DISSERTATION

FIRE DISTURBANCE BELOWGROUND: UNTANGLING CONSEQUENCES FOR SOIL FOOD WEBS AND ORGANIC MATTER

Submitted by

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ABSTRACT

FIRE DISTURBANCE BELOWGROUND: UNTANGLING CONSEQUENCES FOR SOIL FOOD WEBS AND ORGANIC MATTER

Soils and the ecological communities they house provide a diverse array of ecosystem services including the provisioning of food and fiber, decomposition and nutrient cycling, water filtration, and the maintenance of terrestrial biodiversity. These complex belowground communities, and therefore the ecosystem processes they regulate, are increasingly threatened by fire due to climate, land use, and management changes. Fires can have profound effects on the physical and chemical soil environment, with consequences for soil biological communities. Fires cause mortality of soil organisms during the disturbance event, change the soil pH, and alter the quantity and quality of soil organic matter (SOM). In particular, fires transform organic matter into pyrogenic carbon (PyC), a recalcitrant material with a dense aromatic structure and long residence times in soils. In natural ecosystems, soil food webs interact with PyC produced after a fire. In agroecosystems, PyC, in the form of biochar, is also used as a tool to manage soil carbon and fertility. Given the widespread effects of fire on biological, chemical, and physical components of the soil, and the importance of soil communities for the provisioning of ecosystem services, understanding the consequences of fire disturbance for soil food webs and organic matter is an important research objective.

My dissertation leverages several different scientific inquiry approaches to understand the consequences of disturbance and management for the ecology of soils. I take a multifaceted approach by considering soil organisms, food webs, and organic matter in the context of fire disturbance and agricultural management. I begin by presenting results from a meta-analysis investigating the effect of fire on soil biota biomass, abundance, richness, evenness, and diversity. Overall, I found a pervasive negative effect of fire on soil microorganisms and conclude that soil fauna are more resistant to fire than

soil microorganisms. Then, I present results from a field study investigating the effect of fire frequency on soil food web structure, function, stability, and resilience in an oak-pine savanna. Here, I found that while soil biota biomass and food web function did not differ with fire frequency, food web structure, stability, and resilience did. In particular, soil food webs at intermediate fire frequencies (4-year fire return interval) were the least stable and least resilient to fire. Thereafter, I consider the consequences of fire for SOM composition through the lens of PyC. I seek to understand where and why PyC persists in soils at a continental scale by using multiple analytical techniques to quantify PyC across Europe. I found that PyC may contribute a smaller component of soil organic carbon than previously thought and that organic carbon is the best predictor of PyC at a continental scale. I then consider how agricultural management and PyC in the form of biochar, impacts soil food webs in a semi-arid corn agroecosystem. I did not find any measurable effects of biochar on soil food web structure or function. I conclude that the long-term impact of historical land management on soil food webs far outweighs any impact of short-term management practices involving biochar. I then use this field study as an opportunity to integrate scientific inquiry in middle school classrooms. I present a collection of classroom activities co-developed with secondary educators that lead students to investigate the effect of biochar on soils and plants.

I conclude by discussing the themes, patterns, and ideas that emerge from the preceding chapters. I found that the responses of soil ecological communities to disturbance are highly context dependent. This context dependency leads to hidden, unexpected, and even contradictory patterns. I end by reflecting on how completing this work has informed my non-linear approach to science.

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many other courses - thank you for giving me the space to find my way as an educator. My graduate experience has been richer because of it.

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DEDICATION

to my parents,

Armin and Christina,

for their tireless support of even my wildest ideas.

TABLE OF CONTENTS

Chapter 1: Introduction

Soils are among Earth's most vital resources. Soils control a diverse array of ecosystem services including the provisioning of food and fiber, decomposition and nutrient cycling, water filtration, and the maintenance of terrestrial biodiversity (Smith et al., 2015). Soils house diverse microbial and faunal communities that mediate the many ecological functions that give rise to these ecosystem services (Bardgett and van der Putten, 2014). However, these complex belowground communities, and therefore the ecosystem processes they regulate, are increasingly threatened by global change, disturbances, and land management decisions (Coyle et al., 2017). Fire is one such disturbance that is of increasing relevance due to climate, land use, and management changes. Ecosystems worldwide are experiencing more frequent, severe, and often unprecedented fires as the world warms (Flannigan et al., 2009; Mack et al., 2011; Moritz et al., 2012). Fires can have profound effects on soil biota with ecosystem-wide consequences (Pressler et al., 2018). Understanding the degree to which fire alters soil community structure, function, and dynamics is necessary to determine whether shifting fire regimes will have long-term implications for ecosystem function.

The influence of fire on soils extends beyond just soil communities. Fires fundamentally alter the physical and chemical soil environment in ways that are likely to regulate responses of the soil community in the weeks, months, and even years after a fire event (Hart et al., 2005). Fires alter the quantity and quality of soil organic matter (SOM; Gonzalez-Perez et al., 2004) and transform organic matter into pyrogenic carbon (PyC), a recalcitrant material with a dense aromatic structure (Knicker, 2011). Understanding the amount, distribution, and role of PyC in terrestrial ecosystems is of increasing importance both as fire regimes change and as we manage soil carbon stocks in an attempt to mitigate adverse effects of climate change.

PyC has recently become the subject of one such soil carbon management strategy. Biochar, the man-made analog of PyC, has been applied as an organic amendment in agricultural soils in an effort to

increase soil carbon storage, while simultaneously improving soil fertility (Lehmann, 2007). However, the results of adding biochar to soils have been inconsistent, revealing the context-dependent nature of biochar-soil interactions (Jeffery et al., 2011). Just as soil communities interact with PyC in fire-affected ecosystems, so too they respond to biochar addition in agroecosystems. Disentangling the effects of biochar on soil ecological processes is an important research aim as biochar continues to be applied to soils as a climate mitigation strategy.

Studying soil food webs and organic matter provides an opportunity to answer fundamental ecological questions about how communities respond to disturbance while also allowing us to evaluate the outcomes of soil management strategies. Given this context, my dissertation research takes a multifaceted approach to answer a question of global relevance: what are the consequences of disturbance and management for the ecology of soils? My dissertation attempts to answer this question by considering several soil ecology disciplinary elements in different disturbance and management contexts using a suite of scientific inquiry practices.

In the paragraphs to come, I share an example from the paleontology and paleoart community to illustrate the importance of taking such a multifaceted approach to scientific inquiry. I then describe a more topical example from the history of soil ecology. Given this setting, I return to my central research question and describe the implications of my approach for the studies included in my dissertation. I integrate the disciplinary elements, disturbance and management contexts, and scientific practices of each study into a conceptual table of contents and outline how each chapter fits within this framework.

There is more than one way to reconstruct a dinosaur

We can learn an important lesson about the roles of evidence, theory, and multifaceted approaches to scientific inquiry from the paleontology and paleoart community. Paleoartists use primary evidence from the fossil record to reconstruct and portray dinosaur morphology and behavior. Most paleoartists draw dinosaurs with strict scientific rigor by basing their designs solely on fossilized remains. That is, paleoartists rely heavily on one single piece of evidence to envision an entire dinosaur. However, the

fossil record is incomplete and very rarely preserves soft tissue or muscle morphology. For decades, dinosaurs have been depicted in their "shrink-wrapped" form. Shrink-wrapped dinosaurs are drawn without fat, cartilage, feathers, or any other features not explicitly preserved in the fossil record. Dinosaur depictions, and therefore our ideas about their morphology and behavior, are then bounded by what we know for certain from the fossil record. These views, however, are unlikely to accurately represent the dinosaurs that roamed the planet.

Recently, paleoart has undergone a renaissance of evidence-based speculation, driven by the paleontologists and paleoartists John Conway, C.M. Koseman, Darren Naish, and Scott Hartman (Conway et al., 2013). In their book, *All Yesterdays*, Conway and colleagues blend hard evidence from the fossil record, observations of modern animals, and artistic speculation to create compelling, thought-provoking depictions of dinosaur morphology and behavior. These "speculative art" pieces, as they are so called, have stimulated hypothesis generation and theoretical advances in the paleontology community that expand beyond the empirical limits of the fossil record.

At the end of their book, Conway and co-authors conduct a thought experiment. They imagine they have no prior knowledge of modern animals. Then, they attempt to draw modern animals based on only the evidence they would gain from the fossilized remains of those animals. Consider what might happen if you draw an elephant based only on its skeleton. Conway and colleagues did just that. The picture that emerges is quite profound: an elephant without its most iconic feature, the trunk. Now, consider a similar thought experiment for soil ecology. Without any additional knowledge or evidence, how much would the DNA content of a given soil sample really tell us? Would we be able to make inferences about the soil ecological community from this one type of evidence alone? It quickly becomes clear that we can only make meaning from genetic information given our understanding genes, proteins, cells, organisms, and the fundamentals of how biological systems work. Evidence is only meaningful in the context of other evidence.

Ecologists are not bound to any one source of evidence the way paleontologists are limited to the fossil record. We have the ability to draw inference from multiple, interacting lines of evidence. We blend

empirical and theoretical approaches by applying experimental, observational, synthesis, and modeling techniques to triangulate patterns, processes, and emergent properties in nature. While one approach would lead us to a shrink-wrapped view of ecological communities, we have conceptual and analytical tools to move beyond such a constrained outlook and into a systematic perspective of the natural world.

The theoretical conundrum of soil organic matter

Especially in the study of soils, one line of evidence, one fact, one piece of data, or one method, often leaves us with an unsatisfying view of the form and function of soils. Soils are heterogeneous and interdisciplinary by nature, requiring the use of varied approaches to study them. Despite this, there are instances in the history of soil ecology where our understanding of soils has been defined, and therefore limited, by a single approach. The study of SOM is a striking and recent example.

For over 200 years, soil scientists studied SOM using technical variations of a single method: the alkaline extraction (Lehmann and Kleber, 2015). The procedure aims to isolate components of SOM by mixing soil samples with a sodium hydroxide solution with a very high pH. This process renders organic compounds much more soluble in water that can then be sequentially precipitated out. The remaining humic acids, fulvic acids, and humin were thought to characterize SOM. There are technical flaws of the alkaline extraction method that we now know result in SOM components that do not accurately represent SOM in natural ecosystems. But perhaps the biggest setback of our reliance on the alkaline extraction method was the development of theory that perpetuated these methodological flaws. For centuries, SOM methodologies drove theory, rather than the other way around.

It has only been in the last decade that the introduction of new spectroscopic techniques have allowed us to study SOM in situ, outside of the constraints of the alkaline extraction technique. The results have sparked a paradigm shift in our understanding of SOM and a complete reversal of prior theories of SOM formation and stabilization. Traditional theories were based on the fundamental idea that large, recalcitrant macromolecules comprised the most stable and persistent SOM (Lehmann and Kleber, 2015). Emerging theories now suggest the opposite: small, microbially-processed monomers are the precursors

to stable and persistent SOM (Cotrufo et al., 2013; Lehmann and Kleber, 2015). Further, we now recognize that SOM persistence is controlled by ecosystem properties, rather than just biochemical recalcitrance (Schmidt et al., 2011). Our study of SOM is a clear example that relying on only one approach to scientific inquiry, particularly in complex, heterogeneous systems like soils, can, and does, impede scientific progress.

A multifaceted approach to soil ecology

I began my dissertation research in the middle of the SOM paradigm shift in soil ecology. As a result, I have been acutely aware of the pitfalls of relying too heavily on one approach to make inferences in ecology. The multifaceted approach I have taken in my dissertation research is a direct reflection of my "upbringing" in the modern field of soil ecology. Together, the speculative art revolution in paleoart and our recent paradigm shift in SOM illustrate the two fundamental principles that define my approach to ecological research:

(1) there is more than one way to understand an ecosystem

(2) the most compelling discoveries lie at the confluence of different approaches to scientific inquiry

Guided by these principles, the chapters to follow describe studies that focus on several disciplinary elements in soil ecology, exist within diverse disturbance and management contexts, and employ a suite of scientific inquiry approaches (Figure 1.1). The disciplinary elements include soil microorganisms and fauna, soil food webs, and SOM. I study these disciplinary elements in the context of single disturbance events, disturbance regimes, and management systems. I use observational, experimental, modeling, and synthesis approaches to conduct the research and then consider how we might design K-12 educational activities to reflect these authentic science practices. Each study asks a more focused question and therefore makes disciplinary-specific contributions. When considered together, each study also contributes to answering the central question of my dissertation research.

Chapters 2, 3, and 4 consider each of the three disciplinary elements (soil microorganisms and fauna, soil food webs, and SOM) in the context of fire disturbance. I begin with a systematic synthesis of the effect of fire on soil microorganisms and fauna in Chapter 2. Here, I use a meta-analytical approach to investigate how fire affects soil biota biomass, abundance, richness, evenness, and diversity by compiling literature estimates from 131 publications. I use this synthesis effort to make generalizations about how fire affects soil communities while also highlighting knowledge gaps that need to be addressed to improve our understanding of how soil communities will respond to shifting fire regimes. In Chapter 3, I address some of these knowledge gaps by studying how fire regimes affect soil food web structure, function, and dynamics in an oak-pine savanna ecosystem. Here, I take an experimental approach by sampling soils in a pre-existing fire frequency field experiment and apply an energetic food web modeling framework to evaluate the consequences of fire frequency for soil food webs. In Chapter 4, I consider how fire alters SOM composition through the lens of PyC. I take an observational approach to understand where and why PyC persists in soils at a continental scale by using multiple analytical techniques to quantify PyC across Europe.

Chapters 5 and 6 consider disciplinary elements presented in earlier chapters outside the context of fire disturbance. In Chapter 5, I investigate the consequences of agricultural management and biochar amendment for soil food webs using experimental and modeling approaches. In Chapter 6, I present a collection of classroom activities co-developed with secondary educators that reflects the soil ecology research conducted in Chapter 5. I use the effect of biochar on soils and plants as a case study to demonstrate one approach to designing authentic soil science inquiry experiences in K-12 classrooms. In Chapter 7, I conclude by discussing the themes, patterns, and ideas that emerge from the preceding chapters. I also take the opportunity to reflect how completing this work contributed to my scientific philosophy and approach.

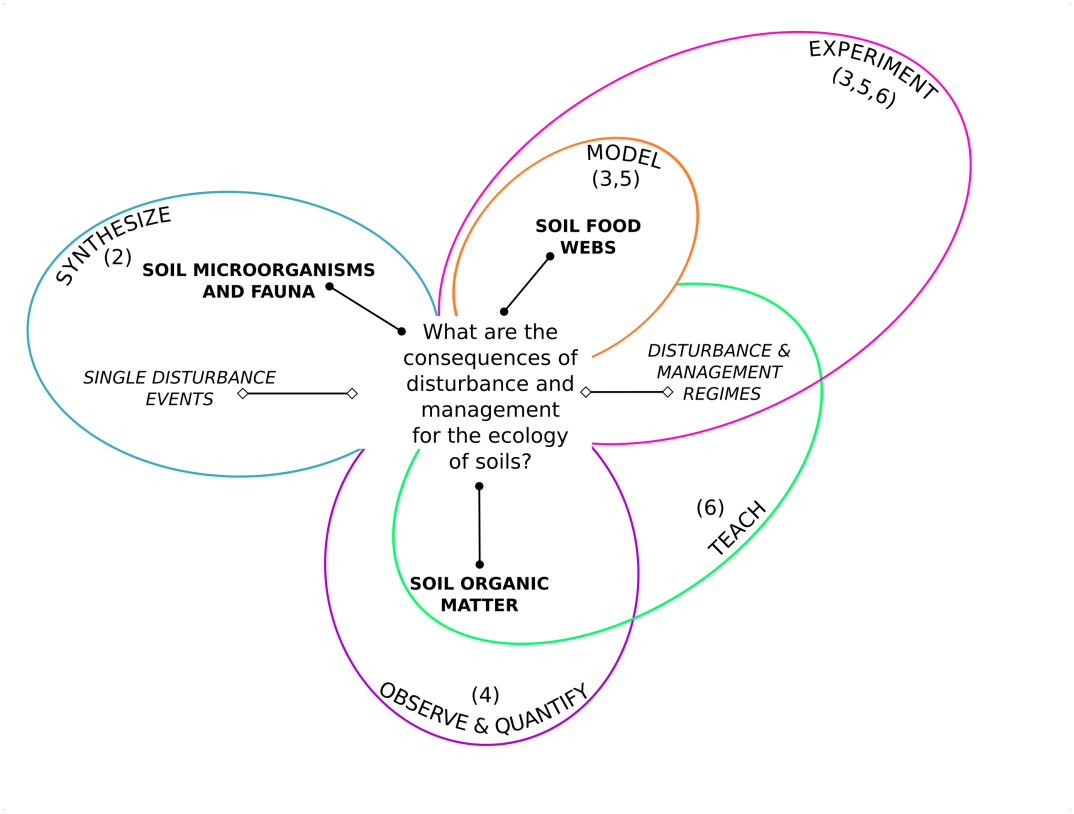

Figure 1.1 Conceptual table of contents for my dissertation. The central question lies at the center of the diagram, with each of three soil ecology disciplinary elements in bold and represented by double bulleted line. The disturbance and management contexts are italicized and represented by open diamond ended lines. Ellipses represent approaches to scientific inquiry including to synthesize (blue), observe and quantify (purple), experiment (pink), model (orange), and teach (green) that I used to answer the central question. Numbers in parentheses correspond with dissertation chapters that fall within each ellipse.

Chapter 2: Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna[1]

Introduction

Fire is an important natural disturbance in many ecosystems (Bond and Keeley, 2005; Sugihara et al., 2006). Global fire regimes have and continue to shift due to human influences on land use (Marlon et al., 2008) and environmental conditions conducive to fire such as increased lightning strikes (Romps et al., 2014), increased net primary productivity (NPP; Nemani et al., 2003), longer fire seasons (Westerling et al., 2006) and a warmer and drier atmosphere (IPCC, 2014). Changing fire regimes have become a significant component of global change (Flannigan et al., 2013; Flannigan et al., 2009, Moritz et al., 2012) and have feedback potential that may exacerbate climate change (Levine et al., 1995). Given that fire frequency is a long-term driver of soil carbon (C) and nitrogen (N) stocks (Soong and Cotrufo, 2015; Pellegrini et al., 2018), it is critical to understand the consequences of novel fire regimes for ecosystem structure, function, and dynamics.

The literature is replete with studies on the impact of fire on plant communities and the importance of the linkages between plants and soil biota for recovery from disturbance (Bond and Keeley, 2005; Wardle et al., 2004; Hart et al., 2005). However, the focus on microorganisms has precluded a comprehensive view of entire soil biological communities and their functions to fire (Zaitsev et al., 2016). Disturbances can exert positive, negative, and neutral effects on soil organisms that are often species specific (Coyle et al., 2017). From the resistance and resilience framework (Holling, 1973; Holling and Gunderson, 2002), resistant soil communities might exhibit a neutral response to a perturbation and maintain their biomass, abundance, and composition after disturbance while resilient communities change due to disturbance but return to their original structure after some period of time (Allison and Martiny, 2008). Resistance

[1] Published in *Oikos* with J.C. Moore and M.F. Cotrufo in 2018

requires the survival of individuals via stress tolerance and plasticity (Shade et al., 2012). Resilience depends on population and community persistence, the presence of dormant groups, and dispersal and migration from undisturbed areas (Shade et al., 2012).

Soil biological communities are at the nexus of understanding how ecosystem processes will respond to changing disturbance regimes. Soil organisms play a critical role in ecosystem C and N cycling by regulating decomposition (Brussaard, 1998; Seastedt, 1984; Wall et al., 2008) and contributing one of the largest C fluxes from the biosphere to the atmosphere, through respiration (IPCC, 2014). Soil fauna are recognized as important mediators of belowground biogeochemical cycling (Wardle et al., 2004; Wall and Bardgett, 2012), and both trophic (Moore et al., 1988) and non-trophic (DeAngelis, 2016; Eisenhauer, 2010) community dynamics. However, few studies on the impacts of fire have included both soil microorganisms (bacteria, fungi, and Archaea) and soil micro- and mesofauna (e.g., Protozoa, nematodes, microarthropods) in an integrative manner.

