	10-20	25.9 ± 0.9b	28.7 ± 0.9a	*	24.5 ± 0.8	24.5 ± 0.8	24.5 ± 0.8	
	2020		25.7 ± 0.9a	26.6 ± 0.9a	NS	20.5 ± 1.7a	26.3 ± 1.7a	26.7 ± 1.7a	NS
	2018	20-50	69.5 ± 3.7a	71.6 ± 3.2a	NS	66.5 ± 3.6	66.5 ± 3.6	66.5 ± 3.6	
	2020		65.1 ± 3.7a	69.1 ± 3.2a	NS	57.8 ± 7.6a	72.8 ± 7.6a	73.6 ± 7.6a	NS
	2018	50-80	__	__		72.1 ± 3.0	72.1 ± 3.0	72.1 ± 3.0	
	2020		__	__		67.4 ± 7.2a	79.7 ± 6.5a	72.2 ± 6.5a	NS
	2018	0-10	2.4 ± 0.1a	2.1 ± 0.1b	*	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	
	2020		2.7 ± 0.1a	2.2 ± 0.1b	**	2.2 ± 0.1a	2.3 ± 0.1a	2.1 ± 0.1a	NS

Soil total nitrogen (Mg N ha^{-1})	2018	10-20	1.8 ± 0.05a	1.8 ± 0.05a	NS	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	
	2020		1.8 ± 0.05a	1.7 ± 0.05a	NS	1.6 ± 0.1a	1.5 ± 0.1a	1.5 ± 0.1a	NS
	2018	20-50	3.1 ± 0.1a	3.1 ± 0.1a	NS	2.9 ± 0.09	2.9 ± 0.09	2.9 ± 0.09	
	2020		3.1 ± 0.1a	2.9 ± 0.1b	*	2.7 ± 0.2	2.6 ± 0.2a	2.5 ± 0.2a	NS
	2018	50-80	__	__		2.3 ± 0.07	2.3 ± 0.07	2.3 ± 0.07	
	2020		__	__		2.1 ± 0.1a	1.9 ± 0.1a	1.8 ± 0.1a	NS

[1] Values from 2018 represent stocks measured over the period of 2012-2018, and values from 2020 represent stocks measured over the period of 2018-2020. These values include 2012 estimates as a covariate due to initial treatment differences at the plot level.

[2] Values from 2018 represent baseline stock measurements at the block level, and values from 2020 represent stocks measured over the period of 2018-2020.

Appendix 2 Table 3. Annual least-squares means ± standard error (SE) of aboveground biomass removed from control and treatment plots from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values for treatments within year indicated by different letters; *P* > 0.05 (non-significant; NS), *P* ≤ 0.05 (*) , *P* ≤ 0.01 (**).

		IP			SNP[2]			
Variable	Year	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Biomass C	2018	1.66 ± 0.11a	1.18 ± 0.11b	**	0.51 ± 0.14a	0.58 ± 0.14a	0.36 ± 0.14a	NS
removed	2019	1.13 ± 0.11a	0.96 ± 0.11a	NS	0.62 ± 0.15a	0.52 ± 0.14a	0.40 ± 0.14a	NS
(Mg C ha^{-1})	2020	1.80 ± 0.11a	1.34 ± 0.11b	**	0.37 ± 0.15a	0.36 ± 0.15a	0.40 ± 0.15a	NS

[1] Biomass C removed estimated at 90% of peak standing aboveground biomass

[2] For SNP, n = 3 in 2020

Appendix 2 Table 4. Annual least-squares means ± standard error (SE) of cumulative soil respiration from control and treatment plots from SNP: semi-native pasture (n = 6). Significant p-values for treatments within year indicated by different letters; *P* > 0.05 (non-significant; NS), *P* ≤ 0.05 (*) , *P* ≤ 0.01 (**).

		SNP			
Variable	Year	Organic N	Inorganic N	Control	*P*
Cumulative soil	2018	4.7 ± 0.56a	4.9 ± 0.55a	4.7 ± 0.55a	NS
CO$_2$ flux (Mg C	2019	4.0 ± 0.56a	4.2 ± 0.55a	4.3 ± 0.58a	NS
ha^{-1})	2020	3.1 ± 0.56a	3.3 ± 0.58a	3.5 ± 0.58a	NS

Appendix 3 Table 1. Annual least-squares means ± standard error (SE) of aboveground plant responses to control and treatments from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values for treatments within year indicated by different letters; *P* > 0.1 (non-significant; NS), *P* ≤ 0.1 (*) , *P* ≤ 0.05 (**), and *P* ≤ 0.01 (***).