Soil communities can be impacted by fire both directly through mortality during the disturbance (Certini, 2005) and indirectly through physical, chemical, and biological changes to the soil environment. Such changes include organic matter loss through combustion (Gonzalez-Perez et al., 2004) and erosion (Cotrufo et al., 2016; Rumpel et al., 2009; Shakesby 2011), modification of organic matter quality through the addition of pyrogenic organic matter (Bird et al., 2015; Gonzalez-Perez et al., 2004; Knicker, 2007), alterations in plant community composition and subsequent inputs (Hart et al., 2005), loss of soil structure (Mataix-Solera et al., 2011), changes in albedo with consequences for soil moisture dynamics (Beringer et al., 2003; Jin and Roy, 2005; Jin et al. 2012), and increases in pH (Certini, 2005) – all of which have consequences for soil organisms. Simultaneously, loss of soil organism abundance and diversity during and after fire may change soil food web dynamics and alter the resiliency of entire communities and ecosystems post disturbance. If the effects of fire on soil communities are substantial, shifting fire regimes should have long-term implications for ecosystem function.

We conducted a comprehensive meta-analysis of the literature to answer the following guiding questions: (1) To what extent are soil biota biomasses, abundances, and diversity resistant and resilient to

fire disturbance? (2) What are the important controls on soil biota responses to fire? We then integrate our findings into a conceptual framework that highlights important mechanisms driving soil biota responses to fire. We also discuss knowledge gaps in the literature and recommend research priorities for understanding the consequences of changing fire regimes for soil communities and ecosystem processes.

Material and Methods

Data acquisition

We conducted a systematic meta-analysis of all relevant peer-reviewed publications to investigate the impact of fire on soil microorganisms, microfauna, and mesofauna. Three successive levels of filtering were used to select studies for consideration. First, given the large number of potentially relevant studies, we limited our search to scientific journal articles that were archived in the Web of Science Core Collection database. The final search date was February 20, 2017 and included only publications through 2016. We limited our search to include only peer-reviewed English or English-translated journal articles and excluded book chapters, conference proceedings, talks and presentations, posters, and unpublished datasets from the meta-analysis. With this first level of restrictions we searched the Web of Knowledge Web of Science Core Collection database (www.webofknowledge.com) using multiple Boolean search combinations of one of the following fire keywords: fire, wildfire, burn, pyrogenic organic matter; and all of the following soil biota keywords: soil organism, soil microorganism, soil microbe, soil biota, soil animal, soil biodiversity, soil fauna, soil flora, arthropod, microarthropod, nematode, protozoa, bacteria, fungi, acari, collembola, dipluran, proturan, symphylan, archaea, pauropoda, enchytraeid, microbial community. Given that their unique life histories warrant a separate synthesis effort, we considered macrofauna (e.g., surface dwelling arthropods, snails, slugs, earthworms) outside the scope of our study.

The initial search yielded 6,058 studies. Next, we used the following a priori acceptance criteria for studies to be included in the meta-analysis before filtering downloaded records: (1) Studies must measure the response of one or more soil organism (see Table 1) to fire; (2) Measured response variables must

include either biomass, abundance, richness (e.g., number of species, number of operational taxonomic units), evenness (e.g., Pielou's eveness, Simpson's equitability, Shannon evenness), or diversity indices (e.g., Shannon, Simpson, McIntosh); (3) Studies must compare the response(s) of said soil organism(s) between either burned and non-burned, or pre-fire and post-fire areas; (4) Studies must report mean responses, standard deviation or standard error, and sample size.

Lastly, of the remaining studies, we only included those that measured euedaphic soil organisms (i.e., those that spend the majority of their lifespan in the soil habitat). Thus, studies investigating the only effects of fire on litter dwelling and soil surface dwelling organisms were considered outside the scope of our meta-analysis. We included both organic and mineral soils in our analysis. Additionally, when studies presented both microbial biomass C and N, only biomass C was included as it was the more commonly reported of the two parameters. Because some studies only measured microbial biomass using methods that were not able to differentiate between bacterial biomass and fungal biomass (e.g., chloroform fumigation), "microbial" responses were considered separately from bacterial and fungal responses in the analysis. Similarly, studies that reported changes in fungal communities by means of root colonization (%) were not included and have been synthesized elsewhere by Dove and Hart (2017).

After filtering the initial study set with the above criteria, 131 studies published from 1988 to 2016 remained for further analysis (Table 2.1). Response data were taken directly from tables, figures, and written text. Data were extracted from figures using ImageJ software (Schneider et al., 2012). Within the software, axis scales were defined, and data point locations were measured using the measure function. We extracted both means and standard deviations or standard errors. Additional information that was gathered from the studies included: sample size, response parameter, units, treatment type, time since fire, method of soil biota quantification, qualitative characterizations of fire severity (e.g., light, moderate, or severe), type of fire (e.g., wild vs. prescribed), biome, depth of sampling, page number, table or figure number. We categorized the studies into general biome classes (e.g., forest, grassland) rather than regional biomes (e.g., temperate forest, boreal forest) given that an insufficient number of studies reported higher trophic levels (e.g., nematodes, arthropods) or specified the abundance and diversity parameters.

Meta-analysis statistics

All meta-analysis computations were done in R (R Core Team 2015) using the metafor package (Viechtbauer, 2010). We conducted a hierarchical nested meta-regression to account for studies with repeated observations either across time or space (Koricheva et al., 2013). While two is the absolute minimum number of studies required for meta-analysis (Valentine et al., 2010), we only conducted meta-analysis when three or more studies were available. Soil organism by parameter groups (e.g., biomass, abundance) that had less than three studies were only included in "all studies" analyses (Table 2.2). To ensure normality in all analyses, we utilized the natural log of the response ratio as the effect size (Hedges et al., 1999) as follows:

$$[1] \qquad \ln RR = \ln(X_B) - \ln(X_{NB})$$

where B is the mean of the burned site and NB is the mean of the non-burned site. We back-transformed the model estimates from the log scale to the linear scale as a measure of percent change due to fire to facilitate interpretation as follows:

$$[2] \qquad \text{mean \% change} = (e^b - 1) \cdot 100\%$$

where b is the lnRR model estimate. We first conducted a mixed effects meta-regression on each study to determine an average effect size of fire on each soil organism and parameter. We then used these average effect sizes from each study to conduct a global mixed effects meta-regression on all studies to determine the overall effect of fire on each soil organism and parameter. Taken together, these results were used to guide our interpretation of the effects of fire on the soil community. For example, effect sizes that were not significantly different from zero could be interpreted as a neutral response (e.g., resistant to the fire) or high resilience inasmuch as the group recovered within the time between the fire

event and sampling. Likewise, groups that exhibited significantly negative effect sizes might represent non-resistant and non-resilient groups. We conducted a separate nested meta-regression for the 14 studies that measured both fungal and bacterial biomass to determine whether the effect of fire on one group was consistently greater than the effect on the other. We ran the global meta-regression model on the ratio of fungi and bacteria effect sizes, wherein a ratio greater than 1 indicates that the effect of fire on fungi is greater than bacteria. To keep our estimate conservative, we used the larger of the two standard errors of the effect sizes from the meta-regression model of the single studies of bacteria and fungi as inputs into the global meta-regression.

To investigate the effect of biome, type of fire, depth, and quantification method on the effect of fire on soil organisms, we conducted a mixed effects meta-regression with study as the random effect and biome, type of fire, depth and their interactions as categorical moderators. Due to lack of data for each categorical variable and limited statistical power, we did not test the three-way interaction of biome, type of fire, and soil depth. For this analysis, we binned sampling depth from ground surface into three categories: surface ($\leq$ 5 cm), subsoil ($>$ 5 cm), and unknown (sampling depth not reported). We did not analyze depth as a continuous variable because soils were primarily sampled using cores and analyzed across the depth range of the core (e.g., 0 – 5 cm or 0 – 20 cm). For studies of richness, evenness, and diversity where the number of observations was limited, we reduced the full model to focus on main effects and interactions that address questions of interest. We conducted an omnibus test of parameters for models that resulted in significant moderators to determine differences between parameter categories (e.g. surface vs. subsoil).

To investigate the effect of time since fire on the response of soil biota biomass and abundance to fire, we fit linear mixed effects meta-regression models with study as the random effect and natural log response ratios weighted by variance. We included all observations in studies with multiple observations because time since fire ranged widely both within and between studies. Soil biota richness, evenness, and diversity estimates, as well as microbial abundance, were excluded from the time since fire regression

analysis due to insufficient observation numbers. We interpreted positive linear trends of negative effect sizes to represent resilience.

We assessed publication bias using a regression test for funnel plot asymmetry of the global mixed effects meta-regression model described above (Egger et al., 1997). Groups of studies that showed evidence of funnel plot asymmetry ($p \leq 0.05$) were considered to have publication bias and were not included in our interpretation.

Results

Distribution of the literature

The majority of studies (75%) included in our meta-analysis focused on soil microorganisms (e.g., bacteria, fungi, microbes), whereas a much smaller percentage (25%) considered soil fauna (e.g., protozoa, nematodes, arthropods). While no studies looked at Archaea alone, they are included in microbial biomass estimates. Studies investigating biomass and abundance were more common (93%) than studies examining aspects of diversity (7%). While studies were distributed across different biomes, the majority of studies were conducted in forests (66%), shrublands (13%), and grasslands (12%). Studies investigating prescribed fires (59%) were more common than wildfires (41%). Sampling depth and soil biota quantification methods varied widely across all studies (Table 1.1).

Table 1.1. Studies used in meta-analysis of response of soil biota to fire including the soil organism, response parameters, biome, type of fire, sampling depth from ground surface (cm), soil biota quantification method, and number of observations reported in each publication.

Soil Organism	Response Parameters	Biome	Type of Fire	Sampling Depth (cm)	Quantification Method	No. obs	Reference
Arthropod	Abundance	Forest	Prescribed	6	Tullgren, Direct count	4	(Haimi et al., 2000)
Arthropod	Abundance	Forest	Prescribed	5	Direct count	10	(Dress and Boerner, 2004)
Arthropod	Abundance	Forest	Prescribed	3, 6	High Gradient Apparatus	4	(Berch et al., 2007)

Arthropod	Abundance	Forest	Prescribed	NA	Berlese, direct count	2	(Camann et al., 2008)
Arthropod	Abundance	Forest	Prescribed	NA	Tullgren, Direct count	2	(Brennan et al., 2009)
Arthropod	Abundance	Forest	Prescribed	NA	Tullgren, Direct count	1	(Dechene and Buddle, 2009)
Arthropod, Enchytraeid	Abundance	Forest	Prescribed	15	Tullgren, Direct count	58	(Malmstrom et al., 2009)
Arthropod	Abundance	Forest	Prescribed	5	Tullgren, Direct count	3	(Grabczynska et al., 2009)
Arthropod	Abundance	Forest	Wild	15	Tullgren, Direct count	152	(Broza and Izhaki, 1997)
Arthropod	Abundance	Forest	Wild	2	Tullgren, Direct count	40	(Gongalsky and Persson, 2013)
Arthropod	Abundance	Forest	Wild	12	Tullgren, Direct count	1	(Zaitsev et al., 2014)
Arthropod	Abundance	Grassland	Prescribed	5	Berlese, direct count	7	(Barratt et al., 2006)
Arthropod	Abundance	Grassland	Prescribed	20	Tullgren, Direct count	3	(Whitford and Steinberger, 2012)
Arthropod	Abundance	Shrubland	Prescribed	8	Berlese, direct count	28	(Caruso and Migliorini, 2006)
Arthropod	Abundance	Shrubland	Wild	6	Tullgren, Direct Count	1	(Shaw, 1997)
Arthropod	Abundance, Evenness, Diversity	Forest	Prescribed	NA	Tullgren, Direct count	24	(Malmstrom, 2012)
Arthropod	Abundance, Richness	Forest	Prescribed	NA	Tullgren, Direct count	48	(Malmstrom et al., 2008)
Arthropod	Abundance, Richness	Forest	Prescribed	10	Tullgren, Direct count	18	(Gongalsky et al., 2012)
Arthropod	Abundance, Richness	Forest	Prescribed	30	Direct count	2	(Rossi et al., 2010)
Arthropod	Abundance, Richness	Grassland	Prescribed	30	Tullgren, Direct count	6	(Doamba et al., 2014)
Arthropod	Abundance, Richness, Evenness, Diversity	Forest	Prescribed	10	Tullgren, Direct Count	4	(Huebner et al., 2012)
Arthropod	Abundance, Richness, Evenness, Diversity	Forest	Wild	15	High Gradient Apparatus	4	(Cuchta et al., 2012)

Arthropod	Abundance, Richness, Evenness, Diversity	Forest	Wild	12	High Gradient Apparatus	4	(Cuchta et al., 2013)
Arthropod	Richness	Grassland	Prescribed	15	Berlese, direct count	4	(Beyer et al., 2011)
Bacteria	Abundance	Forest	Prescribed	15	Direct count	7	(Song et al., 2004)
Bacteria	Abundance	Forest	Wild	5	Most Probable Number Dilution Series	10	(Acea and Carballas, 1996)
Bacteria	Abundance	Forest	Wild	10	Culturing and direct count	2	(Khodadad et al., 2011)
Bacteria	Abundance	Forest	Wild	5, 15, 25	Direct count	30	(Kim et al., 2004)
Bacteria	Diversity	Forest	Wild	10	DNA	3	(de Carvalho et al., 2016)
Bacteria	Richness, Evenness, Diversity	Forest	Wild	1	DNA	6	(Sun et al., 2016)
Bacteria	Richness, Evenness, Diversity	Grassland	Prescribed	10	DNA	3	(Coolon et al., 2013)
Fungi	Abundance	Agriculture	Prescribed	10, 20, 30	Direct count	9	(Celik et al., 2011)
Fungi	Abundance	Agriculture	Prescribed	0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20	Direct count	11	(Nasim, 2011)
Fungi	Abundance	Forest	Prescribed	15	Direct count	14	(Johnson, 1995)
Fungi	Abundance	Forest	Prescribed	15	Direct count	6	(Dahlberg et al., 2001)
Fungi	Abundance	Forest	Prescribed	NA	DNA	21	(Mah et al., 2001)
Fungi	Abundance	Forest	Wild	10	Direct count	8	(Vilarino and Arines, 1991)
Fungi	Abundance	Forest	Wild	15	PCR ITS–restriction fragment length polymorphism	4	(Jonsson et al., 1999)
Fungi	Abundance	Forest	Wild	NA	Direct count	2	(Jones et al., 2010)
Fungi	Abundance	Forest	Wild	NA	Direct count	1	(Masaphy and Zabari, 2013)
Fungi	Abundance	Forest	Wild	20	Direct count	2	(Moreira et al., 2006)

Fungi	Abundance	Grassland	Prescribed	15	Direct count	44	(Dhillion and Anderson, 1993)
Fungi	Abundance	Shrubland	Wild	15	Direct count	1	(Bellgard et al., 1994)
Fungi	Abundance	Shrubland	Wild	10	Direct count	2	(Rashid et al., 1997)
Fungi	Abundance, Richness	Grassland	Prescribed	25	Direct count	7	(Gibson and Hetrick, 1988)
Fungi	Abundance, Richness, Diversity	Agriculture	Prescribed	15	Direct count	3	(Wang et al., 2010)
Fungi	Abundance, Richness, Diversity	Forest	Prescribed	NA	Direct count	6	(Tuininga and Dighton, 2004)
Fungi	Abundance, Richness, Evenness, Diversity	Forest	Wild	15	Direct count	40	(Longo et al., 2014)
Fungi	Biomass	Forest	Prescribed	10	Direct count	1	(Waters et al., 1994)
Fungi	Biomass	Forest	Prescribed	40	Direct count	6	(Stendell et al., 1999)
Fungi	Biomass	Forest	Wild	1, 3, 5	Direct count	6	(Akema et al., 2009)
Fungi	Richness	Forest	Wild	10	Direct count	3	(Violi et al., 2008)
Fungi	Richness	Forest	Wild	15	Direct count	6	(Motiejunaite et al., 2014)
Fungi	Richness	Forest	Wild	10	rRNA	4	(Xiang et al., 2015)
Fungi	Richness, Diversity	Forest	Prescribed	30	DNA	4	(Cowan et al., 2016)
Fungi	Richness, Diversity	Forest	Wild	20	DNA	12	(Buscardo et al., 2015)
Fungi	Richness, Evenness, Diversity	Agriculture	Prescribed	10	Direct count	9	(de Azevedo et al., 2014)
Fungi	Richness, Evenness, Diversity	Forest	Prescribed	10	DNA	12	(Oliver et al., 2015)
Fungi, Arthropod	Biomass, Abundance	Shrubland	Prescribed	5	Tullgren, Direct count	42	(Rutigliano et al., 2013)
Fungi, Bacteria	Abundance	Forest	Prescribed	2, 5	Culturing	4	(Garcia-Oliva et al., 1999)
Fungi,	Abundance	Forest	Wild	NA	Direct count	2	(Tateishi et al.,

Bacteria							1989)
Fungi, Bacteria	Abundance	Forest	Wild	5	Culturing	6	(Pourreza et al., 2014)
Fungi, Bacteria	Abundance	Grassland	Prescribed	15	Culturing	48	(Dhillion and Anderson, 1994)
Fungi, Bacteria	Abundance, Biomass	Grassland	Prescribed	15	Quantitative PCR, lipid biomarkers	2	(Docherty et al., 2012)
Fungi, Bacteria	Biomass	Agriculture	Prescribed	10	Direct count	2	(Aleixo et al., 2014)
Fungi, Bacteria	Biomass	Agriculture	Prescribed	0.5, 1	Substrate-Induced Respiration	2	(Rahkonen et al., 1999)
Fungi, Bacteria	Biomass	Forest	Prescribed	5, 20	Chloroform fumigation	26	(Luizao et al., 1992)
Fungi, Bacteria	Biomass	Forest	Prescribed	NA	Chloroform fumigation	1	(Fritze et al., 1994)
Fungi, Bacteria	Biomass	Forest	Prescribed	NA	Phospholipid fatty acids	3	(Baath et al., 1995)
Fungi, Bacteria	Biomass	Forest	Prescribed	5	Chloroform fumigation	4	(Andersson et al., 2004)
Fungi, Bacteria	Biomass	Forest	Prescribed	5, 15	Direct count	12	(Esquilin et al., 2007)
Fungi, Bacteria	Biomass	Forest	Prescribed	0, 5	Phospholipid fatty acids	6	(Overby and Hart, 2016)
Fungi, Bacteria	Biomass	Forest	Wild	5	Substrate-Induced Respiration	5	(Dumontet et al., 1996)
Fungi, Bacteria	Biomass	Forest	Wild	5, 10	Chloroform fumigation	33	(Prieto-Fernandez et al., 1998)
Fungi, Bacteria	Biomass	Forest	Wild	10	Direct count	3	(Esquilin et al., 2008)
Fungi, Bacteria	Biomass	Grassland	Prescribed	5	Chloroform fumigation	4	(Jensen et al., 2001)
Fungi, Bacteria	Biomass	Plantation, Shrubland	Prescribed	10	Phospholipid fatty acids	8	(Sun et al., 2011)
Fungi, Bacteria	Biomass	Shrubland	Wild	5	Substrate-Induced Respiration	1	(Halvorson et al., 1997)
Fungi, Bacteria	Evenness, Diversity	Forest	Prescribed	NA	Culturing	20	(Staddon et al., 1997)
Fungi, Bacteria	Richness, Evenness	Forest	Wild	NA	DNA, Quantitative PCR	12	(Kennedy and Egger, 2010)