		IP			SNP			
Variable	Year	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Biomass								
Biomass C (Mg C ha^{-1})	2018	1.84 ± 0.12a	1.31 ± 0.12b	***	0.73 ± 0.17ab	0.77 ± 0.17a	0.49 ± 0.17b	*
	2019	1.25 ± 0.12a	1.07 ± 0.12a	NS	0.98 ± 0.18a	0.85 ± 0.17a	0.86 ± 0.17a	NS
	2020	2.00 ± 0.12a	1.49 ± 0.12b	***	0.69 ± 0.17a	0.62 ± 0.17a	0.79 ± 0.17a	NS
Biomass N[1] (kg N ha^{-1})	2018	67.60 ± 4.81a	48.30 ± 4.81b	***	26.90 ± 7.41a	28.80 ± 7.41a	14.90 ± 7.41b	*, **[1]
	2019	41.20 ± 4.81a	33.70 ± 4.81a	NS	25.40 ± 7.63a	20.80 ± 7.41a	20.40 ± 7.41a	NS
	2020	77.20 ± 4.81a	53.50 ± 4.81b	***	19.80 ± 7.41a	19.30 ± 7.41a	24.60 ± 7.41a	NS
Diversity								
Shannon Index (H)	2018	0.63 ± 0.09a	0.39 ± 0.10b	**	0.81 ± 0.16a	1.05 ± 0.16a	0.80 ± 0.16a	NS
	2019	0.74 ± 0.09a	0.36 ± 0.09b	***	0.89 ± 0.16a	0.94 ± 0.16a	0.74 ± 0.16a	NS
	2020	0.47 ± 0.10a	0.30 ± 0.09a	NS	0.89 ± 0.16a	0.78 ± 0.16a	0.77 ± 0.16a	NS
Species evenness	2018	0.19 ± 0.05a	0.13 ± 0.05a	NS	0.54 ± 0.07a	0.70 ± 0.07a	0.55 ± 0.07a	NS
	2019	0.54 ± 0.05a	0.35 ± 0.05b	***	0.51 ± 0.07a	0.61 ± 0.07a	0.49 ± 0.07a	NS
	2020	0.44 ± 0.05a	0.35 ± 0.05a	*	0.51 ± 0.07a	0.46 ± 0.07a	0.51 ± 0.07a	NS
Species richness	2018	3.25 ± 0.32a	2.71 ± 0.33a	NS	4.42 ± 0.62a	4.92 ± 0.62a	4.42 ± 0.62a	NS
	2019	3.44 ± 0.32a	2.50 ± 0.32b	**	5.42 ± 0.62a	5.17 ± 0.62a	3.81 ± 0.67a	NS
	2020	2.88 ± 0.32a	2.38 ± 0.32a	NS	5.33 ± 0.62a	4.92 ± 0.62a	4.75 ± 0.62a	NS

[1] Organic N significant at P = 0.07, Inorganic N significant at P = 0.03

Appendix 3 Table 2. Annual least-squares means ± standard error (SE) of root and soil responses to control and treatments from two field experiments. IP: improved pasture; SNP: semi-native pasture. Significant p-values for treatments within year indicated by different letters; *P* > 0.1 (non-significant; NS), *P* ≤ 0.1 (*) , *P* ≤ 0.05 (**), and *P* ≤ 0.01 (***).