Fungi, Bacteria, Microbial	Abundance	Forest	Wild	5	Culturing	8	(Marozas et al., 2013)
Fungi, Bacteria, Microbial	Abundance, Biomass	Forest	Wild	5	Chloroform fumigation	156	(Mabuhay et al., 2006)
Fungi, Bacteria, Microbial	Abundance, Biomass	Forest	Wild	5	Culturing, Chloroform fumigation, Phospholipid fatty acids	7	(Barcenas-Moreno et al., 2011)
Fungi, Bacteria, Microbial	Biomass	Forest	Wild	15	Fatty acid methyl esters	8	(Hamman et al., 2007)
Fungi, Bacteria, Microbial	Biomass	Forest	Wild	2.5, 5	Phospholipid fatty acids	24	(Lombao et al., 2015)
Fungi, Bacteria, Microbial	Biomass	Grassland	Prescribed	15	Phospholipid fatty acids, Chloroform fumigation	12	(Zhang et al., 2013)
Fungi, Bacteria, Microbial	Biomass	Grassland	Wild	20	Phospholipid fatty acids	6	(Wang et al., 2016)
Fungi, Bacteria, Microbial	Biomass	Plantation	Wild	5	Culturing, chloroform fumigation	6	(Kara and Bolat, 2009)
Fungi, Bacteria, Microbial	Biomass	Shrubland	Prescribed	5	Phospholipid fatty acids	5	(Barreiro et al., 2015)
Fungi, Bacteria, Microbial	Biomass	Shrubland	Prescribed	2, 5	Phospholipid fatty acids, Chloroform fumigation, Ergosterol	14	(Barreiro et al., 2016b)
Fungi, Bacteria, Microbial	Biomass, Abundance	Shrubland	Wild	5	Culturing, Chloroform fumigation, Phospholipid fatty acids	28	(Barcenas-Moreno et al., 2016)
Fungi, Bacteria, Microbial, Protozoa	Biomass, Abundance	Forest, Grassland	Wild, Prescribed	2.5, 5	Chloroform fumigation, Fatty acid methyl esters	36	(Fultz et al., 2016)
Fungi, Microbial	Abundance	Shrubland	Wild	NA	Culturing	16	(Cilliers et al., 2005)
Fungi, Microbial	Biomass	Forest	Prescribed	5	Chloroform fumigation, Direct count	40	(Rutigliano et al., 2002)

Fungi, Microbial	Biomass	Forest	Wild	NA	quantitative PCR, chloroform fumigation	6	(Waldrop and Harden, 2008)
Fungi, Microbial	Biomass	Forest	Wild	2	Ergosterol, Phospholipid fatty acids	3	(Barreiro et al., 2016a)
Fungi, Microbial	Biomass	Forest	Wild	15	Chloroform fumigation, Ergosterol	6	(Koster et al., 2016)
Fungi, Microbial	Biomass	Shrubland	Prescribed	5	Chloroform fumigation, Direct count	32	(Rutigliano et al., 2007)
Fungi, Microbial	Biomass, Evenness, Diversity	Forest	Wild	5	Direct count, Chloroform fumigation	12	(Martin-Pinto et al., 2006)
Microbial	Abundance	Forest	Prescribed	15	Phospholipid fatty acids	4	(Rau et al., 2008)
Microbial	Biomass	Agriculture	Prescribed	20	Chloroform fumigation	4	(Basilio Azevedo et al., 2015)
Microbial	Biomass	Forest	Prescribed	5	Substrate-Induced Respiration	10	(D'Ascoli et al., 2005)
Microbial	Biomass	Forest	Prescribed	10	Phospholipid fatty acids	4	(Gundale et al., 2005)
Microbial	Biomass	Forest	Prescribed	NA	Chloroform fumigation	1	(Swallow et al., 2009)
Microbial	Biomass	Forest	Prescribed	20	Substrate-Induced Respiration	1	(Bogorodskaya et al., 2014)
Microbial	Biomass	Forest	Prescribed	2.5	Chloroform fumigation	14	(Palese et al., 2004)
Microbial	Biomass	Forest	Prescribed	10	Chloroform fumigation	1	(Guo et al., 2015)
Microbial	Biomass	Forest	Prescribed	10, 20, 40, 60, 80	Chloroform fumigation	10	(Liu et al., 2015)
Microbial	Biomass	Forest	Prescribed	10	Chloroform fumigation	4	(Shen et al., 2016)
Microbial	Biomass	Forest	Wild	10	Chloroform fumigation	1	(Smith et al., 2008)
Microbial	Biomass	Forest	Wild	15	Chloroform fumigation	16	(Jiang et al., 2012)
Microbial	Biomass	Forest	Wild	5	Chloroform fumigation	2	(Heydari et al., 2016)
Microbial	Biomass	Forest	Wild, Prescribed	15	Chloroform fumigation	6	(Grady and Hart, 2006)

Microbial	Biomass	Grassland	Prescribed	15	Chloroform fumigation	10	(Liu et al., 2007)
Microbial	Biomass	Grassland	Prescribed	10	Chloroform fumigation	1	(Harris et al., 2008)
Microbial	Biomass	Grassland	Prescribed	10	Phospholipid fatty acids	1	(Jangid et al., 2010)
Microbial	Biomass	Grassland	Prescribed	10	Chloroform fumigation	6	(San Emeterio et al., 2016)
Microbial	Biomass	Shrubland	Prescribed	15	Chloroform fumigation	6	(Liu et al.,2010)
Microbial	Biomass	Shrubland	Prescribed	5	Chloroform fumigation, Substrate-induced respiration	8	(Fonturbel et al., 2012)
Microbial	Biomass	Shrubland	Wild	2	Chloroform fumigation	1	(Barreiro et al., 2010)
Microbial	Biomass	Shrubland	Wild	4	Chloroform fumigation	1	(Dannenmann et al., 2011)
Microbial	Biomass	Shrubland	Wild	15	Chloroform fumigation	5	(Hanan et al., 2016)
Microbial	Biomass, Diversity	Agriculture	Prescribed	18.5	DNA	2	(Sul et al., 2013)
Microbial	Biomass, Diversity	Shrubland	Prescribed	0, 2	Chloroform fumigation, Average well colour development	36	(Fonturbel et al., 2016)
Microbial	Richness, Evenness	Forest	Prescribed	10	DNA	4	(Brown et al., 2013)
Microbial, Arthropod, Nematode, Protozoa, Enchytraeid	Biomass, Abundance, Richness	Forest	Wild	15	Chloroform fumigation, Direct count, Baermann, Tullgren	11	(Gongalsky et al., 2016)
Nematode	Abundance	Forest	Prescribed	20	Baermann, Direct count	12	(McSorley, 1993)
Nematode	Abundance	Forest	Prescribed	10	Baermann, Direct count	40	(Pen-Mouratov et al., 2012)
Nematode	Abundance, Diversity	Forest	Wild	10	Baermann, Direct count	2	(Cerevkova and Renco, 2009)
Nematode	Biomass, Abundance, Richness, Diversity	Forest	Wild	10	Baermann, Direct count	12	(Renco and Cerevkova, 2015)

Responses of soil biota to fire

Overall, fire had a strong, statistically significant negative effect on biomass (Figure 2.1a; $p < 0.0001$) and abundance (Figure 2.1b; $p < 0.0001$) estimates of all soil organisms available for meta-analysis except for arthropod abundance ($p = 0.94$). Fire had the greatest overall impact on fungal biomass (-96%; $p < 0.0001$), but the effects of fire on bacterial (-90%; $p < 0.0001$) and overall microbial biomass (-44%; $p = 0.025$), and bacterial (-96%; $p < 0.0001$) and nematode (-88%; $p = 0.005$) abundance were all negative and significantly different than zero (Figure 2.1). The effect of fire on microbial abundance (-99%) was greater than that of microbial biomass (-44%), but not significantly different from zero (Figure 2.1; $p = 0.07$). For studies that measured both fungal and bacterial biomass, the effect of fire on fungal biomass was significantly greater than the effect on bacterial biomass (Fungi effect size: Bacteria effect size = 1.62; $p < 0.0001$). Despite differences in quantification approaches and units, the overall effects of fire on soil organism biomass and abundance were similar (-1.60 ± 0.22 vs. -2.07 ± 0.29 respectively; Figure 2.1).

The effect of fire on fungal (-98%) and arthropod (-97%) richness, fungal (-99%) and arthropod (-99%) evenness, and fungal (-99%), bacterial (-93%), arthropod (-99%) and microbial (-99%) diversity was comparable across all groups, with the exception of bacterial richness (-71%). Fire had a strong negative effect on fungal and arthropod richness (Figure 2.2a, $p < 0.0001$), evenness (Figure 2.2b, $p < 0.0001$), and diversity (Figure 2.2c, $p < 0.0001$), but had no significant effect on bacterial richness ($p = 0.45$) or diversity ($p = 0.09$). In general, the effect sizes of fire on soil organism richness, evenness, and diversity (Figure 2.2) were greater than those on biomass and abundance (Figure 2.1).

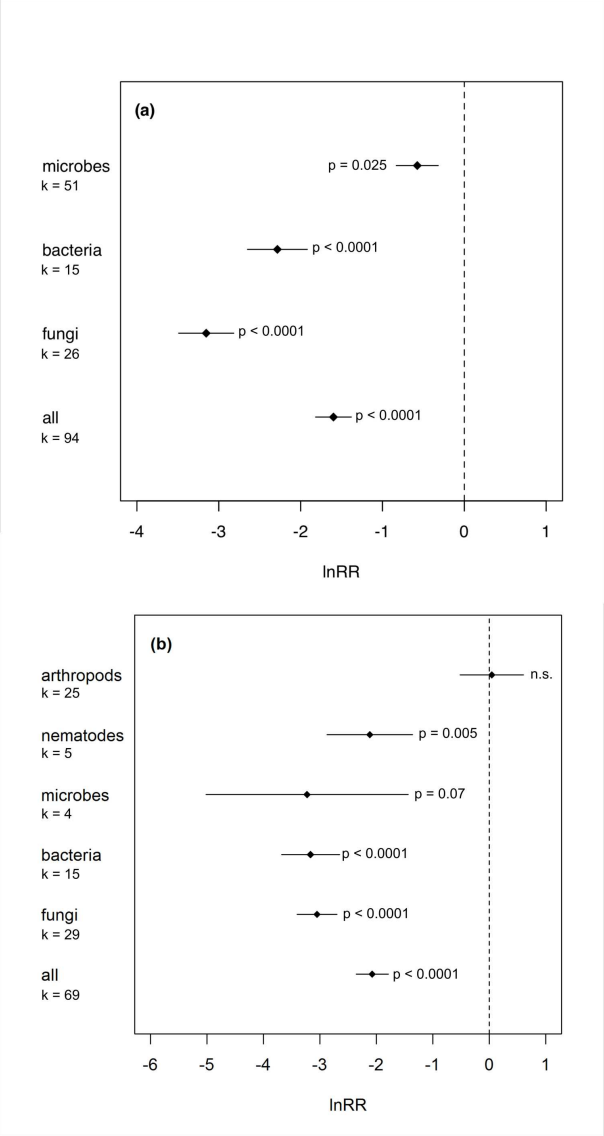

Figure 2.1. Effect of fire on soil biota biomass (a) and abundance (b). lnRR (see EQ 1) is the estimated overall effect size ± standard error from meta-regression model. k is the number of observations included in each analysis; p-values indicate whether lnRR is significantly different from zero (n.s. is not significant). lnRR reported for all studies ("all") include additional studies of nematodes, Protozoa, and enchytraeids analysis in addition to groups presented in figure as there were too few to conduct separate meta-analyses ($k < 3$).

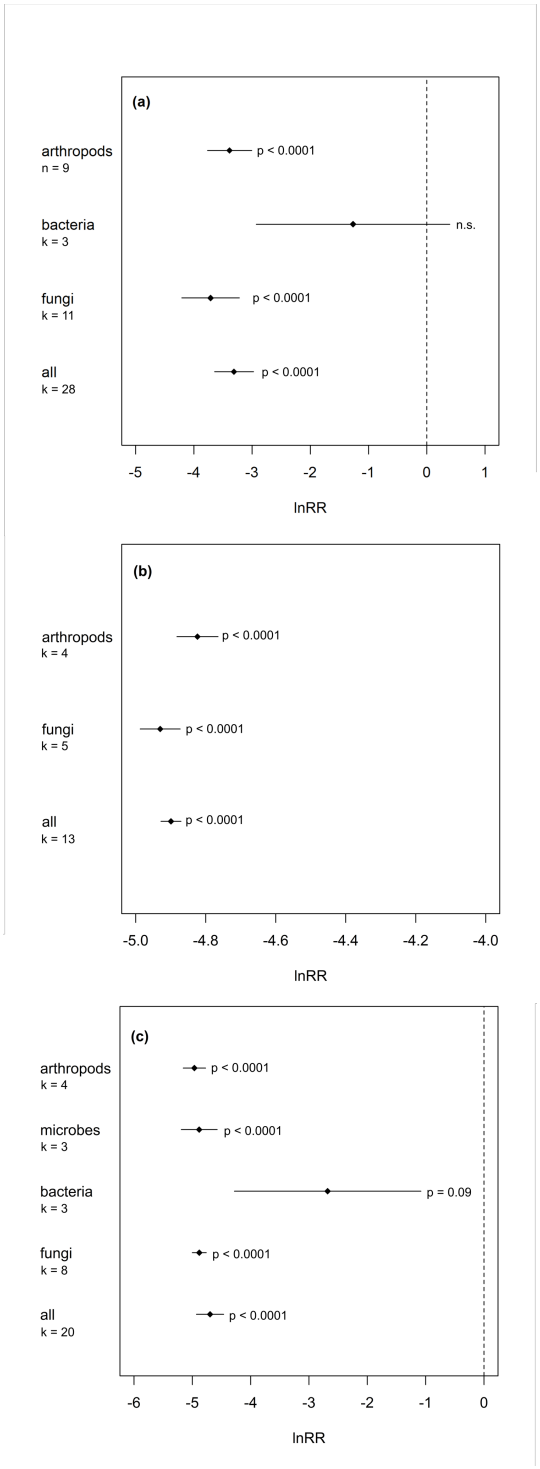

Figure 2.2 Effect of fire on soil biota richness (a), evenness (b), and diversity (c). lnRR (see EQ 1) is the estimated overall effect size ± standard error from meta-regression model. k is the number of observations included in each analysis; p-values indicate whether the lnRR is significantly different from zero (n.s. is not significant). lnRRs for "all" studies were derived as described in Figure 2.1.

Controls on soil biota responses to fire

Biome, burning depth and fire type alone were not good predictors of the response of soil biota biomass and abundance to fire (Table 2.2). Two exceptions include differences in bacterial and microbial biomass responses between surface and subsoils (Table 2.2). The effect of fire on bacterial biomass was greater in surface than subsoils (-2.34 ± 1.21 vs. 0.07 ± 1.60, respectively; $p = 0.02$). Additionally, the effect of fire on microbial biomass across sampling depth depended on fire type, with prescribed fires resulting in a greater reduction of microbial biomass in subsoils and wildfires having a greater negative effect in surface soils (Figure 2.3). We found that prescribed fires had a greater negative effect on fungal biomass in forests than wildfires, while the effects of wildfires and prescribed fires were similar in grasslands and shrublands (Figure 2.4). While the overall interaction between biome and fire type is significant for bacterial biomass (Table 2.2), the omnibus test of parameters did not reveal any significant trend. The significant effect of the interaction between fire type and burning depth on fungal abundance (Table 2.2) was driven by effect sizes in the "unknown" burning depth category, and therefore could not be interpreted in an ecological context.

Biome was often a good predictor of fire effects on richness, evenness, and diversity (Table 2.2). Biome alone was a significant predictor of arthropod richness, while the effect of fire on fungal richness, evenness, and diversity across different biomes depended on the fire type (Table 2.2). Prescribed fires, rather than wildfires, resulted in a greater reduction in fungal richness (-3.47 ± 0.93 vs. -3.31 ± 0.67, respectively; $p < 0.0001$), evenness (-5.13 ± 0.12 vs. -4.86 ± 0.06, respectively; $p < 0.0001$), and diversity (-5.05 ± 0.21 vs. -4.74 ± 0.23, respectively: $p < 0.0001$) in forests. Arthropod richness was reduced more in grasslands (-5.81 ± 0.92; $p < 0.0001$) than in forests (-3.30 ± 0.63; $p < 0.0001$). Burning depth alone was also a significant predictor of fungal evenness, but this was driven by studies with an "unknown" burning depth category and therefore could not be further interpreted (Table 2.2). While many of the main effects or interactions were significant when compiling all studies (i.e., all soil biota groups), the significance of these effects is largely driven by one soil biota group with a greater number of

observations included in the meta-analysis (i.e., fungi). As a result, we do not attempt to use results of the all studies category to suggest generalizability across soil biota groups.

Table 2.2. Influence of biome, fire type, burning depth, and quantification method on effect of fire on soil organisms. k is the number of observations for each meta-analysis. A dash (-) signifies groups that were not analyzed because they did not meet the minimum study number (3). Values represent p-values of mixed effects meta-regression for each soil organism and parameter group. Significant effects are bolded ($p \leq 0.05$).

			Moderators						
Soil Organism	Parameter	*k*	Biome	Fire Type	Burning Depth	Biome* Fire Type	Fire Type* Burning Depth	Biome* Burning Depth	Method
Fungi	Biomass	29	0.59	0.19	0.23	**0.02**	0.48	0.30	0.11
	Abundance	30	0.77	0.79	0.95	0.70	**0.03**	0.35	0.09
	Richness	11	0.43	0.72	0.97	**0.02**	0.45	-	-
	Evenness	5	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	-	-	-
	Diversity	8	**<0.0001**	0.93	0.23	**<0.0001**	0.14	-	-
Bacteria	Biomass	15	0.54	0.84	**0.005**	**0.01**	0.89	0.25	0.93
	Abundance	16	0.96	0.96	0.84	0.96	0.87	0.65	0.86
Microbes	Biomass	56	0.64	0.44	**0.04**	0.97	**0.04**	0.80	0.70
Nematodes	Abundance	5	-	0.86	-	-	-	-	-
Arthropods	Abundance	25	0.57	0.89	0.71	0.56	0.88	0.32	-
	Richness	9	**<0.0001**	0.44	0.21	0.39	-	-	-
	Evenness	4	0.08	0.90	0.98	-	-	-	-
	Diversity	4	0.18	0.83	0.98	-	-	-	-

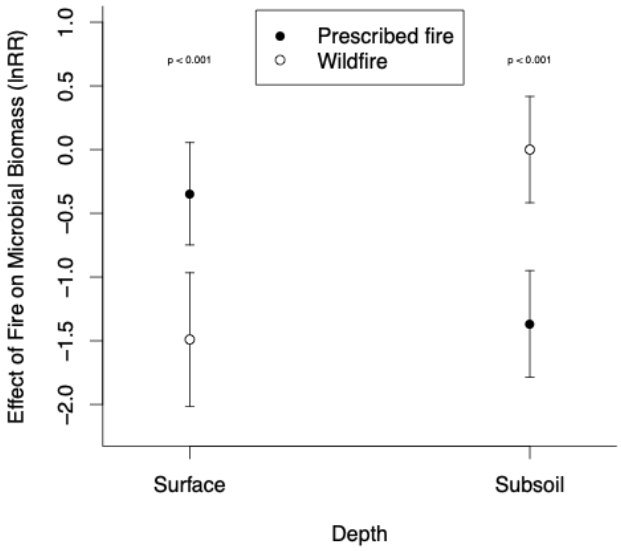

Figure 2.3 Effect of prescribed fire and wildfire on microbial biomass in surface and subsoils. lnRR (see EQ 1) is the estimated overall effect size ± standard error from meta-regression model. P-values indicate whether lnRR is significantly different from zero.

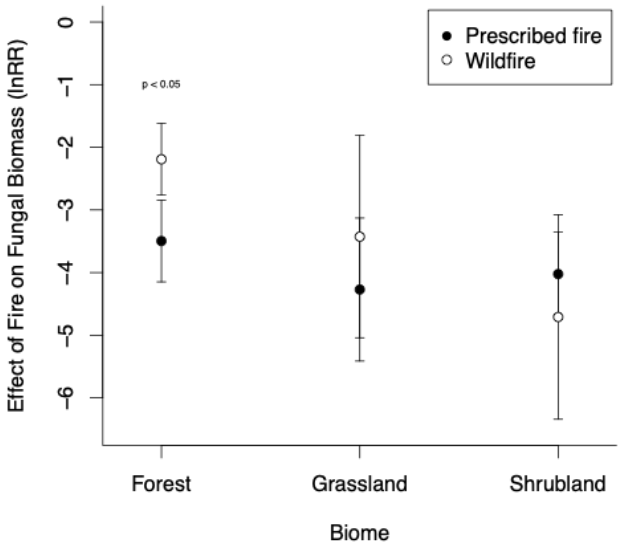

Figure 2.4 Effect of prescribed and wildfire on fungal biomass in forests, grasslands, and shrublands. lnRR (see EQ 1) is the estimated overall effect size ± standard error from meta-regression model. P-values indicate whether lnRR is significantly different from zero.

Soil biota responses over time since fire

The studies included in the meta-analysis sampled soils between immediately after the fire to 62 years, with an average time since fire of 2 years. Based on the results of linear mixed effects meta-regressions, we found no clear temporal trend of the effect of fire on fungal biomass (marginal $r^2 = 0.06$, $p = 0.72$, $df = 100$), fungal abundance (marginal $r^2 = 0.05$, $p < 0.001$, $df = 233$), bacterial biomass (marginal $r^2 = 0.002$, $p = 0.89$, $df = 8$), bacterial abundance (marginal $r^2 = 0.000007$, $p < 0.001$, $df = 182$), microbial biomass (marginal $r^2 = 0.00002$, $p = 0.87$, $df = 269$) or arthropod abundance (marginal $r^2 = 0.0001$, $p = 0.57$, $df = 348$) over time since disturbance. However, we found a weak, positive relationship between nematode response to fire and time since fire (marginal $r^2 = 0.13$, $p = 0.09$, $df = 5$), suggesting that the effect of fire on nematode abundance decreased with increasing time since fire.

Publication bias

Our meta-analysis focused on published peer-reviewed journal articles and some degree of publication bias against results archived elsewhere is unavoidable. Within our dataset, the regression test for funnel plot asymmetry (Egger et al., 1997) revealed evidence for publication bias in studies of nematode abundance ($p < 0.0001$; $k = 5$), bacterial richness ($p = 0.0005$; $k = 3$), and microbial diversity ($p = 0.01$; $k = 3$). These groups also had a low number of studies available for meta-analysis and the results were therefore not included in our interpretation.

Discussion

Soil mesofauna are more resistant to fire than soil microorganisms

The significant negative effect of fire was consistent across microbial taxa and parameters considered in this meta-analysis suggesting that soil microbial communities are not resistant to fire. These results align with expectations from the literature and previous syntheses that soil microbial communities can be both directly and indirectly negatively affected by fire (Dooley and Treseder, 2012; Dove and Hart,

2017). The primary direct effect of fire is mortality of soil organisms during the event, but it is difficult to separate direct effects from indirect effects using a meta-analysis approach. We found that, across all studies, the effect of fire on fungal biomass is greater than its effect on bacterial biomass (Figure 2.1a). This trend remains consistent when considering only the 14 studies that measured both bacterial and fungal biomass suggesting that bacteria are more resistant to fire than fungi. However, when considering abundance, our results show that fire affects both fungal and bacterial abundance to a similar degree (Figure 2.1b) likely due to differences in quantification methods for fungi and bacteria (e.g., sporocarps vs. culturing). The effect of fire on microbial abundance was greater than that of microbial biomass, but this observation is based on only four studies of microbial abundance and likely depends on differences in quantification methods for abundance and biomass. With respect to fungal richness, our results (Figure 2.2a) are consistent with those of a recent synthesis of fungal diversity in fire-affected ecosystems (Dove and Hart, 2017), confirming that fungal richness decreases with fire. These findings confirm the widely held view that fungi are more sensitive to fire than bacteria. Previous evidence suggests that this is due to both the lower thermal tolerance of fungi and, for mycorrhizal fungi, the mortality of plant hosts during fire (Neary et al., 1999).

Unlike fungi, bacterial richness was not significantly different between burned and unburned sites (Figure 2.2a). However, only three studies of bacterial richness were available for meta-analysis and we found evidence for publication bias. Thus, our understanding of how fire affects bacterial richness remains unresolved. In general, far fewer studies investigated the effect of fire on aspects of belowground diversity than biomass or abundance measures (25% vs. 75% of studies, respectively). While we found that the overall effect of fire on belowground diversity is strongly negative, this finding is based on a limited number of studies and additional research is needed to validate this negative effect across different taxa and ecosystems.

Our results indicate that the effect of fire on soil mesofauna (nematodes and arthropods) is weaker than for microorganisms (bacteria, fungi, microbes), but far fewer studies were available for mesofauna than for microorganisms (Figure 2.1). There are clear differences in the morphologies, physiologies and

ecologies between microorganisms and soil mesofauna that could explain these results. Soil mesofauna, particularly arthropods, are larger, possess greater agility and tend to occupy higher trophic positions than microorganisms. Soil arthropods appear to be either resistant to or highly resilient to fire as their abundance did not significantly increase or decrease with fire (Figure 2.1b). Soil arthropod richness, evenness, and diversity did decrease significantly with fire (Figure 2.2) but the number of studies available is limited (Table 2.1). While it has been shown that soil arthropods have differential susceptibilities to fire and asynchronous recovery times (Malmstrom et al., 2009), we were not able to discern this effect in our dataset. Soil arthropods are more agile than other invertebrates and have the ability to move about soil pore spaces in search of more favorable conditions. However, dispersal ability is species specific and distance traveled belowground tends to be low (Lehmitz et al,. 2012). Some taxa (e.g., oribatid mites) are more heavily armored than others (e.g., Collembola), which results in greater protection against increased temperatures during fire (Malmstrom, 2008). Soil arthropod communities may be resistant to fire because they are able to either withstand or escape high soil temperatures by migrating to unburned patches or deeper into the soil. The spatial heterogeneity of soil after a fire is an important control on soil arthropod community recovery with unburned refugia harboring greater abundance and diversity of soil arthropods (Gongalsky and Zaitsev, 2016). Soil arthropods that survive in unburned patches or deeper in the soil profile may serve as colonizing populations for recovering arthropod communities (Zaitsev et al., 2014). Depending on the rate of recolonization, this may result in no net difference in arthropod abundance estimates between burned and non-burned plots by the time of sampling.

Omnivory is common among soil arthropods (Moore et al., 1988) and thus arthropods have a wide array of prey options available which may allow for greater survival as prey resources become limiting after a fire. When considered in a food web context, the resistance of soil arthropods to fire may result in an increase in top down pressure on their microbial prey that may exacerbate microbial sensitivity to fire and contribute to their limited recovery. However, few studies investigate the effect of fire on microorganisms, fauna and their interactions in a food web context. Thus, the implications of fire-

resistant soil arthropods for top down controls on microbial communities remains speculative and unresolved.

Soil microorganisms are not resilient to fire

Not only is the negative effect of fire on soil biota generally consistent across taxa and biomes, the response remains consistent over time since disturbance. We found a weak relationship ($r2 = 0.13$, $p = 0.09$, $df = 5$) between nematode abundance response and time since fire, but these data are subject to publication bias. For all other groups, we found no clear temporal signal of soil biota recovery after fire suggesting that microbial communities are not particularly resilient to fire. The majority of the studies included in this meta-analysis sampled soil biological communities within 10 years after fire (two years on average), but even a decade after fire, we find no consistent evidence for a trend towards recovery of soil biological communities. While a few studies found a positive effect on soil biota at some time points, the majority of response ratios were negative, regardless of soil biota group or time since fire. The negative effect of fire on soil biota biomass and abundance may persist over a decade, as observed in both arthropod (Malmstrom, 2012) and fungal communities (Treseder et al., 2004). Our results align with meta-analyses by Dove and Hart (2017) and Dooley and Treseder (2012) who found limited evidence of recovery of fungal richness and microbial biomass in less than 10 years. A recovery trend of microbial biomass was found in Boreal forests (Dooley and Treseder, 2012) and fungal richness across sites (Dove and Hart, 2017), but these effects were not realized until 10-20 years or more after the fire. If soil biological communities are not resilient to fire within a decade, the predicted increase in fire frequency (Moritz et al., 2012) may further hinder the recovery of soil communities and the important ecosystem processes they regulate.

Biome, burning depth and fire type are poor predictors of soil biota responses

Our synthesis provides some evidence for ecosystem and fire parameters to drive soil biota responses to fire. However, biome, burning depth, fire type, and quantification method were overall inadequate

predictors of soil biota responses (Table 2.2). Many of the other physical, chemical, and biological mechanisms that likely control soil biota responses to fire (e.g., pH, organic matter quantity and quality, soil temperature and moisture, plant community composition, soil food web interactions) were not measured or reported consistently across the literature and thus could not be included as moderators in our meta-analysis. Instead, discussions of variation in soil biota responses to fire in the literature have focused primarily on differences in ecosystem types (Dooley and Treseder, 2012; Dove and Hart, 2017), fire severity (Gongalsky, 2006; Malmström, 2010), fire type (Dove and Hart, 2017), and measurement technique (Dooley and Treseder, 2012; Dove and Hart, 2017). Although biome, burning depth, and fire type could not explain much of the variation in soil biota responses (Table 2.2), a few patterns emerged from our analyses that serve as starting points for future research.

We found that fire had a stronger negative effect on bacterial biomass in surface soils than in subsoils. This result confirms previous observations that burning depth plays an important role in determining the degree to which soil biological communities suffer direct mortality due to fire (Neary et al., 1999). Depending on fire severity, some fires may only burn the top few centimeters of the soil surface (Neary et al., 1999) and samples that are taken down to greater depths may dilute the signal of the response of soil microbial communities to fire.

We found that microbial biomass is more susceptible to wildfire in surface soils, whereas the effect of prescribed fires is greater in subsoils (Figure 2.3). The observed decrease in microbial biomass in subsoils after prescribed fires could be driven by changes in the fungal community. In forests, we found that prescribed fires have a greater negative effect on fungal biomass and diversity than wildfires (Figure 2.4). We did not see a difference in the effect of fire on fungal biomass in grasslands and shrublands (Figure 2.4). We speculate that this may be because their management results in similar severity of prescribed burns and wildfires. A number of studies included in the meta-analysis were prescribed fires that were set after clear cutting the forest (e.g., Baath et al., 1995; Mah et al., 2001; Malmstrom et al., 2008). Fires and tree removal during clear cutting shift plant community composition and thus influence the rhizosphere and rhizosphere interactions (Moore, 1988; Wall and Moore, 1999), particularly mycorrhizal associations.

Thus, the greater negative effect of prescribed fire on fungal biomass may be driven by studies investigating post-clearcut burning. Mycorrhizal colonization also decreases after fire when measured in situ (Dove and Hart, 2017) and may explain the decreases in fungal and microbial biomass in both surface soils and subsoils. Regardless of fire type, low severity fires result in high tree survival and shallow depth of burn into mineral soil in which mycorrhizal colonization and diversity can persist (Dahlberg et al., 2001) but we were not able to test an effect of fire severity due to a limited availability of studies. When considering fire regimes, rather than recovery from single fires, our perspective on the resistance and resilience of soil communities changes. Repeated burning overall may not affect fungal richness (Dove and Hart, 2017), but distinct fire-adapted fungal communities do develop in frequently burned forests (two- and three-year intervals; Oliver et al., 2015).

Our analysis found that fire reduces arthropod richness to a greater degree in grasslands than in forests (Table 2.2). Forest litter and soil horizons often have a lower bulk density than grassland soils (Keen et al., 2011), allowing for greater ease of movement for arthropods to traverse deeper into the soil to evade high temperatures during fire. Oribatid mites possess exoskeletons that are more resistant to higher soil temperatures (Malmstrom, 2008) and are common in forests, while less armored microarthropods (e.g., collembola, prostigmatid mites) are common in grasslands (Maraun and Scheu, 2000), though both kinds of microarthropods can be found in either ecosystem. Mycorrhizal associations play a key role in structuring grassland plant communities (Hartnett and Wilson, 2002) and many soil microarthropods are fungal feeders (Hunt et al., 1987; Schneider et al., 2004). The observed reduction in arthropod richness may be a consequence of direct effects of fire on plant communities and their mycorrhizal associations with cascading effects to their microarthropod consumers. However, mycorrhizae also play important roles in both temperate (Read et al., 2004) and tropical forests (Rillig et al., 2001), suggesting that these mechanisms are likely also at play in regulating arthropod richness after fire in forest ecosystems.

Knowledge gaps and research priorities

Our synthesis revealed two major knowledge gaps: (1) the biotic mechanisms by which soil food webs are influenced by fire and, (2) approaches to studying the responses of soil biota to fire in the context of global change. As with much of the soil ecology literature (Coyle et al., 2017), there is a clear gap in our understanding of how higher trophic levels respond to fire (Zaitsev et al., 2016). It is necessary to consider taxa in higher trophic levels and how they interact with primary consumers and the rest of the food web when seeking to understand shifts in ecosystem function to fire. In fact, trophic interactions within the soil food web have been shown as useful predictors for soil organism vulnerability to disturbance (Hedlund et al., 2004). However, our literature review turned up only two studies that considered the effects of fire on Protozoa, and while slightly more studies were found for nematodes and arthropods (Table 2.1), higher trophic levels are underrepresented in the literature in comparison to microorganisms (bacteria, fungi, Archaea). Further, most studies only considered one type of soil organism, and those that did consider more than one did not integrate these findings into a community or food web framework, precluding a strong connection to changes in belowground functioning.

While not the focus of this meta-analysis, an aboveground-belowground linkages framework is a promising approach to improve our understanding of soil biological responses to fire and consequences for ecosystem function. Links between soil organisms and plant communities can be used to predict soil organism vulnerability to disturbance, particularly for soil biota that are directly associated with plants (i.e., mycorrhizae; see Hedlund et al., 2004). Such an aboveground-belowground linkages framework has been evoked as a way to approach restoration of post-fire systems (Kardol and Wardle, 2010). Despite our understanding of the importance of aboveground-belowground linkages and plant-soil feedbacks for understanding community dynamics and ecosystem function (Bardgett and Wardle, 2010), efforts to explain soil biota responses to fire have largely focused on short-term changes to the physical and chemical soil environment. Understanding the consequences of longer term shifts in forest plant communities for soil microbial community responses to fire has been noted as an important research need (Hart et al., 2005), but links to higher trophic levels in soil food webs are seldom discussed.

The majority of the studies included in this meta-analysis only considered the effect of a single fire event on soil biota. While understanding the short-term implications of a single fire on belowground communities is important, the nature of fire regimes (frequency, severity, size, and timing) is shifting as a result of climate change (Flannigan et al., 2013; Flannigan et al., 2009; Moritz et al., 2012). The effect of fire on fungal richness does not differ between single and repeated burns (Dove and Hart, 2017), but distinct fungal communities develop under frequently burned and unburned sites (Oliver et al., 2015). Understanding how fungal communities change under different fire regimes is an important first step. We currently do not have sufficient data to determine the consequences of shifting fire frequency, severity, seasonality, size and duration on the structure and function of the entire belowground community or the physical, chemical and biological interactions that give rise to these responses. Further, fire frequency has important ecosystem consequences for soil C and N stocks (Pellegrini et al., 2018). As with many disturbances, understanding the effect of a single fire on an ecological community and its processes cannot simply be extrapolated additively to multiple, repeat fires. Similarly, fire regimes are not shifting in isolation of other global changes and therefore must be included in investigations of ecosystem responses to global change. In particular, whether fire will render soil communities more or less susceptible to other global changes (e.g., precipitation shifts, warming, nitrogen deposition) remains an open question. When fire is included as a treatment in fully factorial experimental designs investigating other global changes, responses of plant communities and abiotic factors, rather than soil communities, tend to be the focus (e.g. Henry et al., 2006; Koerner and Collins, 2014, but see Allison et al., 2010 and Docherty et al., 2012).

Given the knowledge gaps identified above, we suggest the following avenues for impactful future research at the intersection of fire disturbance ecology and soil biology:

1. Further investigate the effect of fire on soil mesofauna
2. Consider multiple microbial and faunal taxa in a single study and, where possible, the entire soil food web.
3. Identify biotic mechanisms for recovery after fire, both for the soil food web and the interactions

between plant and soil communities, and determine in which biomes and under what conditions biotic or abiotic mechanisms are the most important drivers of soil communities after fire.

4. Investigate how disturbance regimes, rather than single disturbances, restructure belowground communities, and identify consequences for ecosystem processes.
5. Utilize both experimental and observational approaches to explore the interactive effects of fire with other global changes.

Moving forward, soil ecologists could take a more comprehensive view of belowground communities by considering both food web interactions and aboveground-belowground linkages that may influence the response of recovery of soil biota to fire. This approach can be readily applied to other types of disturbance. Studies that link responses of soil communities to changing fire regimes with ecosystem processes such as decomposition and biogeochemical cycling will bring us closer to predicting and quantifying the consequences of climate-induced shifts in fire regimes for ecosystem function.

Chapter 3: Latent dynamic properties: Fire frequency alters soil food web structure and stability, but not biomass and function, in an oak-pine savanna[2]

Introduction

Fire is an important disturbance in many ecosystems because it removes biomass, changes plant community composition, alters resource quantity and quality, and drives the evolution of fire adapted communities (Bond and Keeley, 2005). Climate, land use, and management changes driven by human activity are altering the nature of fire regimes with consequences for ecosystem processes. Climate change has resulted in conditions that promote greater fire frequency and severity including increased ignition via lightning strikes (Romps et al., 2014), excess resources to burn due to elevated net primary productivity (NPP) (Nemani et al., 2003) and accumulation of fuel and understories due to past fire suppression efforts (Bowman et al., 201), and warmer, drier atmospheric conditions (IPCC, 2014) that result in longer fire seasons (Westerling et al., 2006). Such changes are affecting the frequency, severity, timing, seasonality, size, and duration of fires around the globe (Moritz et al., 2012; Flannigan et al., 2013). Given the importance of fire for structuring and maintaining ecosystems, understanding the consequences of changing fire regimes for ecosystem structure, function, and dynamics is a clear research need.

Soils are a key component of ecosystems because they connect aboveground and belowground systems by regulating water, cycling nutrients, and providing structural support for plants (Binkley and Fisher, 2012). Fires have profound effects on the physical, chemical, and biological components of soils (Certini, 2005). Fires alter the physical soil environment through loss of soil structure and changes to bulk density (Mataix-Solera et al., 2011), changes in albedo and moisture dynamics (Beringer et al., 2003; Jin et al., 2012), water repellency (DeBano, 2000; MacDonald and Huffman, 2004), and increased soil

[2] Research conducted in collaboration with P. Coppick, G.W.T. Wilson, M.F. Cotrufo, and J.C. Moore

temperatures (Certini, 2005). Fires also affect the biology of soils through altering soil microbial and faunal biomass, abundance, and community composition (Pressler et al., 2018; Hart et al., 2005). Further, fires impact the chemical soil environment by changing soil organic matter quantity and quality through combustion (González-Pérez et al., 2004; Knicker, 2011) and erosion (Rumpel et al., 2009; Shakesby, 2011; Cotrufo et al., 2016), alterations to mineralogy (Ulery et al., 1996; Yusiharni and Gilkes, 2012), and an increase in pH (Certini, 2005). Fire can alter soil to such a degree that it has even been proposed as a soil forming factor (Certini, 2014).

Soil pH plays an important role in controlling biomass, abundance and diversity of soil biological communities (Räty and Huhta, 2003; Rousk et al., 2010). Soil pH increases after fire due to the denaturation of organic acids, the release of base cations, and the addition of pyrogenic carbon (PyC) to soils (Certini, 2005; Knicker, 2011). PyC is the product of incomplete combustion of organic matter (Bird et al., 2015). PyC is characterized by a polycyclic aromatic hydrocarbon structure that is chemically recalcitrant and can remain in the soil for centuries to millennia (Bird et al., 2015). PyC can affect the soil environment by altering water and nutrient dynamics (Liang et al., 2006; Knicker, 2007) and by serving as a substrate for soil microbial communities (Santos et al., 2012). As fire frequencies increase, soil pH will increase and PyC is added to the soil. Such changes to the soil environment may have consequences for the structure, function, and stability of the soil food web.