		Depth	IP			SNP			
Variable	Year	(cm)	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Summer Roots						*Autumn Roots*			
Total biomass C (Mg C ha^{-1})	2018	0-10	2.62 ± 0.22a	2.04 ± 0.22b	*	1.15 ± 0.25a	1.39 ± 0.25a	1.56 ± 0.25a	NS
	2019		2.05 ± 0.22a	1.64 ± 0.22a	NS	__	__	__	
	2020		0.90 ± 0.22a	0.84 ± 0.22a	NS	0.53 ± 0.25a	0.65 ± 0.25a	0.64 ± 0.25a	NS
	2018	10-20	0.57 ± 0.06a	0.54 ± 0.06a	NS	0.37 ± 0.14a	0.47 ± 0.14a	0.71 ± 0.14a	NS
	2019		0.41 ± 0.06b	0.54 ± 0.06a	NS	__	__	__	
	2020		0.38 ± 0.06a	0.43 ± 0.06a	NS	0.42 ± 0.14a	0.32 ± 0.14a	0.40 ± 0.14a	NS
	2018	0-20	3.18 ± 0.23a	2.58 ± 0.23b	**	1.51 ± 0.15a	1.71 ± 0.17a	1.53 ± 0.17a	
	2019		2.46 ± 0.23a	2.18 ± 0.23a	NS	__	__	__	
	2020		1.28 ± 0.23a	1.27 ± 0.23a	NS	0.95 ± 0.15a	0.97 ± 0.15a	1.04 ± 0.15a	
Coarse root biomass C (Mg C ha^{-1})	2018	0-10	2.48 ± 0.23a	1.91 ± 0.23b	*	0.99 ± 0.24a	1.20 ± 0.24a	1.39 ± 0.24a	NS
	2019		1.93 ± 0.23a	1.49 ± 0.23a	NS	__	__	__	
	2020		0.37 ± 0.23a	0.34 ± 0.23a	NS	0.23 ± 0.24a	0.31 ± 0.24a	0.30 ± 0.24a	NS
	2018	10-20	0.51 ± 0.05a	0.47 ± 0.05a	NS	0.29 ± 0.13a	0.37 ± 0.13a	0.63 ± 0.13a	NS
	2019		0.36 ± 0.05a	0.47 ± 0.05a	NS	__	__	__	
	2020		0.20 ± 0.05a	0.24 ± 0.05a	NS	0.23 ± 0.13a	0.17 ± 0.13a	0.20 ±0.13a	NS
	2018	0-20	2.99 ± 0.23a	2.38 ± 0.23b	**	1.27 ± 0.11a	1.35 ± 0.13a	1.22 ± 0.13a	NS
	2019		2.29 ± 0.23a	1.95 ± 0.23a	NS	__	__	__	
	2020		0.57 ± 0.23a	0.58 ± 0.23a	NS	0.46 ± 0.11a	0.48 ± 0.11a	0.50 ± 0.11a	NS
Fine root biomass C (Mg C ha^{-1})	2018	0-10	0.13 ± 0.03a	0.13 ± 0.03a	NS	0.16 ± 0.07a	0.20 ± 0.07a	0.17 ± 0.07a	NS
	2019		0.12 ± 0.03a	0.15 ± 0.03a	NS	__	__	__	
	2020		0.53 ± 0.03a	0.51 ± 0.03a	NS	0.30 ± 0.07a	0.34 ± 0.07a	0.35 ± 0.07a	NS
	2018	10-20	0.05 ± 0.02a	0.07 ± 0.02a	NS	0.08 ± 0.02a	0.09 ± 0.02a	0.07 ± 0.02a	NS
	2019		0.05 ± 0.02a	0.07 ± 0.02a	NS	__	__	__	
	2020		0.19 ± 0.02a	0.18 ± 0.02a	NS	0.18 ± 0.02b	0.15 ± 0.02b	0.20 ± 0.02a	NS

2018	0-20	0.21 ± 0.05a	0.32 ± 0.05a	NS	0.24 ± 0.09a	0.29 ± 0.09a	0.24 ± 0.09a	NS
2019		0.17 ± 0.05a	0.22 ± 0.05a	NS	__	__	__	
2020		0.72 ± 0.05a	0.69 ± 0.05a	NS	0.49 ± 0.09a	0.49 ± 0.09a	0.54 ± 0.09a	NS

Appendix 3 Table 3. Annual least-squares means ± standard error (SE) of PLFA biomarker responses (nmol g^{-1}) to control and treatments in the spring from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6)[1]. Significant p-values ($P \leq 0.1$) indicated by different letters; $P > 0.1$ (non-significant; NS), $P \leq 0.1$ (*) , $P \leq 0.05$ (**), and $P \leq 0.01$ (***).