The effects of fire on the physical and chemical soil environment are well documented, but our understanding of how fire affects belowground communities is still developing (Pressler et al., 2018). Soil food webs mediate many important ecosystem processes including decomposition and nutrient cycling (Binkley and Fisher, 2012). Soil microbial communities (bacteria, Archaea, and fungi) directly control decomposition through the catabolic mineralization of soil organic matter, while soil fauna play a more indirect role in fragmenting litter and controlling microbial population turnover through predation (Paul, 2014). Most evidence suggests that soil microorganisms are negatively affected by fire, while soil fauna are more resistant, however, we have a limited view of how soil fauna respond to fire as these taxa are not well represented in the literature (Zaitsev et al., 2016; Pressler et al., 2018). Studying the effect of fire on

microbial communities, without considering fauna, and vice versa reveals an incomplete picture of soil biological responses to fire, but most studies do not integrate across taxa or in a community or food web framework (Pressler et al., 2018). Increased fire frequency is likely to have negative consequences for soil food web recovery after fire as most available studies suggest that biomass, abundance and diversity of soil communities can remain altered up to a decade or more after fire (Malmstrom et al. 2008; Pressler et al., 2018; Dooley and Treseder, 2012; Dove and Hart, 2017). If fire frequencies increase across this decadal threshold, soil food webs may experience long-lasting shifts in community structure and function.

Few studies have considered soil food web resilience and stability in the context of fire, despite the recognition that soil food web stability is vulnerable to other perturbations including agricultural management practices, land use and drought (Moore 1994; de Vries et al. 2012). At the same time, much of the soil ecology literature has focused on the effects of single fires, rather than fire regimes, despite the fact that fire frequency drives soil carbon (C) and nitrogen (N) stocks in broadleaf forests and savanna grasslands (Soong and Cotrufo, 2015; Pellegrini et al., 2018). Given that soil communities mediate soil C and N dynamics, understanding their responses to changing fire regimes, rather than single fires, is needed. Predicting the impact of shifting disturbance regimes on any ecological community requires that we go beyond simply measuring the resistance of organismal biomass and functions to a single disturbance event, and instead consider the dynamic properties these systems including stability and resilience in the context of disturbance regimes.

To address these knowledge gaps, we take a fire-regime perspective by investigating the effects of long-term controlled fire frequency on soil food webs. To understand how fire regimes affect soil food web structure, function, and stability, we sampled a long-term fire return interval (FRI) experiment during peak aboveground biomass, rather than immediately after the fire. The following questions drove our research: (1) How does FRI affect the soil environment? (2) How does FRI alter soil food web biomass, structure, function, stability, and resilience? (3) What are the relationships between the soil environment and soil food web structure, function, stability, and resilience across the FRI gradient?

We hypothesize that FRI will alter the physical and chemical soil environment with consequences for soil food web biomass, structure, function, and stability. We hypothesize that pH will decrease with FRI because fires increase soil pH (Certini 2005). We hypothesize that PyC content will be highest at intermediate FRIs where time between fires allows biomass to accumulate, but fires still occur frequently enough to produce substantial amounts of PyC. With respect to the soil community, we hypothesize that the biomass of fungi and their consumers will increase with FRI relative to bacteria and their consumers because fungi are more sensitive to fire than bacteria (Pressler et al., 2018). Given this, we hypothesize greater C and N mineralization rates as a result of higher bacterial biomass at low FRIs. We hypothesize that soil food web complexity will increase with FRI because fewer disturbances allow for more trophic interactions to develop. We hypothesize that all soil food webs along the FRI gradient are stable overall, but that their degree of instability and resilience differs by fire frequency. We hypothesize that soil food webs with intermediate FRIs (2-year and 4-year) are less stable and less resilient than food webs at either low (1-year) or high (30-year) FRIs. Finally, we hypothesize clear relationships between soil food web structure, function, stability, and resilience and soil pH because it is an important driver of soil microbial and faunal abundance and diversity (Rousk et al., 2010), and PyC because it alters the soil physical and chemical environment (Knicker, 2007) and some bacteria may use PyC as a substrate in soils even despite low soil concentrations (Santos et al., 2012; Soong and Cotrufo, 2015).

Materials and Methods

Study system and experimental design

Our study took place at a long-term FRI manipulation experiment at the Pushmataha Forest Habitat Research Area in Clayton, Oklahoma (Lat: 34.527, Long: -95.35). Dominant plant species at the site include *Pinus echinata*, *Quercus stellata*, and *Carya tomentosa* in the overstory and *Andropogon gerardii*, *Schizachyrium scoparium*, *Panacium spp.*, *Carex spp.*, and *Scleria spp.* in the understory (Feltrin et al., 2016). Soils include the Carnasaw (fine, mixed, semi active, thermic, Typic Hapludults)

and Stapp Series (fine, mixed, active, thermic Aquic Hapludults) with stony fine sandy loam textures (Feltrin et al., 2016). The experiment was established in 1984 in an oak-pine savanna ecosystem. The experimental design consists of 8,000 - 16,200 m^2 treatment plots with three replicates of the following FRI treatments for a total of 12 plots in a completely randomized design: 1-year, 2-year, 4-year, and 30-year (non-burned since 1984). Prior to initiating the fire treatments, all experimental plots were harvested for pine and thinned. The appropriate plots are burned in March each year.

Soil sampling

On September 18, 2015, we collected soil samples from all 12 of the treatment plots. We collected 2 soil cores (5.5 cm diameter) in 6 sampling locations that were randomly selected from 10 subplots along transects for a total of 144 soil cores to a depth of 10 cm. When we were unable to reach the 10 cm depth due to the stony nature of the soils, we recorded the depth of the core and adjusted our biomass calculations accordingly. One of each paired soil core was kept intact for arthropod extraction (n = 72). The other cores for nematode, protozoa, bacteria, fungi, and gravimetric soil moisture were bulked together with one other randomly selected subplot in the same treatment plot for a total of 3 samples per plot (n = 36). Additional soil samples were taken to 10 cm depth for phospholipid and neutral lipid fatty acid analyses following the same subplot bulking approach. All non-arthropod samples were sieved to 2 mm before further analysis. For laboratory procedures that could not be done on site (pH, C, N, and PyC), sieved samples were further bulked to 1 sample per plot (n = 12) in order to meet USDA regulatory requirements for transporting quarantined soil.

Physical & chemical soil analyses

Gravimetric soil moisture was collected from sieved samples by oven drying at 105 ºC for 48 hours. Pulverized, oven dried soil samples were analyzed on the LECO True-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA) for %C and %N. We quantified soil bulk density using the soil core method on intact

cores used for microarthropod extraction with rocks removed. Soil pH was measured on a 10 g subsample in a 1:1 soil to water slurry using a Thermo Scientific Expandable Ion Analyzer (EA 940).

Pyrogenic carbon analysis

Pyrogenic carbon (PyC) was quantified using the Benzene Polycarboxylic Acid (BPCA) technique following Wiedemeier et al. (2013) and described in Boot et al. (2014). Briefly, polycyclic aromatic hydrocarbons in 150 - 300 mg of ground soil samples were oxidized with 70% nitric acid for 8 hours at 170 ºC to liberate BPCAs. The samples were filtered through ashless cellulose filters prior to adding phthalic acid as an internal standard. The solution was then filtered through a cation exchange resin, freeze dried, and reconstituted before quantification. BPCAs were quantified using High Performance Liquid Chromatography and a photo diode array detector (Agilent 1100 Series). The BPCAs were separated along a reversed stationary phase column (Waters X-Bridge C18, 3.5um particle size, 2.1mm x 150 mm) using a gradient separation of 25 mL L-1 85% orthophosphoric acid and acetonitrile. Individual BPCA peaks were quantified from a 4-point standard calibration curve of benzenetricarboxylic acids (B3CA: hemimellitic acid, trimellitic acid, trimesic acid), benzenetetracarboxylic acid (B4CA: pyromellictic acid), benzenepentacarboxylic acid (B5CA), and benzenehexacar-boxylic acid (B6CA: mellitic acid).

Soil biota extractions

Microarthropods and nematodes

Microarthropods were heat-extracted from intact soil cores using a modified-Tullgren funnel method (Moore et al., 2000). Microarthropods were collected into 70% ethanol and identified into taxonomic functional groups following Moore et al. (1988). Nematodes were extracted from 2 mm sieved soil samples (20 g) using the Baermann funnel technique (Baermann, 1917). Samples remained in the apparatus for 3 days before nematodes were collected and preserved with formalin. Nematodes were

counted and identified into broad functional groups based on their feeding morphology (Yeates and Coleman, 1982).

Protozoa

Protozoa were extracted from 2 mm sieved soil (10 g) and quantified using the most probable number technique (Darbyshire et al., 1974). Samples were mixed with 90 mL of protozoa growth media and serially diluted by tenfold dilutions to 10-6 mL. Four 0.5 mL subsamples from each dilution were pipetted into consecutive wells of a 24-well tissue culture plate. The protozoa were fed a bacterial mixture of *Enterobacter cloacae*, *Citrobacter freundii*, *Pseudomonas putida*, and *Comamonas testosteroni* (Baas et al., 2016) and incubated at 14 ºC for 5 days. Presence and absence of flagellates, amoebae, and ciliates were recorded for each well. Total protozoan abundance was estimated with the US EPA Most Probable Number estimate program (U.S. EPA, 2013).

Bacteria and fungi

Bacteria and fungi were extracted from 2 mm sieved soil (5 g) and quantified using epiflourescent microscopy (Bloem, 1995), phospholipid fatty acids (PLFAs; (Zelles, 1999)) and neutral lipid fatty acids (NLFAs; Olsson et al. 1995). For the epiflourescent assays, the sample was blended with 45 mL of sterile deionized water and aliquoted (10 ul) onto a 10-well slide. Bacteria slides were stained with 5-(4,6 dichlorotriazin-2-yl) aminoflourescein. Fungi slides were stained with calcofluor fluorescent brightener (Frey et al., 1999). Bacteria and fungi abundance was quantified on an Olympus Photomicrographic Microscope System with a reflected light fluorescent attachment at 490 nm for bacteria and 334-365 nm for fungi. Total fungal biomass was scaled to active biomass (10%) following Ingham & Klein (1984).

For the PLFA and NLFA assays, samples (5 g) were freeze dried and finely ground prior to phase separation. Lipids were extracted from the sample by mixing the soil with a phosphate buffer, methanol, and chloroform, respectively and separating the mixture by centrifugation. Phospholipids, neutral lipids, and glycolipids were separated by solid phase extraction by eluting with chloroform, acetone and

methanol. Phospholipids were hydrolyzed and fatty acids were methylated. The methylated fatty acids were extracted with hexane and evaporated under nitrogen at 37 ºC. Quantification of fatty acids were performed on an Agilent 7890A gas chromatograph with an Agilent 5975 series mass selective detector. Following Olsson et al. (1995) and Cobb and Wilson (2018), the following PLFA and NLFA biomarker associations were used. PLFA biomarkers for gram-positive bacteria were i-15:0, a-15:0, i-17:0, and i-16:0; and for gram-negative bacteria were 16:1w7, cy19:0, 2-OH 16:0, 2-OH 14:0, 3-OH 14:0, cy17:0, 18:1w9. PLFA biomarkers used for arbuscular-mycorrhizal fungi (AMF) were 16:1w5c, 20:1w9, and 22:1w13; and for saprotrophic fungi (SAP) were 18:2w9,12c and 18:1w9c. NLFA biomarkers used for AMF were 16:1w5c; and for SAP were 18:2w9,12c and 18:1w9c.

Modeling soil food web structure, function, and stability

Soil organism abundance estimates were converted to g of biomass C using known weights of soil organisms from the literature and a 50% conversion from total biomass to biomass C as described in Andrés et al. (2016). We used bulk density to convert biomass estimates to a per square meter scale. Bulk density estimates were variable across the subsamples within plots due to high rock content but did not differ significantly between the treatments ($p = 0.10$). We used average bulk density for each plot for the biomass conversions.

We used known feeding preferences and trophic interactions to create a connectedness food web for the soil community (Hunt et al., 1987). We calculated the number of trophic links and mean and maximum food chain lengths using the Cheddar package in R (Hudson Lawrence et al., 2012). We then calculated connectance, the proportion of realized trophic interactions within a food web, as

[1] $$C = 2L / S(S-1)$$

where C is connectance (sensu May, 1972), L is the number of links in the food web, and S is the number of functional groups (Moore and de Ruiter, 2012). We also calculated the linkage density, the average number of links per functional group, as L/S.

We used the connectedness food webs described above to parameterize an energetic food web model to investigate the effect of fire frequency on soil food web function and stability (Moore and de Ruiter, 2012). We ran 1000 Monte Carlo simulations of the model in R to assess the variability across model runs. We removed functional groups that were not found in field samples from the connectedness web for each treatment replicate. For each simulation, the model randomly sampled from gamma distributions defined by the means and standard deviations of soil biota biomasses from field samples. The model uses biomass estimates from the gamma distribution, assimilation efficiencies, production efficiencies, death rates, C:N ratios, feeding preferences, and trophic interactions to estimate C and N mineralization from each functional group within the soil food web as described in Hunt et al. (1987), Moore and de Ruiter (2012), and Andrés et al. (2016). Death rates are based on natural death rates and do not explicitly incorporate the fire-induced mortality. We did not estimate root biomass or detritus but rather assumed that these resources were not limiting and assigned theoretical values of 300 g C m^{-2} and 4000 g C m^{-2}, respectively.

We created food web interaction matrices to assess the stability and resilience of soil food webs as described in de Ruiter et al. (1995), Moore and de Ruiter (2012), and Andres et al. (2016). The elements in the matrix represent the interactions strengths between functional groups and quantify the effect of each functional group on the dynamics of the other near equilibrium. From this trophic perspective, prey have a positive per biomass effect on the dynamics of predators, whereas predators have a negative per biomass effect on the dynamics of prey. We assumed that if all the eigenvalues of the interaction matrix had negative real parts, then the food web was stable near equilibrium (May 1973). We used the diagonal matrix elements, representing intraspecific interaction strengths, to calculate the minimum intraspecific interaction needed to maintain matrix stability (minimum s-value, Neutel et al., 2002). We estimated the relative stability of each food web by comparing the minimum s-value, where the smaller the value, the more stable the food web matrix. We also estimated resilience for each food web by calculating the return time as the negative reciprocal of the dominant eigenvalue ($RT = -1/\lambda$ max, when $\lambda < 0$) (Pimm and

Lawton, 1977). Last, we compared the proportion of model runs that resulted in dominant eigenvalues with imaginary components which indicates oscillation around equilibrium and can be interpreted as instability.

Statistics

Prior to conducting statistical analyses in R (R Core Team, 2017), we averaged values across pseudoreplicates (n = 72) to analyze data with only three true field replicates (n = 12). We conducted a Type III ANOVA and pairwise comparisons to determine differences between FRI treatments for soil biota biomass estimates, PLFAs, NLFAs, gravimetric soil moisture, bulk density, C, N, pH, BPCA, soil food web structural metrics, C and N mineralization estimates, average minimum S, and average return times. We conducted the same statistical tests on soil biota biomass estimates by trophic groups (bacterial consumers, fungal consumers, herbivores, and predators) and the proportion of total C flux through the bacterial, fungal, and herbivore energetic pathways. We conducted Komogorov-Smirnov tests to determine whether the distributions of minimum S and return time model outputs differed with FRI treatment.

We conducted multiple regression analyses to evaluate relationships between soil chemical characteristics and soil food web structure, function, and stability across the FRI gradient. Response variables were soil food web structural metrics (connectance and linkage density), functional metrics (modeled C and N mineralization), and stability metrics (minimum S and return time). Based on our hypotheses, the predictor variables were FRI, soil pH, total BPCA content, and their interactions. We used backwards model selection based on AIC to determine the best suited model and strongest predictors using dredge() from MuMIN package in R (Barton, 2018). We then removed FRI from the model to determine whether pH or PyC were stronger predictors of each response variable across the FRI gradient.

Results

Physical and chemical soil properties

Soil moisture ($p = 0.18$) and bulk density ($p = 0.10$) did not vary significantly across the FRI treatments (Table 3.1). There was a slight trend towards higher bulk density in the 1-year FRI treatment relative to the 4-year and 2-year FRI treatment, but these differences were not statistically significant ($p = 0.10$, 0.16 respectively; Table 3.1). Soil C (%) varied significantly across the FRI gradient ($p = 0.02$; Table 3.1). The greatest soil C was in the 2-year and 4-year FRI treatments, with the lowest soil C content found in the 1-year FRI treatment (Table 3.1). Soil N (%) varied across the FRI gradient following a similar pattern to soil C ($p = 0.04$; Table 3.1). There was a trend towards higher soil N in the 2-year and 4-year FRI treatments than the 1-year treatment, but these differences were not statistically significant at the $p < 0.05$ level ($p = 0.11$, 0.05 respectively; Table 3.1)).

Fire significantly increased pH at all FRIs relative to the 30-year plots ($p = 0.02$, Table 3.1), but the magnitude of the increase did not differ between the 1-, 2-, and 4-year FRI treatments (Table 3.1). PyC content was significantly different across the FRI treatments ($p = 0.007$) with the highest amount of PyC at the intermediate FRI (2-year) and the lowest at low (1-year) and high (30-year) FRIs (Table 3.1). This pattern remains when considered as a fraction of total soil mass (g BPCA-C kg^{-1} dry mass; Table 3.1) and as a fraction of soil organic carbon (g BPCA-C kg^{-1} organic C; Table 3.1).

Table 3.1. Physical and chemical characteristics of the soil across the FRI treatments. Data are means ± standard error (n = 3). Letters signify differences at the $p < 0.05$ level.

FRI	**1**	**2**	**4**	**30**
Soil Moisture (%)	10.9 ± 0.76 a	10.8 ± 0.93 a	11.5 ± 0.51 a	9.3 ± 0.15 a
Bulk Density ($g\ cm^{-3}$)	0.87 ± 0.06 a	0.65 ± 0.11 a	0.62 ± 0.02 a	0.69 ± 0.03 a
C (%)	2.66 ± 0.24 a	4.48 ± 0.62 b	4.87 ± 0.37 b	3.94 ± 0.24 ab
N (%)	0.17 ± 0.02 a	0.23 ± 0.02 a	0.24 ± 0.01 a	0.19 ± 0.01 a
pH	4.80 ± 0.19 a	4.89 ± 0.13 a	4.78 ± 0.06 a	4.20 ± 0.09 b
Total BPCA-C ($g\ kg^{-1}$)	0.50 ± 0.08 a	1.15 ± 0.13 b	0.84 ± 0.08 ab	0.50 ± 0.13 a
Total BPCA-C ($g\ kg^{-1}$ organic C)	18.41 ± 1.52 ab	26.34 ± 3.68 a	17.31 ± 1.07 ab	12.33 ± 2.43 b

Soil biota biomass

Biomass did not differ significantly across FRI for total bacteria, total fungi, Protozoa, nematodes, or arthropods, with the exception of a slight increasing trend of fungal biomass at the 4-year FRI treatment ($p = 0.11$; Table 3.2). Bacterial consumer ($p = 0.99$), herbivore ($p = 0.52$), and predator ($p = 0.52$) biomass did not differ between FRI treatments (Table 3.2). Fungal consumer biomass did differ between FRI treatments ($p = 0.008$) where high FRIs (4-year and 30-year) had higher fungal consumer biomass than low FRIs (1-year and 2-year; Table 23.).

Table 3.2. Soil biota biomass (g C m^{-2}) across the FRI treatments. Bacteria and fungi biomass estimates are from epiflourescent microscopy method. Data are means ± standard error (n = 3). Letters signify differences at the $p < 0.05$ level.

FRI	**1**	**2**	**4**	**30**
Taxa				
Bacteria	0.003 ± 0.0006 a	0.003 ± 0.0006 a	0.003 ± 0.0006 a	0.002 ± 0.0003 a
Fungi	3.25 ± 0.89 a	3.32 ± 0.51 a	4.64 ± 0.32 a	3.21 ± 0.31 a
Protozoa	0.000007 ± 0.000002 a	0.00001 ± 0.000005 a	0.00002 ± 0.000005 a	0.00001 ± 0.000002 a
Nematodes	0.0003 ± 0.00003 a	0.0004 ± 0.000 a	0.0005 ± 0.0002 a	0.0004 ± 0.00023 a
Arthropods	0.02 ± 0.02 a	0.09 ± 0.08 a	0.01 ± 0.007 a	0.008 ± 0.002 a
Functional Groups				
Bacterial consumers	0.004 ± 0.0007 a	0.004 ± 0.002 a	0.004 ± 0.002 a	0.004 ± 0.0003 a
Fungal consumers	0.002 ± 0.0001 a	0.002 ± 0.0005 a	0.003 ± 0.0005 ab	0.005 ± 0.0007 b
Herbivores	0.0006 ± 0.00007 a	0.001 ± 0.0006 a	0.001 ± 0.0003 a	0.0005 ± 0.00004 a
Predators	0.02 ± 0.02 a	0.09 ± 0.08 a	0.01 ± 0.006 a	0.005 ± 0.0008 a

PLFAs did not differ between FRI treatments for arbuscular mycorrhizal fungi (AMF; $p = 0.93$), saprotrophic fungi ($p = 0.61$), gram-negative bacteria ($p = 0.47$) or gram-positive bacteria ($p = 0.69$). NLFAs did differ significantly between FRI treatments for AMF ($p = 0.006$) but not saprotrophic fungi ($p = 0.08$). AMF NLFAs were highest at the low FRIs (1-year and 2-year; Figure 3.1a) and lowest at the high FRI (4-year and 30-year; Figure 3.1a). A similar trend was observed for saprotrophic fungi, but the results were not statistically significant (Figure 3.1b).

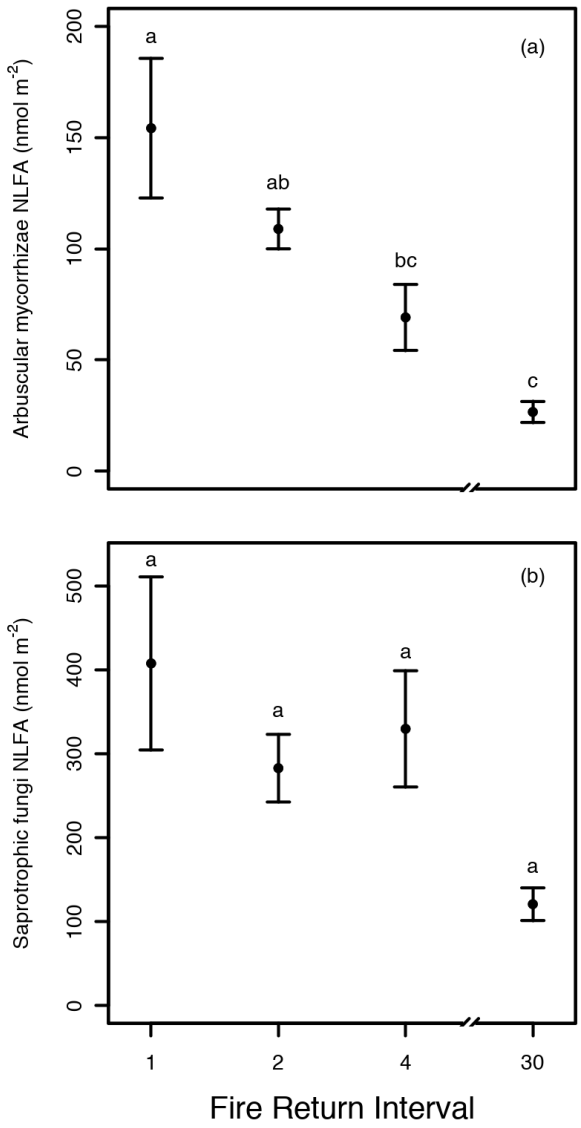

Figure 3.1. Neutral lipid fatty acid estimates (nmol m^{-2}) of arbuscular mycorrhizal (a) and saprotrophic (b) fungi. Data are means ± standard error (n=3). Letters indicate significant differences at the $p < 0.05$ level.

Soil food web structure

Soil food web trophic structure was most complex at the 30-year FRI (Figure 3.2). Collembola were absent in all 4-year FRI replicates and two replicates of 1- and 2-year FRIs; predatory mites were absent in all 1-, and 2-year FRI replicates. The number of functional groups did not differ significantly across the FRI gradient ($p = 0.59$; Table 3.3). Food web connectance varied significantly across the FRI gradient ($p = 0.003$; Table 3.3). Connectance was greatest in the 30-year FRI treatment and lowest in the 1-year and

2-year treatments (Table 3.3). Linkage density varied across the FRI gradient (p = 0.02; Table 3.3). Linkage density was highest at the 30-year FRI treatment and lowest at the 1-year and 2-year FRI treatments (Table 3.3). The number of trophic links (p =0.09), mean food chain length (p = 0.64), and maximum food chain length (p = 0.40) were not significantly different across the FRI gradient (Table 3.3).

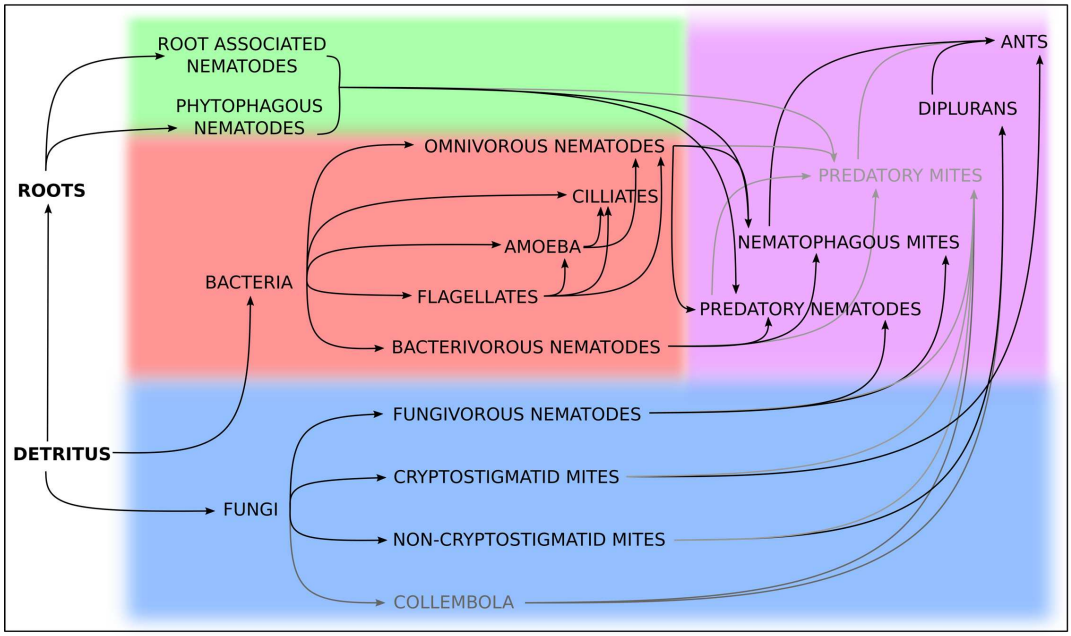

Figure 3.2. Soil food web trophic structure. Colors represent energetic pathways: herbivore pathway in green, bacterial pathway in red, fungal pathway in blue, predators in purple. Functional groups absent from burned food webs are shown in gray. All groups are present in all 30-year FRI replicates. Collembola are absent in all 4-year FRI replicates and two replicates of 1- and 2-year FRIs; Predatory mites are absent in all 1-, and 2-year FRI replicates.

Table 3.3. Soil food web structural metrics for each FRI treatment. Data are means ± standard error (n=3). Letters signify differences at the $p < 0.05$ level. Functional groups (S) is the number of functional groups, connectance (C) is the proportion of realized links, linkage density is the average number of feeding links per species, trophic links (L) is the number of trophic links, mean and max food chain lengths (FCL) are the average and maximum number of length of all food chains, respectively.

FRI	**1**	**2**	**4**	**30**
S	16.7 ± 0.88 a	17.0 ± 1.15 a	18.0 ± 0.58 a	18.0 ± 0.58 a
C	0.21 ± 0.001 a	0.21 ± 0.001 a	0.23 ± 0.01 ab	0.26 ± 0.006 b
LD	1.63 ± 0.09 a	1.65 ± 0.11 a	1.93 ± 0.17 ab	2.18 ± 0.03 b
L	35.0 ± 2.91 a	27.3 ± 3.76 a	28.3 ± 4.16 a	39.3 ± 1.76 a
Mean FCL	4.92 ± 0.29 a	5.24 ± 0.13 a	5.22 ± 0.45 a	5.50 ± 0.26 a
Max FCL	7.3 ± 0.33 a	7.3 ± 0.33 a	8.0 ± 0.58 a	8.3 ± 0.33 a

Soil food web function

We found no evidence for differences in soil food web C ($p = 0.73$) and N ($p = 0.74$) mineralization with FRI treatment. C mineralization estimates ranged from 7.75 to 26.25 g C m^{-2} yr^{-1} and N mineralization estimates ranged from 0.77 to 2.63 g C m^{-2} yr^{-1}. C and N mineralization did not differ with FRI treatment in the bacterial ($p = 0.96$, 0.95), fungal ($p = 0.72$, 0.72), herbivore ($p = 0.51$, 0.51), or predator ($p = 0.49$, 0.49) energetic pathways, respectively.

We did not find any evidence for differences in the total flux of C through the soil food web with FRI treatment ($p = 0.73$). Total C flux estimates ranged from 4022 to 4075 g C m^{-2} yr^{-1}. Total C flux did not differ with FRI treatment in bacterial ($p = 0.92$), fungal ($p = 0.63$), or predator ($p = 0.55$) energetic pathways. However, total C flux through the herbivore pathway did differ with FRI treatment ($p = 0.05$), with a 4x greater amount of C flux in the 4-year FRI than the 1-year ($p = 0.05$). Similarly, the proportion of total C flux through the herbivore energetic pathway relative to bacterial and fungal pathways was significantly greater in the 4-year FRI than the 1-year ($p = 0.009$), but the relative C flux through the herbivore pathway was small compared to the other energetic pathways (1.7% vs. 45-52%).

Soil food web stability and resilience

All model runs for all food webs resulted in eigenvalues with negative real parts, indicating that the food webs are stable near equilibrium. We found no differences between average return times ($p = 0.61$) across FRI treatments but did find significant differences between average minimum S values ($p = 0.03$). Average minimum S was lowest at the 2-year FRI, and highest at the 4-year FRI. Kolmogorov-Smirnov tests for differences in nonparametric distributions revealed significant differences between all minimum S ($p < 0.0001$; Figure 3.3a) and return time ($p < 0.0001$; Figure 3.3b) distributions for all FRI treatments. The proportion of model runs with eigenvalues with imaginary components was 0.007 and 0.003 for the 2-, and 4-year FRI treatments, compared to 0 and 0.001 for the 30- and 1-year FRI treatments, respectively.

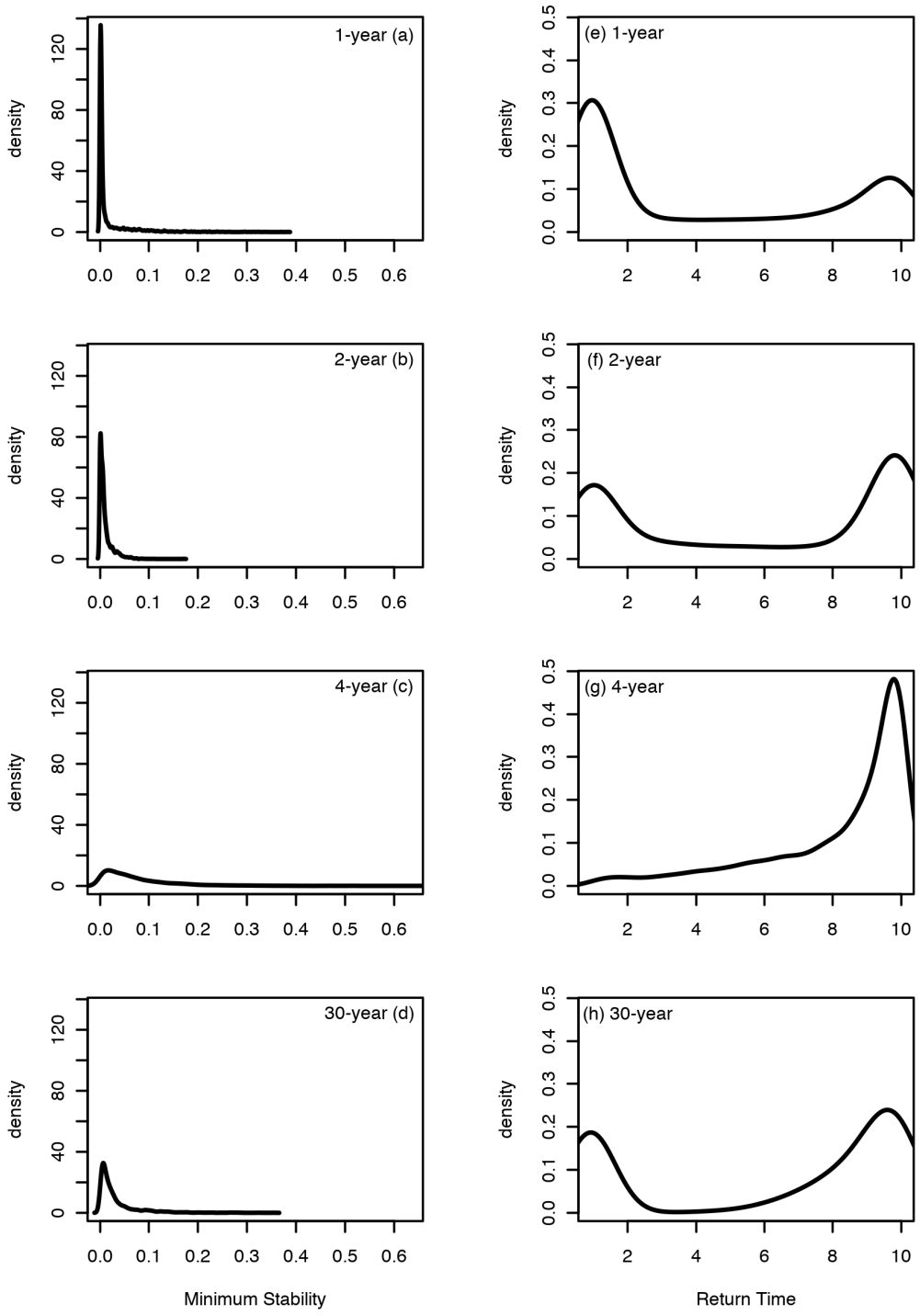

Figure 3.3. Density distributions of soil food web minimum stability (a-d) and return time (e-h) for 1-year (a,e), 2-year (b,f), 4-year (c,g), and 30-year (d,h) fire return intervals. Distributions display data from 1000 model simulations for each of three replicates within fire return interval treatments.

Relationships between soil food webs and the soil environment

The connectance regression models with the lowest AIC included only FRI (AIC = -66.8), FRI and pH (AIC = -65.4), and FRI and PyC (AIC = -64.8). When FRI was removed from the model, pH ($p = 0.18$) was a stronger explanatory variable of connectance in this ecosystem than PyC ($p = 0.93$). When considered across the entire FRI gradient, connectance significantly decreased as pH increased ($R2 = 0.60$, $p = 0.003$; Figure 3.4a) while PyC and connectance were not related ($R2 = 0.11$, $p = 0.28$; Figure 3.4b). The linkage density regression models with the lowest AIC included only FRI (AIC = -0.1), FRI and pH (AIC = 1.3), and only pH (AIC = 1.4). When FRI was removed from the model, pH ($p = 0.06$) was a stronger predictor of linkage density than PyC ($p = 0.29$). Across the FRI gradient, linkage density decreased with pH ($R2 = 0.48$, $p = 0.01$; Figure 3.4c) whereas PyC and linkage density were not related ($R2 = 0.09$, $p = 0.33$; Figure 3.4d).

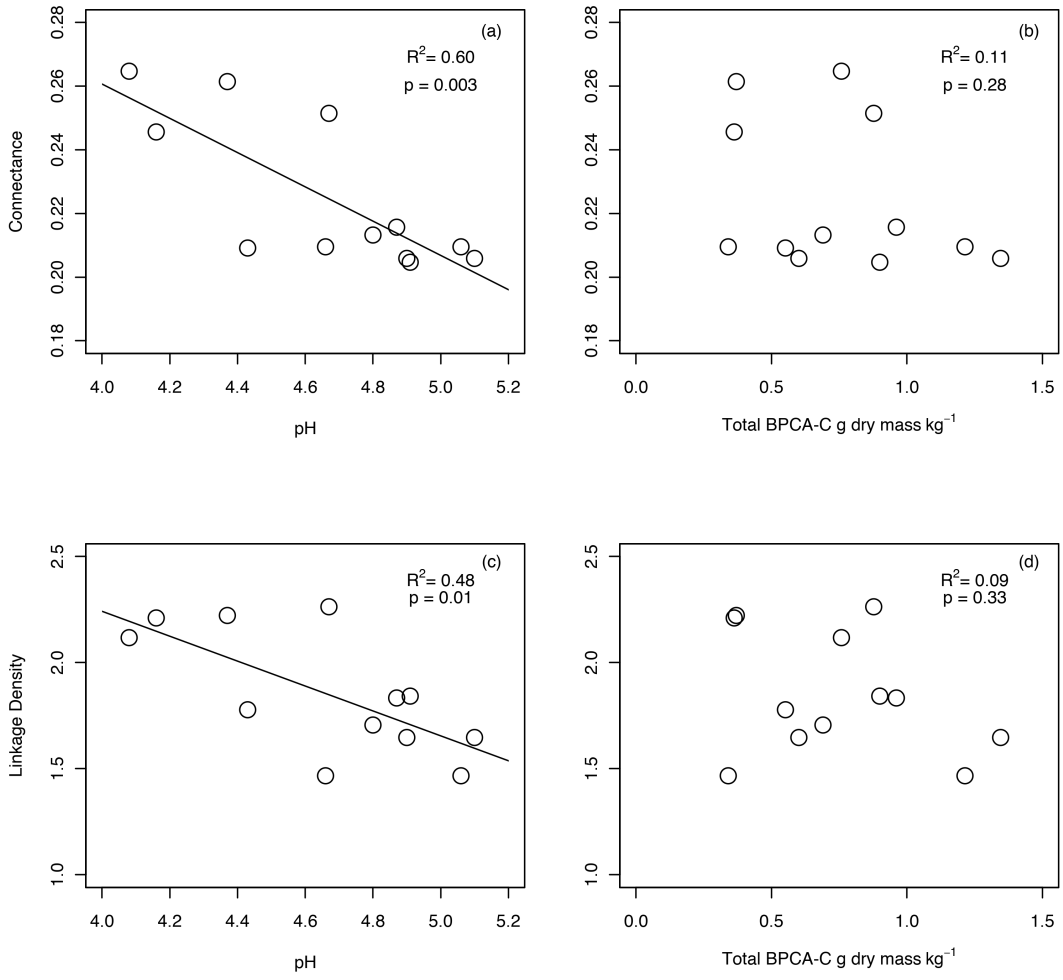

Figure 3.4. Soil food web connectance (a,b) and linkage density (c,d) as a function of soil pH (a,c) and pyrogenic carbon content (b,d) (n = 12).

The total C mineralization regression models with the lowest AIC included FRI, pH and their interaction (AIC = 70.7), FRI and pH (AIC = 71.2) and FRI, pH, PyC and the interaction of pH and PyC (AIC = 71.7). When FRI was removed from the model, neither pH (p = 0.96), PyC (p = 0.81), nor their interaction (p = 0.81) were significant predictors of C mineralization. The total N mineralization regression models with the lowest AIC included FRI, pH and their interaction (AIC = 15.6), FRI and pH (AIC = 16.0), and FRI, pH, PyC and the interaction of pH and PyC (AIC = 16.5). When FRI was removed from the model, neither pH (p = 0.96), PyC (p = 0.81) nor their interaction (p = 0.81) were significant

predictors of N mineralization. The total C flux through the food web regression models with the lowest AIC included FRI, pH and their interaction (AIC = 95.9), FRI and pH (AIC = 96.3) and FRI, pH, and PyC (AIC = 96.7). When FRI was removed from the model, neither pH ($p = 0.91$), PyC ($p = 0.86$) or their interaction ($p = 0.85$) were significant predictors of total C flux.