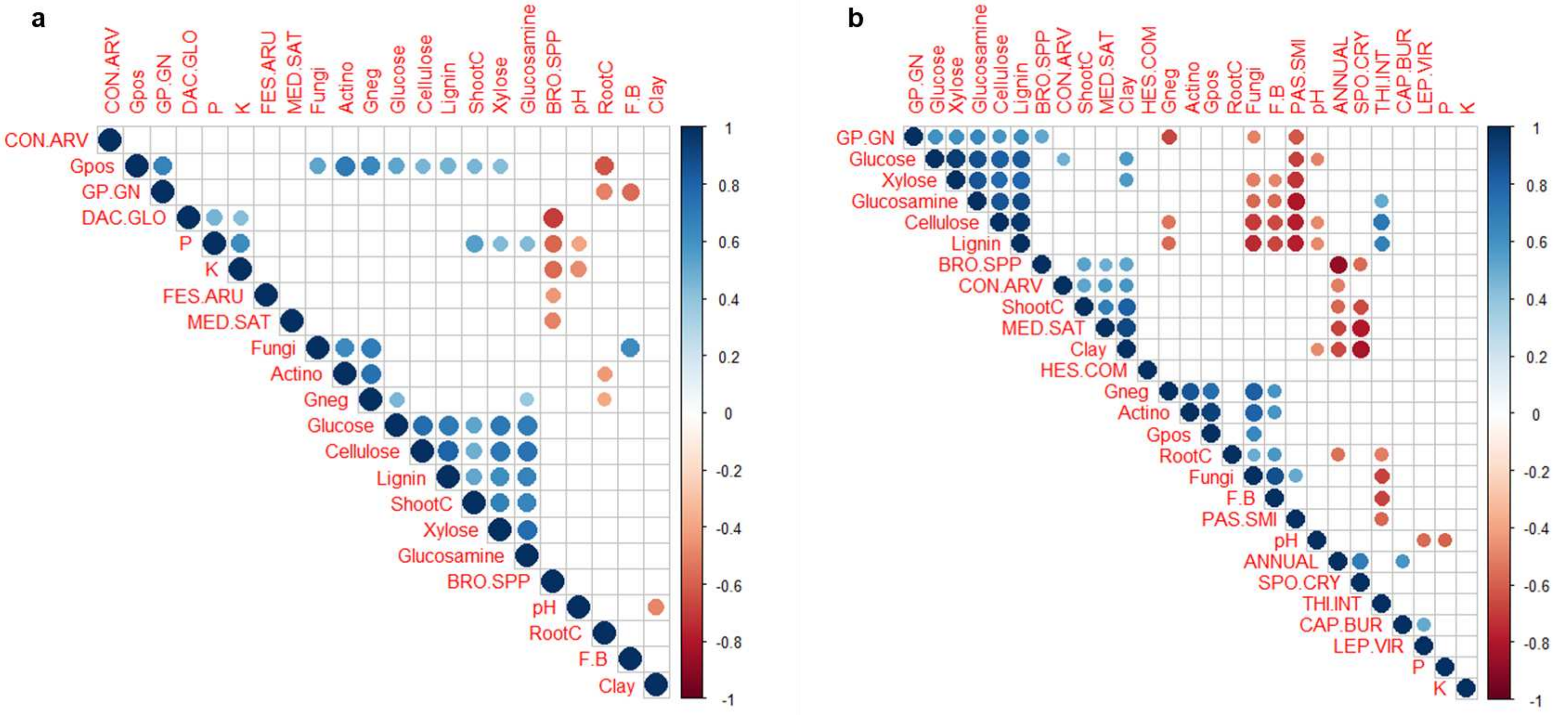
		IP			SNP[1]			
Variable	Year	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Functional group								
Total bacteria (nmol g^{-1})	2019	267 ± 33a	194 ± 36a	NS	94 ± 41a	112 ± 41a	91 ± 41a	NS
	2020	563 ± 33a	473 ± 33b	*	368 ± 41a	294 ± 41a	306 ± 41a	NS
Total fungi (nmol g^{-1})	2019	31.7 ± 9.0a	32.3 ± 9.0a	NS	6.7 ± 4.6a	4.7 ± 4.6a	5.4 ± 4.6a	NS
	2020	63.1 ± 8.3a	46.1 ± 8.3a	NS	19.9 ± 4.6a	23.5 ± 4.6a	16.6 ± 4.6a	NS
Total actinomycetes (nmol g^{-1})	2019	52.0 ± 9.8a	36.8 ± 9.8a	NS	20.6 ± 9.5a	22.9 ± 9.5a	19.2 ± 9.5a	NS
	2020	120.4 ± 9.8a	94.6 ± 9.8b	*	87.7 ± 9.5a	62.1 ± 9.5a	63.6 ± 9.5a	NS
Gram positive bacteria (nmol g^{-1})	2019	79.8 ± 18.0a	64.2 ± 18.0a	NS	33.3 ± 19.0a	38.9 ± 19.0a	31.4 ± 19.0a	NS
	2020	238.0 ± 16.8a	208.1 ± 16.8a	NS	162.6 ± 19.0a	132.9 ± 19.0a	124.5 ± 19.0a	NS
Gram negative bacteria (nmol g^{-1})	2019	117.7 ± 15.5a	96.2 ± 16.7a	NS	35.2 ± 16.2a	45.9 ± 16.2a	36.4 ± 16.2a	NS
	2020	161.1 ± 16.7a	148.5 ± 15.5a	NS	91.5 ± 16.2a	76.9 ± 16.2a	96.5 ± 16.2	NS
Ratios								
Fungi:Bacteria	2019	0.14 ± 0.02a	0.17 ± 0.02a	NS	0.06 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.01a	NS
	2020	0.11 ± 0.02a	0.14 ± 0.02a	NS	0.06 ± 0.01a	0.07 ± 0.01a	0.06 ± 0.01a	NS
Gram Positive:Gram Negative	2019	0.86 ± 0.14a	0.68 ± 0.14a	NS	1.18 ± 0.45a	0.89 ± 0.45a	1.08 ± 0.45a	NS
	2020	1.07 ± 0.14a	1.12 ± 0.14a	NS	1.41 ± 0.50a	1.18 ± 0.50a	1.39 ± 0.45a	NS
Biomass								
Total microbial biomass (nmol g^{-1})	2019	504 ± 70a	474 ± 70a	NS	218 ± 63a	232 ± 63a	223 ± 63a	NS
	2020	1037 ± 70a	936 ± 70a	NS	600 ± 63a	576 ± 63a	372 ± 78a	NS