The minimum stability regression model with the lowest AIC was the full model including FRI, pH, PyC and all interactions (AIC = -82.5). When FRI was removed from the model, neither pH ($p = 0.89$), PyC ($p = 0.99$), nor their interaction ($p = 0.99$) were significant predictors of minimum S. The return time regression model with the lowest AIC included FRI, pH, PyC, and the interactions of FRI and pH, and pH and PyC (AIC = 64.4). When FRI was removed from the model, neither pH ($p = 0.47$), PyC ($p = 0.30$), nor their interaction ($p = 0.33$) were significant predictors of return time.

Discussion

Fire frequency alters soil chemical environment

Fire changes the physical and chemical soil environment to such a degree that it has been proposed as a soil forming factor (Certini, 2014). In this oak-pine savanna, fire frequency shifted the chemical nature of the soil through pH and soil organic matter composition. We found evidence for significant alterations of soil pH, soil C, and PyC due to fire (Table 3.1). Our finding that fire increases soil pH (Table 3.1) confirms our hypothesis and is well established in the literature (Certini, 2005). However, we expected the degree to which fire increases pH to depend on fire frequency as observed in other oak savannas (Tester, 1989). Our data suggest that there may be a saturation point between 4 and 30-year FRIs beyond which additional fires do not continue to increase soil pH, but without data from intermediate FRIs, this conclusion remains speculative. Recent evidence suggests that fire frequency drives substantial losses in soil C and N in savanna grasslands on decadal timescales (Pellegrini et al., 2018). However, annual burning in temperate tallgrass prairie does not affect soil C and N (Kitchen et al., 2009). Our results align with data from the tallgrass prairie. We did not find any differences in soil C or N between annually

burned and infrequently burned (30-year FRI) sites (Table 3.1). Fire frequency did not affect soil N at intermediate FRIs, but did increase soil C at the 2- and 4-year FRIs (Table 3.1). Our data confirmed our hypothesis that PyC would be greatest at intermediate FRIs (Table 3.1). Fire frequency is a known local driver of PyC content in soils (Czimczik and Masiello, 2007). Low FRIs do not allow for enough biomass accumulation to produce substantial PyC and allow for combustion of PyC in subsequent fires (Santin et al. 2013). At high FRIs, fires simply do not occur frequently enough to produce substantial amounts of PyC that accumulates in soils (Czimczik and Masiello, 2007). Similar patterns have been observed in boreal forest mineral soils (Czimczik et al., 2004).

With respect to the soil physical environment, we found no differences in soil moisture or bulk density between the FRI treatments (Table 3.1). Both bulk density and soil moisture are controlled by soil organic matter with bulk density decreasing and soil moisture increasing with soil organic matter content (Brady and Weil, 2010). The loss of soil organic matter to combustion during fire can have negative effects on water storage capacity and bulk density (Úbeda and Outeiro, 2009). Further, soil moisture and pore space play important roles in controlling soil microbial and faunal biomass and activity (Paul, 2014). However, our sampling date was 6 months after the fire and soil moisture was more likely driven by recent precipitation rather than fire frequency. Given that soil moisture and bulk density did not differ between FRI treatments, we conclude that the effects of FRI on soil food webs were in fact due to fire, not soil moisture or bulk density.

Soil biota biomass is conserved across the fire frequency gradient

Single fires can have substantial negative consequences for soil organismal abundance, biomass, and diversity and can alter soil communities for decades (Treseder et al., 2004, Malmström, 2012, Pressler et al., 2018). Soil microorganisms (bacteria, fungi, and Archaea) often decrease in abundance and biomass after fire, with fungi being more sensitive to fire than bacteria (Pressler et al., 2018). Repeat fires can also negatively affect soil communities, but results differ between ecosystems. In a tallgrass prairie, annual burning has been shown to reduce microbial biomass C and N (Ajwa et al., 1999). In a temperate oak

woodland, the biomass of gram negative bacteria, but no other microbial functional groups, decreased with fire frequency (Williams et al., 2012). Contrary to our expectation and previous findings, we found little evidence for a reduction in biomass of bacteria, fungi, Protozoa, nematodes, and arthropods with FRI treatment (Figure 3.3). Depth to burn may be one explanation for why we did not find the decrease in soil biota biomass with fire that is common in the literature (Pressler et al., 2018). It is possible that our sampling depth of 10 cm may have diluted any decreases in soil biota biomass in surface soil directly impacted by fire. Dry mineral soil is a good insulator and soil temperature profiles during a fire decline sharply with depth (DeBano et al., 1979). This may be particularly pronounced in the savanna sites (1-, 2-year FRIs) where soil temperatures often do not increase below 5 cm during a fire (Miranda et al., 1993). Taken together, it is likely that soil organisms present below the maximum burning depth may not have been affected by the fire and dominated the signal in our samples.

The amount of time between the fire event and sampling plays an important role in the measured response of soil biota to fire. In an African savanna, soil microbial biomass was significantly higher in burned plots 12 days after fire, but this effect did not last beyond 6 months (Andersson et al., 2004). Our study took a fire regime perspective and did not capture this immediate temporal response as we sampled almost 6 months after the fire. In another similar oak-pine savanna, Ponder et al. (2009) found an increase in gram positive and gram negative bacterial PLFAs and a decrease in fungal PLFAs after fire (Ponder et al., 2009). We found no differences in bacteria or fungal PLFAs between our unburned (30-year) and annual burned treatments.

While total bacterial and fungal biomass estimates from epifluorescent microscopy did not differ with FRI (Table 3.2), the NLFA assay revealed clear differences in AMF. Contrary to our hypothesis that fungi would be more sensitive to fire and therefore more abundant at high FRIs, AMF NLFAs were highest at the lowest FRIs and decreased with increasing FRI (Figure 3.1a). These results reflect the dominate plant communities across the FRI continuum. Low FRI sites are characterized by C4 grasses that associate with AMF species, whereas high FRIs are dominated by woody trees and shrubs that do not associate with AMF (Feltrin et al., 2016). Saprotrophic fungi are not in direct symbiosis with plants and their NLFAs

better reflect the pattern observed for total fungal biomass with no significant differences between FRI treatments (Figure 3.1b).

Nematodes and arthropods can be negatively affected by fire but are overall more resistant to fire than soil microorganisms (Pressler et al., 2018). Here, our data show little evidence of differential impacts of fire on soil microorganisms and fauna as the biomasses of both groups were retained across the FRI gradient. We did not find any evidence for changes in protozoa biomass with fire (Table 3.2) but like much of the soil ecology literature, our understanding of how protozoa respond to fire is limited (Pressler et al., 2018).

Soil food web complexity increases with fire return interval

Our study is one of the few to investigate the effect of fire on the entire soil food web. Previous work has focused on specific taxa and functional groups (Pressler et al. 2018), but a food web approach is particularly important because fires may alter feeding structure and interactions, but not abundance and biomass estimates as has been observed in soil nematode communities (Butenko et al., 2017). In this oak-pine savanna, bacterial consumer, herbivore and predator biomass did not differ across the FRI gradient but fungal consumer biomass was highest in low FRIs (Table 3.2). If bottom up effects of prey biomass on consumer biomass were driving these results, we would expect patterns of fungal consumer biomass to align with those of their fungal biomass prey across the FRI gradient. However, fungal consumer biomass and fungal biomass do not follow the same pattern across the FRI gradient, suggesting that fungal consumers responses to fire as likely controlled by abiotic factors, such as pH, rather than predator-prey interactions. Some species of cryptostigmatid mites prefer more acidic soils (pH ~ 4.1-4.4; van Straalen and Verhoef, 1997). Here, cyptostigmatid mites accounted for a large proportion of fungal consumer biomass and the decrease in fungal consumer biomass with increasing fire frequency was driven by the loss of cryptostigmatid mites as soil pH crossed their optimum threshold.

Shifts in fungal consumer biomass translated into significantly different soil food web structure along the FRI gradient (Table 3.3). The number of functional groups within the soil food web did not differ

across the FRI gradient (Table 3.3), but the presence of some groups and therefore the nature of their interactions did change. In agreement with our hypothesis, we found the greatest food web complexity (connectance and linkage density) at the infrequently burned sites (4- and 30-year FRI). This corresponds to a loss of predators (predatory nematodes and mites) and mesofauna (fungivorous arthropods – particularly the Collembola) that reduced soil food web complexity at the frequently burned sites (1- and 2-year FRI). Fungal feeders and their predators are thought to be more resistant but less resilient to disturbance than organisms in the bacterial energy channel (de Vries et al., 2012; Hedlund et al., 2004). Given the repeated fire disturbance at 1-year and 2-year FRIs, the loss of consumers and predators in the fungal pathway aligns with results from other disturbances (e.g., drought; de Vries et al., 2012).

Soil food webs are functionally similar along fire frequency gradient

We found some evidence for shifts in soil food web structure with fire frequency (Table 3.3), but these changes did not translate into meaningful shifts in soil food web function. Contrary to our hypothesis that modeled C and N mineralization estimates would be greatest at low FRIs, mineralization did not differ between the FRI treatments. When considering C dynamics within the food web, the total C flux through the herbivore pathway did significantly increase relative to the bacterial and fungal pathways in the 4-year FRI, perhaps suggesting a shift in food web compartmentalization. However, C fluxes through the herbivore pathway were quite low overall (< 2% of the total flux through herbivore, fungal, and bacterial pathways). Together, this suggests that even though the soil food webs differed in complexity and composition, they were functionally similar with respect to C and N cycling across the FRI gradient. Generalism and omnivory are common belowground, leading to high functional similarity among detrital food webs (Setälä et al., 2005). In a survey of 58 soil food webs associated with individual plants, Bezemer et al. (2010) also found that soil food webs were structurally unique, but functionally redundant with respect to C and N cycling. However, other evidence suggests that after disturbance, functional similarity of microbial communities may not be as common (Allison and Martiny, 2008).

Soil respiration has been shown to increase (Knapp et al., 1998), decrease, or remain unchanged after fire in both forest and grassland ecosystems (Dooley and Treseder, 2012). Previous reviews have found that soil respiration tracks microbial biomass, both decreasing after fire, but these effects were based on single fire events in boreal and temperate forests and did not occur following grassland fires (Dooley and Treseder, 2012). Our results align with these findings as we did not find any evidence of changes in modeled C mineralization with fire frequency as plant cover shifted from oak-pine-dominated to grass-dominated. In an African savanna, nitrifying bacteria were stimulated by fire and soil nitrate levels were elevated relative to the control within the first 3 months post fire (Andersson et al., 2004). Here, we did not observe an effect on fire on modeled C or N mineralization rates, likely because our sampling investigated the effects of the fire regime and did not capture immediate short-term effects of a single fire.

Soil food webs are least stable and resilient at intermediate fire return intervals

Our modeling exercise revealed that soil food webs in the 4-year FRI possessed the least stable and least resilient architectures of all soil food webs along the FRI gradient. We found that the distribution of minimum s, a measure of food web stability, from 1000 simulations was wider for the 4-year FRI soil food webs than for any other FRI (Figure 3.3a). While the 1-year, 2-year, and 30-year FRI food webs had the majority of minimum s values very close to zero, a greater proportion of simulations of the 4-year FRI food webs resulted in larger minimum S values. This suggests that the 4-year FRI food webs were relatively less stable than the food webs at either low or high FRIs. For one, the 1-year and 2-year FRI food webs were both the least complex (Table 3.3) and the most stable (Figure 3.3). However, we expected the 30-year FRI food webs to be the most complex and the least stable. While the 30-year FRI food webs were indeed to most complex (Table 3.3), the 4-year FRI food webs were the least stable (Figure 3.3).

We found that return time, an indicator of food web resilience, differed across the FRI treatments. In particular, soil food webs from 1-, 2-, and 30-year FRIs all resulted in bimodal distributions of return times where some proportion of model simulations had long return times (low resilience) and the rest had

short return times (high resilience) (Figure 3.3). The 4-year FRI was a clear exception (Figure 3.3). In these food webs, the majority of model runs resulted in long return times, indicating that the soil food webs in the 4-year FRI treatment are less resilient to perturbations than the food webs at either low or high FRIs.

The soil food webs in the 4-year FRI treatment may be less stable and resilient due to shifts in the dominant energetic pathway within the food web. Namely, the total C flux through the herbivore pathway in the 4-year FRI webs was 4x higher than that of the 1-year FRI. Given that compartmentalization in food web energetic pathways leads to stability (Moore et al., 2005), a reallocation of C from bacterial and fungal energy channels to the herbivore energy channel may have led to the observed relative instability at the 4-year FRI. However, the average proportion of C flux through the herbivore pathway was still relatively low (1.7%) in the 4-year FRI treatment compared to fluxes through the bacterial (53%), and fungal (45%) pathways and therefore did not substantially tip the balance of the bacterial and fungal energy channels.

Soil food web structure alone does not always confer stability and resilience. For example, soil food webs associated with individual plants may be structurally dissimilar but maintain the same degree of food web stability (Bezemer et al., 2010). With respect to disturbance, the resilience of soil food webs to freezing depends more on which species are present than on food web structure, per se (Allen-Morley and Coleman, 1989). Other work suggests that the resilience of soil food webs to drought depends on plant community composition and belowground inputs (de Vries et al., 2012b), and land use (de Vries et al., 2012). From this perspective, the divergent plant communities as a result of fire frequency at our site may be influencing the relationship between soil food web structure and complexity. Plant community composition in the 4-year FRI treatment provides evidence that fire frequency is maintaining these sites in a transitional state between savanna and forest (Feltrin et al., 2016). Understory aboveground net primary productivity (ANPP) and photosynthetically active radiation at the 4-year site spans the range of values for both savanna and forest sites on either ends of the FRI gradient (Feltrin et al., 2016). While the 4-year FRI treatment is considered to be forested, ANPP of the herbaceous understory is more similar to that of

the savanna sites (1- and 2-year FRIs) (Feltrin et al., 2016). Given that the plant community is in a state of transition, it is not surprising that the soil food webs in the 4-year FRI treatment were least stable and least resilient, despite the fact that the 30-year FRI food webs were more complex. The idiosyncratic relationship we observed between soil food web structure and stability is likely based on interactions between disturbance frequency and plant community composition.

Fire return interval and pH are strongest predictors of soil food web structure

The effect of FRI on soil food web structure was driven to a greater degree by the chemical nature of the soil rather than physical properties. Fire increased soil pH regardless of its frequency, while PyC varied with fire frequency (Table 1). Soil pH, in particular, is a stronger predictor of soil food web structure than PyC. pH is often cited as an important control on soil microbial (Fierer and Jackson, 2006; Rousk et al., 2010) and faunal (Wu et al., 2011) biogeography at large geographic scales. In fact, soil pH may be the primary mechanism through which fire frequency influences soil communities especially if repeated fires increase soil pH beyond the survival threshold of some species and taxa (Van Straalen and Verhoef, 1997). Here, the importance of pH for soil food web structure was driven by a decrease in cryptostigmatid mites at as pH increased with fire frequency. Patterns in soil community responses to disturbance may be hidden when considering biomass of taxa (e.g. arthropods), but emerge when organizing soil organisms into trophic groups (e.g. fungal consumers) and considering food web structure (e.g. connectance). Our results demonstrate that soil pH is not only be a driver of soil microbial and faunal community composition (Fierer and Jackson, 2006; Wu et al 2011), but also controls soil food web structure.

Despite the fact that PyC alters soil nutrient retention and water dynamics (Liang et al., 2006) and may serve as a food source for some microorganisms (Santos et al., 2012), PyC did not emerge as an important predictor of soil food web structure along this FRI gradient. PyC can contribute to the increase in soil pH after fire (Glaser et al., 2002) and its effect on soil food web structure may already be accounted for by changes in soil pH. However, PyC does not always have an effect on pH in forest soils

(DeLuca et al., 2006). In this oak-pine savanna, we found little evidence for differences in soil moisture regardless of fire return interval (Table 3.1) which likely explains the lack of difference in modeled C mineralization. In Brazilian savannas, soil respiration was controlled to a greater degree by soil moisture and vegetation, rather than fire regime (Pinto Alexandre de et al., 2002). Soil respiration did not differ between burned and unburned sites in the dry season, but soil respiration was higher in the burned savanna than the unburned savanna during the wet season (Pinto Alexandre de et al., 2002).

Managing fire regimes results in structural and functional similarity belowground

Along this oak-pine savanna continuum, prescribed fires at variable frequencies are an important tool when managing for aboveground primary productivity and community composition. In fact, fire frequency has proven effective in maintaining C4 grass dominated savannas and oak-pine woodlands at this site (Feltrin et al., 2016). In this case, we find that such aboveground-focused management results in structurally and functionally similar belowground communities. The soil chemical environment (Table 3.1) and soil food web complexity (Table 3.3) changed with fire frequency, but the communities had similar soil biota biomass (Table 3.2) and therefore rates of modeled soil C and N mineralization were maintained across the fire frequency gradient. However, soil food webs stability and resilience differed with FRI (Figure 3.3), suggesting that current functional responses may change with future shifts in fire regimes, particularly at intermediate (4-year) fire frequencies. Soil communities are an important foci when managing for soil C and N. Single fires have important short term effects on soil C cycling immediately after the disturbance, but these effects are less pronounced in savanna and grassland ecosystems (Dooley and Treseder, 2012). However, high fire frequencies reduce soil C and N stocks over decades in both savannas and broadleaf forests (Pellegrini et al., 2018). Our results suggest that managing fire frequency to maintain an oak-pine savanna continuum without dramatically altering belowground C and N cycling may be possible. However, other functions of soil physical, chemical and biological components and the potential for instability in these ecosystems cannot be ignored. Future research should focus on connecting changes in soil food web composition to other functional attributes of the

system beyond C and N cycling, such as water dynamics, soil structure, and biodiversity that may be the target of management objectives.

Conclusions

Shifting global fire regimes have the potential to influence soil food web structure, function, and stability with consequences for ecosystem processes. However, our understanding of how soil communities respond to fire is primarily based on the response of soil biota biomass to single fire events, rather than fire regimes. By leveraging a long-term fire frequency experiment in an oak-pine savanna and an integrated food web modeling approach, our study reveals that soil food webs are structurally unique but functionally redundant along an FRI gradient. Soil biota biomass was conserved, despite changes in the soil chemical environment through an increase in pH at low FRIs and an increase in PyC at intermediate FRIs. When only considering the responses of soil biota biomass and C and N cycling, it is tempting to conclude that fire frequency may not have ecologically meaningful effects on soil communities. However, our modeling approach reveals another perspective: fire frequency regulates soil food web stability particularly in sites in transition between a savanna and a forest. Here, soil food webs in the 4-year FRI treatment were less stable and less resilient than those at either low or high FRIs. Soil food web responses to fire did not emerge when considering biomass and function alone. Signals of instability in the 4-year FRI soil food webs have not yet manifested into measurable differences in soil biota biomass across the FRI gradient. However, these latent signals hint towards vulnerable soil food web architectures at the transition between forest and savanna ecosystems. In a broader ecological sense, our study demonstrates the importance of taking advantage of experimental and modeling perspectives to triangulate the responses of communities to shifting disturbance regimes.

Chapter 4: Consequences of fire for soil organic matter: pyrogenic carbon in soils across Europe[3]

Introduction

Understanding and managing for the persistence of soil organic matter (SOM) is key to maintaining ecosystem services provided by soils and mitigating adverse effects of climate change (Smith et al., 2015). At the same time, climate change is altering global fire regimes (Moritz et al., 2012) with consequences for SOM quantity and quality (Gonzalez-Perez et al., 2004). One such consequence is the production of pyrogenic carbon (PyC), a recalcitrant component of SOM that is formed during fires (Bird et al., 2015). Despite the widespread relevance of the global PyC cycle, the magnitude of terrestrial and aquatic pools and fluxes, and the mechanisms that govern PyC persistence in those pools, remains poorly understood.