[1] For 2020 SNP, n = 4

Appendix 3 Table 4. Back transformed annual least-squares means and 95% confidence intervals (CI) of microbial substrate-induced respiration and annual least-squares means ± standard error (SE) of functional evenness and total respiration responses (µg CO_2-C g^{-1} hr^{-1}) to control and treatments from two field experiments. IP: improved pasture (n = 8); SNP: semi-native pasture (n = 6). Significant p-values ($P \leq 0.1$) indicated by different letters; $P > 0.1$ (non-significant; NS), $P \leq 0.1$ (*), $P \leq 0.05$ (**), and $P \leq 0.01$ (***).

		IP			SNP[1]			
Variable	Year	Organic N	Control	*P*	Organic N	Inorganic N	Control	*P*
Substrate								
Lignin	2018	1.05 (0.88 – 1.23)a	0.99 (0.83 – 1.18)a	NS	0.74 (0.58 – 0.96)a	0.74 (0.58 – 0.96)a	0.77 (0.59 – 0.99)a	NS
	2019	0.75 (0.63 – 0.89)a	0.69 (0.58 – 0.82)a	NS	__	__	__	
	2020	1.01 (0.85 – 1.21)a	0.99 (0.83 – 1.18)a	NS	1.10 (0.82 – 1.46)a	0.99 (0.74 – 1.32)a	0.97 (0.73 – 1.29)a	NS
Glucosamine	2018	1.60 (1.35 – 1.91)a	1.38 (1.16 – 1.64)a	NS	0.85 (0.66 – 1.10)a	0.86 (0.67 – 1.11)a	0.76 (0.59 – 0.98)a	NS
	2019	0.91 (0.76 – 1.09)a	0.93 (0.78 – 1.11)a	NS	__	__	__	
	2020	1.42 (1.19 – 1.69)a	1.42 (1.19 – 1.69)a	NS	1.33 (1.00 – 1.77)a	1.33 (1.00 – 1.77)a	1.12 (0.84 – 1.49)a	NS
Xylose	2018	2.61 (2.19 – 3.11)a	2.28 (1.91 – 2.72)a	NS	1.01 (0.79 – 0.88)a	1.12 (0.87 – 1.44)a	0.95 (0.74 – 1.22)a	NS
	2019	1.42 (1.19 – 1.70)a	1.22 (1.02 – 1.45)a	NS	__	__	__	
	2020	2.73 (2.29 – 3.26)a	2.21 (1.85 – 2.64)b	*	1.90 (1.43 – 2.54)a	2.06 (1.54 – 2.74)a	1.54 (1.15 – 2.05)a	NS
Cellulose	2018	0.99 (0.83 – 1.18)a	0.96 (0.81 – 1.15)a	NS	0.70 (0.55 – 0.91)a	0.77 (0.60 – 0.99)a	0.73 (0.57 – 0.95)a	NS
	2019	0.64 (0.53 – 0.76)a	0.61 (0.51 – 0.73)a	NS	__	__	__	
	2020	0.99 (0.83 – 1.18)a	0.97 (0.82 – 1.16)a	NS	1.02 (0.76 – 1.35)a	0.95 (0.71 – 1.27)a	1.14 (0.86 – 1.52)a	NS
Glucose	2018	3.25 (2.73 – 3.88)a	2.63 (2.20 – 3.13)b	*	1.04 (0.80 – 1.33)a	1.18 (0.92 – 1.52)a	1.09 (0.84 – 1.40)a	NS
	2019	1.54 (1.29 – 1.84)a	1.58 (1.33 – 1.89)a	NS	__	__	__	NS
	2020	3.44 (2.89 – 4.11)a	3.50 (2.93 – 4.17)a	NS	2.38 (1.79 – 3.18)a	2.49 (1.87 – 3.31)a	1.64 (1.23 – 2.18)b	*
Evenness								
Functional evenness	2018	4.06 ± 0.12a	4.23 ± 0.12a	NS	4.85 ± 0.17a	4.73 ± 0.17a	4.78 ± 0.17a	NS
	2019	4.21 ± 0.12a	4.13 ± 0.12a	NS	__	__	__	
	2020	3.74 ± 0.12a	3.89 ± 0.12a	NS	4.43 ± 0.20a	4.26 ± 0.20a	4.53 ± 0.20a	NS
Total respiration								
Microbial substrate-induced respiration	2018	9.73 ± 0.61a	8.56 ± 0.61b	**	4.44 ± 0.86a	4.92 ± 0.86a	4.42 ± 0.86a	NS
	2019	5.60 ± 0.61a	5.28 ± 0.61a	NS	__	__	__	
	2020	9.95 ± 0.61a	9.34 ± 0.61a	NS	8.58 ± 1.01a	9.08 ± 1.01a	7.15 ± 1.01a	NS

[1] For 2020 SNP, n = 4

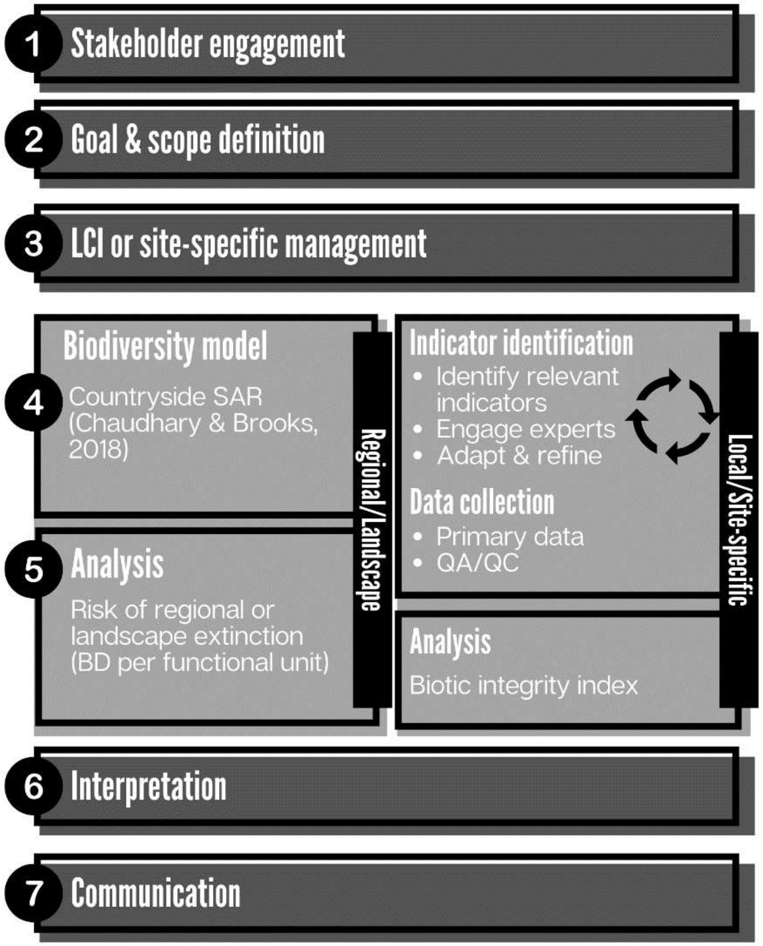


Appendix 3 Figure 1. Correlograms of species and environmental variables in a) improved pasture (IP) and b) semi-native pasture (SNP) during a three-year experiment near Loveland, CO. Significant Spearman-rank correlations shown at $P \leq 0.01$. Plant species: ANNUAL (*Bromus secalinus*, *B. tectorum*), BRO.SPP (*B. commutatus*, *B. inermis*), CON.ARV (*Convolvulus arvensis*), CAP.BUR (*Capsella bursa-pastoris*), DAC.GLO (*Dactylis glomerata*), FES.ARU (*Festuca arundinacea*), HES.COM (*Hesperostipa comata*), LEP.VIR (*Lepidium virginicum*), MED.SAT (*Medicago sativa*), PAS.SMI (*Pascopyrum smithii*), SPO.CRY (*Sporobolus cryptandrus*), THI.INT (*Thinopyrum intermedium*).

Appendix 3 Table 5. Environmental loadings and Spearman-ranks correlations from the Non-Metric Multidimensional Scaling (NMDS) analysis for improved pasture (IP) and semi-native pasture (SNP). Significant variables correlated with NMDS axes are in bold (*P* ≤ 0.05).

Variables	IP				SNP			
	NMDS 1	NMDS 2	R^2	*P*	NMDS 1	NMDS 2	R^2	*P*
Soil properties								
pH	-0.98	-0.17	0.03	0.48	0.46	-0.88	0.14	0.15
P (g kg^{-1})	0.53	0.85	0.05	0.35	0.60	0.80	0.09	0.30
K (g kg^{-1})	0.91	0.42	0.04	0.44	-0.48	-0.87	0.01	0.88
Clay (%)	0.95	-0.30	0.05	0.36	-1.00	-0.01	0.49	**0.001**
Microbial-induced substrate respiration (µg CO_2-C g^{-1} hr^{-1})								
Glucose	0.98	-0.20	0.33	**0.001**	-0.91	0.42	0.41	**0.001**
Cellulose	0.87	-0.49	0.19	**0.01**	-0.53	0.85	0.25	**0.03**
Xylose	0.99	0.12	0.19	**0.01**	-0.90	0.45	0.38	**0.002**
Glucosamine	0.80	0.61	0.20	**0.007**	-0.71	0.70	0.42	**0.001**
Lignin	0.99	-0.10	0.13	**0.05**	-0.43	0.90	0.39	**0.001**
Plant C (Mg C ha^{-1})								
Shoot	0.89	0.45	0.26	**0.001**	-0.99	-0.07	0.32	**0.006**
Root	-0.91	0.40	0.34	**0.002**	-0.69	-0.72	0.13	0.17

APPENDIX 4 CHAPTER 4



Appendix 4 Figure 1. Flow diagram of the process for completing a biodiversity assessment according to FAO (2020). The specific approaches used in this study are described in step 4 and 5.

Appendix 4 Table 1. Description of cow and calf management at Buck Island Ranch for improved pasture (IMP) and semi-native pasture (SN).

Pasture	Year	Animal use days ha^{-1}	Total weaned calf weight (kg)
IMP	2014	59.7	301,776
	2015	64.6	253,199
	2016	81.0	253,781
	2017	72.0	268,624
	2018	54.6	222,248
	2019	52.4	237,935
SN	2014	33.3	168,036
	2015	32.8	128,523
	2016	34.0	106,581
	2017	22.3	83,043
	2018	24.8	101,129
	2019	27.4	124,452

Appendix 4 Table 2. Yield data by pasture type and mean and 95% confidence intervals of land occupation characterization factors (potential species loss m^{-2}) for disaggregated and aggregated taxa from Chaudhary and Brooks (2018).

Pasture	Land classification	Yield (kg LW ha^{-1})	Mammals (CF ± 95% CI)	Birds (CF ± 95% CI)	Amphibians (CF ± 95% CI)	Reptiles (CF ± 95% CI)	Plants (CF ± 95% CI)	Mean (CF ± 95% CI)
Improved (IMP)	Pasture: Intense use	139.4	7.24 x 10^{-12} (5.90 x 10^{-12} – 8.64 x 10^{-12})	2.29 x 10^{-11} (1.94 x 10^{-11} – 2.67 x 10^{-11})	8.37 x 10^{-12} (7.60 x 10^{-12} – 9.40 x 10^{-12})	1.78 x 10^{-11} (9.11 x 10^{-12} – 2.68 x 10^{-11})	3.38 x 10^{-10} (1.69 x 10^{-10} – 5.14 x 10^{-10})	3.57 x 10^{-13} (2.64 x 10^{-13} – 4.56 x 10^{-13})
Semi-native (SN)	Pasture: minimal use	52.0	6.92 x 10^{-12} (5.60 x 10^{-12} – 8.36 x 10^{-12})	2.23 x 10^{-11} (1.88 x 10^{-11} – 2.62 x 10^{-11})	8.36 x 10^{-12} (7.58 x 10^{-12} – 9.39 x 10^{-12})	1.15 x 10^{-11} (4.31 x 10^{-12} – 2.00 x 10^{-11})	2.19 x 10^{-10} (8.02 x 10^{-11} – 3.84 x 10^{-10})	3.10 x 10^{-13} (2.26 x 10^{-13} – 4.07 x 10^{-13})

Appendix 4 Table 3. Biodiversity categories, selected indicators, and datasets used to create the biotic integrity index (BII) for the two pasture types at the MacArthur Agroecology Research Center (MAERC) and the reference system located at the Kissimmee Prairie Preserve State Park (KPPSP).

Category	Indicator	MAERC			Reference System[1]		
		Dataset	Years	Reference	Dataset	Years	Reference
1. Habitat protection	Percentage of semi-natural habitat in the landscape, i.e., hammocks and wetlands	Landsat 8 & National Agricultural Imagery Program (NAIP)	2018	This study	KPPSP 2019 Management Plan	__	Table 5
2. Habitat change	Livestock stocking rate (head acre^{-1} yr^{-1})	LCI	2014-2018	This study	University of Florida, Institute of Food and Agricultural Sciences	__	Hersom (2005)
3. Wildlife conservation	Non-native and native mammal sightings (sum of mean days month^{-1})	Game camera array	2016-2018	This study	KPPSP 2019 Management Plan and expert opinion	__	Addendum 5
4. Invasive species	Invasive plant abundance (% incidence)	Vegetation surveys	2016-2019	This study	KPPSP 2019 Management Plan	__	Table 3

5. Pollution and aquatic biodiversity	Aquatic plant, invertebrate, and vertebrate diversity (sum of mean abundance)	Wetland diversity sampling	2006	Medley et al. (2015)	Kissimmee River Restoration and Other Kissimmee Basin Initiatives		Koebel et al. (2020)
6. Off-farm feed	Imported feed (kg m^{-2} yr^{-1})	LCI	2014-2018	This study	Expert opinion	—	—
7. Landscape-scale heterogeneity	β dispersion of plant cover	Plant diversity transects	2016-2019	This study	Expert opinion and literature	—	—

[1] Kissimmee Prairie Preserve State Park

Appendix 4 Table 4. A description of the data collection method used for each indicator in the biotic integrity index (BII) for the two pasture types at the MacArthur Agroecology Research Center (MAERC) and the reference system located at the Kissimmee Prairie Preserve State Park.

Category	Indicator	MAERC	Reference System[1]
		Description	Description
1. Habitat protection	Habitat protection (%)	Using data products from Landsat 8 satellite records and the National Agricultural Imagery Program (NAIP), a 26-band image was created and used to classify land cover at the MAERC. Expert knowledge was used to create training points for a supervised classification algorithm. The training points included 150 points that were representative of the four land cover types at the MAERC. An existing wetlands shapefile was used to cross-validate the supervised classification algorithm.	Converted the acres of natural and altered land cover types listed in Table 5 into hectares and then percent area.
2. Habitat change	Habitat change (head acre^{-1} year^{-1})	Estimated stocking density (head acre^{-1}) averaged across pasture type annually	Assumed the lowest recommended stocking rate

3. Wildlife conservation	Wildlife conservation (sum of days month-1)	Three-year average of game camera array data. A detailed description of the methodology and dataset is available in Tabak et al. (2019).	Cross-referenced the list in Addendum 5 and assumed similar number of sitings as the semi-native pasture in lieu of a comparable dataset. This estimate is likely conservative.
4. Invasive species	Invasive alien species (% incidence)	Annual pasture exotic plant species abundances collected from 2016-2019.	Assumed the percent area treated for exotic plants equivalent to percent incidence at the site in lieu of comparable data.
5. Pollution and aquatic biodiversity	Aquatic biodiversity (species abundance)	Aquatic biodiversity dataset accessed from Medley et al. (2015).	Used the number of aquatic species reported in Koebel et al. (2020). This estimate is likely conservative.
6. Off-farm feed	Off-farm feed (kg m-1 yr-1)	Annual supplemental feed imported to the MAERC and delivered to each pasture.	Using expert opinion we assumed no off-farm feed imported in this system.
7. Landscape-scale heterogeneity	Landscape scale conservation (index)	Vegetation survey data collected from 2016-2019. β dispersion of plant cover estimated from plant species richness. A description on the metric can be found in Anderson et al. (2006).	In lieu of comparable data, we estimated the fraction of natural area in the landscape as a proxy.

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[1] Kissimmee Prairie Preserve State Park

Appendix 4 Table 5. The mean, inverse, and normalized values for each indicator included in the biotic integrity index for improved pasture (IMP), semi-native pasture (SN), and the reference system (REF) located at the Kissimmee Prairie Preserve State Park.

Category	Indicator	Weighting factor	Mean value			Inverse value			Normalized value		
			IMP	SN	REF	IMP	SN	REF	IMP	SN	REF
1. Habitat protection	Habitat protection (%)	0.143	13.8	27.0	87.0	__	__	__	0.0	0.2	1.0
2. Habitat change	Habitat change (head acre^{-1} year^{-1})	0.143	1.2	0.6	0.1	0.82	1.65	3.48	0.0	0.3	1.0
3. Wildlife conservation	Wildlife conservation (sum of days month-1)	0.143	55.7	72.4	72.4	__	__	__	0.0	1.0	1.0
4. Invasive species	Invasive alien species (% incidence)	0.143	24.0	8.0	2.0	0.04	0.12	0.50	0.0	0.2	1.0
5. Pollution and aquatic biodiversity	Aquatic biodiversity (species abundance)	0.143	200.0	211.7	106.0	__	__	__	0.9	1.0	0.0
6. Off-farm feed	Off-farm feed (kg m-1 yr-1)	0.143	0.04	0.02	0.00	22.2	61.7	100	0.0	0.5	1.0
7. Landscape-scale heterogeneity	Landscape scale conservation (index)	0.143	0.2	0.4	0.9	__	__	__	0.0	0.3	1.0