PyC is a biochemically recalcitrant component of SOM that is produced from the incomplete combustion of organic matter during a fire (Bird et al., 2015). PyC is a thermochemically altered material with a highly condensed polycyclic aromatic hydrocarbon structure (Knicker, 2011; Bird et al., 2015). The chemical structure of PyC is heterogeneous and variable depending on the origin of the organic matter and production conditions (e.g. temperature, oxygen, duration) (Knicker, 2011; Bird et al., 2015). PyC is not an inert material, but its complex molecular composition does resist microbial degradation relative to other more labile forms of SOM (Kuzyakov et al., 2009). As a result, PyC can persist in soils for centuries to millennia with implications for global carbon cycling (Bird et al., 2015). Therefore, understanding the distribution and dynamics of PyC in soils is a critical research need in terrestrial biogeochemistry.

[3] Research conducted in collaboration with M.F. Cotrufo, C. Boot, S. Abiven, and E. Lugato

Efforts to estimate the amount and distribution of PyC in soils have primarily focused on ecological factors that govern fire dynamics and therefore PyC production (Czimczik and Masiello, 2007). At local ecosystem scales, patterns of PyC distribution in soils can often be explained by fire frequency, severity, and fuel load (Czimzcik and Masiello, 2007; Santin et al., 2016; see chapter 3). PyC distribution in soils at a larger spatial scale may be controlled more by its persistence and stabilization, than by production. That is, the places where PyC accumulates in soils, and the mechanisms that govern its persistence, may depend more on the physical and chemical soil environment (Czimzcik and Masiello, 2007), the presence of PyC degrading microbial communities (Santos et al., 2012), and topography and erosion (Cotrufo et al., 2016; Abney et al., 2018) than on production during fires.

PyC is subject to the same mechanisms of stabilization in soils as other forms of non-pyrogenic organic matter. Historically, biochemical recalcitrance was thought to be the most important control on SOM stabilization and persistence in soils (Lehmann and Kleber, 2015). From this perspective, we would expect PyC to persist in soils based solely on its molecular complexity, condensed aromatic structure, and high inherent biochemical recalcitrance. Indeed, much evidence suggests that PyC can remain in soils for centuries to millennia (Bird et al., 2015). However, our understanding of the mechanisms that control SOM stabilization has expanded beyond just biochemical recalcitrance alone. Recent theoretical advances in SOM dynamics have revealed the importance of microbial degradation, physical protection in aggregates, and chemical protection through organo-mineral complexes for the persistence of SOM (Schmidt et al., 2011; Cotrufo et al., 2013; Lehmann and Kleber, 2015). Further, SOM is subject to topographic effects and erosion that modulate its distribution and persistence at the catchment scale (Berhe et al., 2018). Together, SOM, and therefore PyC, persistence is a function of the ecosystem and its physical, chemical, and biological properties (Schmidt et al., 2011). Therefore, predicting the distribution of PyC at regional and global scales requires that we consider not just the factors that control PyC production, but also the drivers of PyC persistence.

PyC in soils has been suggested to be between 5-15% of soil organic carbon (SOC) based on findings from marine and riverine sediments (Masiello and Druffel, 1998; Hockaday et al., 2007; Santin et al.,

2016). A recent meta-analysis found that PyC contributes 13.7% of SOC on average, and can reach up to 60% (Reisser et al., 2016). However, this estimate is primarily based on studies focused on understanding PyC distribution and dynamics in post-fire soils. This bias towards sampling recently burned sites may have led us to overestimate the amount of PyC in soils. Accurate estimations of the proportion of PyC in SOM becomes particularly important as we attempt to extrapolate PyC dynamics to the global scale. For example, accounting for PyC in carbon cycling models has been shown to decrease predicted CO_2 emissions associated with warming temperatures by up to 24% because of the long mean residence time of PyC in soils (Lehmann et al., 2008).

Here, we seek to improve our estimates of soil PyC and better understand the patterns of and controls on PyC distribution in soils. The objectives of this study are to: (1) quantify PyC in soils at a continental scale from variable soil types regardless of fire history, (2) leverage the relationship between molecular marker and spectroscopic techniques to predict PyC in additional samples, and (3) investigate the environmental factors that control PyC distribution in soils. To achieve this, we acquired soils from across Europe and measured PyC using both a precise analytical chemistry technique (benzene polycarboxylic acids, BPCA) and a high throughput spectroscopic technique (Diffuse Reflectance Infrared Fourier Transform Spectroscopy, DRIFTS). We expect our PyC estimates to be lower than previous estimates given that our soil samples originate from a large geographic range and have not necessarily been recently burned. We then use the relationship between BPCA and DRIFTS to broaden our ability to make inference across a large spatial scale. Based on previous calibrations between the two methods (Cotrufo et al., 2016), we expect a strong relationship between the DRIFTS spectral signal and BPCA content of soils. We anticipate texture (% clay and % sand) to be the strongest predictors of PyC in soils. We expect pH and cation exchange capacity (CEC) to be useful, but less robust, predictors of PyC in soils. We do not anticipate a clear signal between PyC content and land cover because PyC can persist in soils for millenia and current land uses do not necessarily reflect the conditions under which PyC was formed.

Materials and Methods

Sample collection and database subsetting

We used a subset of samples from the LUCAS Topsoil database for analysis. The LUCAS Topsoil database includes ca. 22,000 soil samples (0-20 cm) from well described sites across Europe (Orgiazzi et al., 2018). The database includes standard soil characterizations for each sample including texture, organic carbon content (OC), pH, calcium carbonate (CaCO3), nitrogen (N), phosphorous (P), potassium (K), cation exchange capacity (CEC), and land cover. Of the 18,571 mineral soils (organic carbon < 200 g kg^{-1}) included in the LUCAS database, we took a representative subset of 397 samples using the strata function and srswor method in the sampling R package (Tillé and Matei, 2016). The original dataset was stratified based on land cover (cropland - annual crops, cropland - permanent crops; woodland; shrubland; grassland; other) and FAO texture classes (coarse, medium, medium fine, fine, very fine) with random samples selected within each stratum. The resulting 397 samples were analyzed with Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) as described below. The DRIFTS subset was then further subsetted to acquire 102 samples to be analyzed for benzene polycarboxylic acids (BPCAs) as detailed below. The DRFITS subset was stratified based on sand percentage and organic carbon content and the BPCA subset was then determined in R using the stratified random sampling approach described above. Density distributions of clay, sand, and organic carbon content in the original LUCAS database, DRIFTS subset, and BPCA subset are shown in Figure 4.1. Prior to all further analysis, samples were sieved, ground, and oven dried at 105 °C.

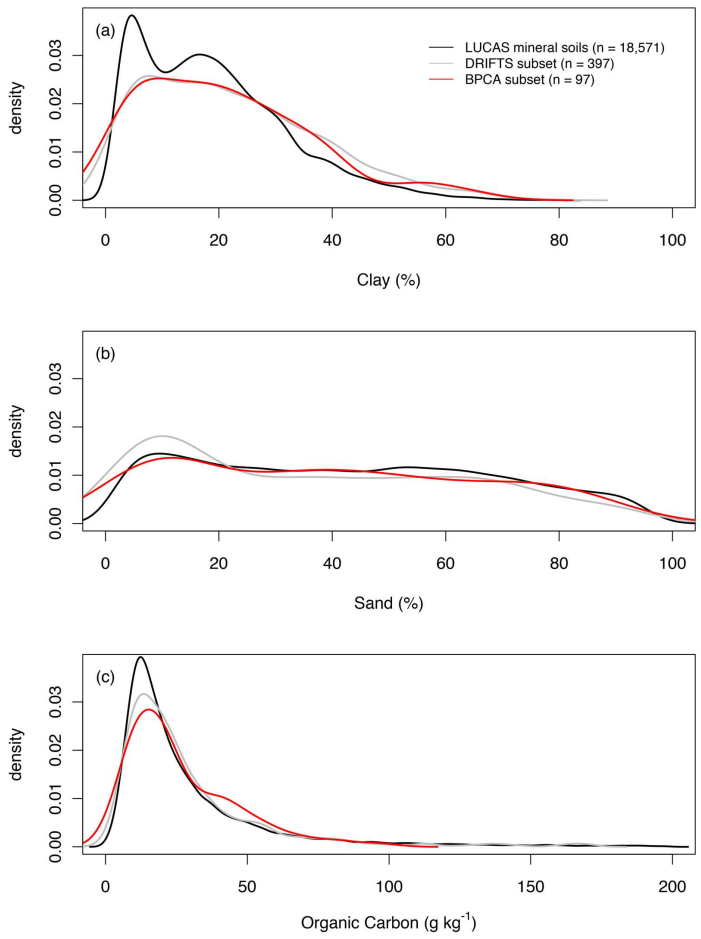

Figure 4.1. Density distributions of clay (%) (a), sand (%) (b), and organic carbon content (g kg^{-1}) (c) of mineral soil samples included in original LUCAS database (black), subset used for DRIFTS analysis (gray), and subset used for BPCA analysis (red). Samples sizes are given in the legend.

BPCA and PyC quantification

To quantify PyC, we measured benzene polycarboxylic acids (BPCAs), a molecular marker of PyC, following Wiedemeier et al. (2013) and Boot et al. (2015). BPCAs were liberated from polycyclic aromatic hydrocarbons in 150 - 300 mg of ground soil samples by oxidizing samples with 70% nitric acid at 170 ºC for 8 hours. The samples were filtered through ashless cellulose filters and a phthalic acid internal standard was added. The solution was further filtered through a cation exchange resin before it was freeze dried and reconstituted for quantification. BPCAs were quantified by High Performance Liquid Chromatography on a Shimadzu LC-20AD with a prominence diode array detector. The BPCAs

were separated along two consecutive reversed stationary phase columns (Waters X-Bridge C18, 3.5um particle size, 2.1mm x 150 mm) using a gradient separation of 25 mL L^{-1} 85% orthophosphoric acid and acetonitrile. We used a 4-point standard calibration curve of benzenetricarboxylic acids (B3CA), benzenetetracarboxylic acid (B4CA), benzenepentacarboxylic acid (B5CA), and benzenehexacarboxylic acid (B6CA) at 5 uM, 25 uM, 50 uM, and 100 uM to quantify individual BPCA peaks. We re-digested and quantified samples where internal standard recoveries were poor (< 70%). After a second round of digestion, samples that continued to return low internal standard recoveries were removed leaving a total of 94 BPCA samples for further analysis. We calculated BPCA-C from total BPCA in solution by multiplying by the C proportion of molecular weight for each BPCA molecule. The BPCA method only captures a portion of the total PyC in soils. To address this, several conversion factors have been suggested: 2.27 by Glaser et al. (1998), 4 by Ziolkowski et al. (2011), and 4.5 by Brodowski et al. (2005). To capture the range in conversion factors, we present estimates of PyC from BPCA-C using both the Glaser et al. (1998) and the Brodowski et al., (2005) conversion factors.

Mid-infrared spectroscopy

We used Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) to acquire mid-infrared spectra from all soil samples. Spectra were collected on a Thermo Scientific Nicolet iS50 Fourier Transform Infrared Spectrometer with a DRIFTS attachment. For each sample, we collected reflectance on 128 scans between 400 and 4000 cm^{-1} wavelengths at 4 cm^{-1} resolution. We collected spectra from a chernozem standard every 25 samples to ensure there was no substantial drift between samples (Hammes et al., 2007). Prior to using the spectra in further statistical analysis, we applied a rubberband baseline correction on the organic portion of the reflectance spectra (wavelengths < 2500 cm^{-1}) with the hyperSpec package in R (Beleites and Sergo, 2018).

Statistics

BPCA - DRFITS calibration

We used partial least squares regression (PLSR) to calibrate the baseline corrected DRIFTS spectra to the BPCA-C data using the plsr package in R (Mevik et al., 2016). We conducted a separate PLSR for total BPCA-C (g kg^{-1}), B6CA-C (g kg^{-1}), and B5CA-C (g kg^{-1}). Early attempts to calibrate the model using only DRIFTS spectra resulted in very poor predictions of BPCA-C. Adding organic carbon content (g kg^{-1}) as a predictor greatly improved the BPCA-C predictions. All PLSRs presented here use baseline corrected spectra (< 2500 cm^{-1}) and organic carbon content to predict BPCA-C for each sample. Prior to running each PLSR, we removed outliers and zeros from the BPCA-C datasets. We determined the most parsimonious number of components for the PLSR model by adding components only when the root mean squared error of prediction (RMSEP) continued to decrease, resulting in 7, 8, and 7 components for total BPCA-C, B6CA-C, and B5CA-C, respectively. We used the most robust model (total BPCA-C) to estimate total BPCA-C for all 397 samples in the DRIFTS subset. We then converted these estimated total BPCA-C values to PyC using conversion factors from Glaser et al. (1998) and Brodowski et al. (2005).

Drivers of PyC distribution

The following analyses were conducted separately on the measured PyC values (measured with BPCA; n = 94) and the estimated PyC values (estimated from BPCA-DRIFTS PLSR; n = 397). We conducted these analyses on PyC values that were derived from BPCA-C data using the most conservative conversion factor of 2.27 based on Glaser et al. (1998). Based on the linear nature of the model, the PLSR estimated some negative PyC values which were removed prior to further analysis. To evaluate possible drivers of PyC distribution in soils, we conducted multiple regression on log10 transformed PyC data using OC (g kg^{-1}), sand (%), silt (%), clay (%), nitrogen (N; g kg^{-1}), pH (measured in water), and CEC (cmol(+) kg^{-1}) as predictors. We could not include OC in the multiple regression of estimated PyC because it was used to train the PLSR model. Therefore, we conducted three separate

multiple regressions: (1) measured PyC including all predictor variables, (2) measured PyC including all predictor variables except OC, and (3) estimated PyC including all predictor variables except OC. We used backwards selection based on AIC to identify the 2 best models for each of the three multiple regressions. We investigated whether PyC differed by soil textural class (an integrated measure of sand, silt, and clay content; e.g. silt loam) by conducting a type 3 ANOVA on log10 transformed PyC data. To assess whether PyC differed by land cover, we conducted type 3 ANOVA on log10 transformed PyC data in g kg^{-1} dry mass and g kg^{-1} SOC.

Results

Quantifying PyC in soils

In the 94 samples in the BPCA subset, total BPCA-C ranged from 0 to 3.12 g kg^{-1} with a mean of 0.35 g kg^{-1} and 1 sample with 0 g kg^{-1}. B6CA-C ranged from 0 to 1.91 g kg^{-1} with a mean of 0.13 g $kg^{-1}$and 6 samples with 0 g kg^{-1}. B5CA-C ranged from 0 to 1.00 g kg^{-1} with a mean of 0.16 g kg^{-1} and 3 samples with 0 g kg^{-1}. Total BPCA-C accounted for 1.2% of SOC on average and ranged from 0 to 10.4% SOC. When we converted total BPCA-C to PyC using the Glaser et al. (1998) conversion, we found that PyC comprised 2.7% of SOC on average and ranged from 0 to 23.5% SOC. When we converted total BPCA-C to PyC using the Brodowski et al. (2005) conversion, we found that PyC comprised 5.4% of SOC on average and ranged from 0 to 46.6% SOC. Total BPCA-C was most strongly correlated with OC and N content (Table 4.1).

Table 4.1. Correlation coefficients of measured total BPCA-C (g kg^{-1}) with soil environmental variables.

	OC (g kg^{-1})	**Sand (%)**	**Silt (%)**	**Clay (%)**	**N (g kg^{-1})**	**pH**	**CEC (cmol(+) kg^{-1})**
BPCA-C (g kg^{-1})	0.62	-0.08	0.12	-0.002	0.54	-0.19	0.22

Estimating PyC in soils

Overall, the calibration between BPCA-C and DRIFTS spectra was not as robust as achieved by Cotrufo et al. (2016). Estimating BPCA-C from DRIFTS spectra alone was not possible with this dataset. In order to achieve reasonable BPCA-C estimations, we included OC as a predictor in the PLSR model. Using this approach, the PLSR model was strongest for total BPCA-C, explaining 62% of the variance (Figure 4.2). The model tended to underestimate high total BPCA-C values, and the variability was quite large throughout.

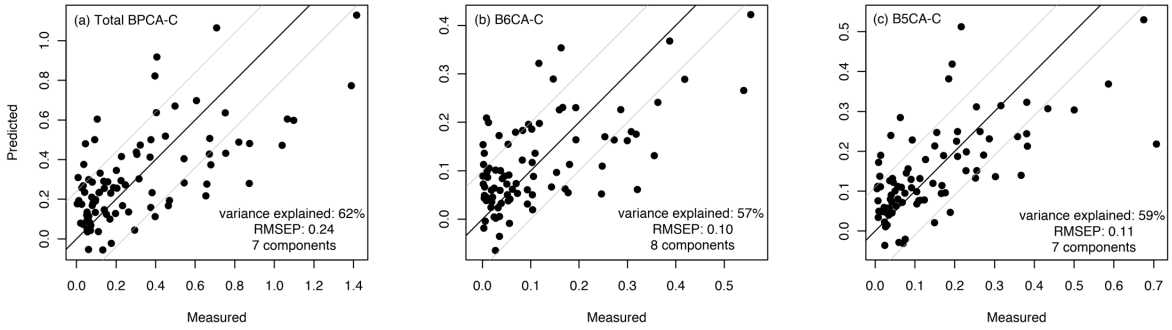

Figure 4.2. Relationships between measured values and predicted values from partial least squares regression model for Total BPCA-C (a), B6CA-C (b), and B5CA-C (c) in g kg^{-1}. Variance explained, root mean squared error of prediction (RMSEP) and number of components are presented for each model. Black lines indicate where measured and predicted values are equal; gray lines represent the 1:1 line ± one RMSEP.

We used the total BPCA-C PLSR model to estimate BPCA-C for all 397 DRIFTS samples. The distributions of the measured (by BPCA) and estimated (by BPCA-DRIFTS PLSR) total BPCA-C values were very similar (Figure 4.3). The range of the measured values was larger than that of the estimated values because we removed outliers from the measured BPCA-C prior to training the PLSR model. Given the linear nature of the PLSR model, some negative BPCA-C values were estimated, but these were removed prior to further analysis.

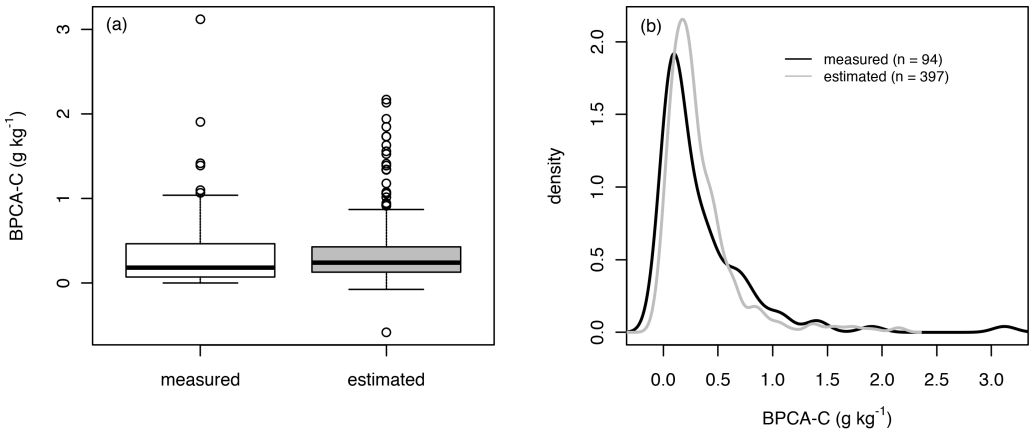

Figure 4.3. Boxplot (a) and density plot (b) of distributions of BPCA-C (g kg^{-1}) values measured by BPCA (black) and estimated with BPCA-DRIFTS PLSR (gray).

Drivers of PyC distribution in soils

We identified the two best multiple regression models for explaining the variance in (1) measured PyC with all predictors, (2) measured PyC with all predictors excluding OC and (3) estimated PyC with all predictors excluding OC (Table 4.2). When considering only measured PyC data, OC was by far the most important predictor of PyC content in soils (Table 4.2). In fact, adding clay and CEC to the model could only explain an additional 2% of the variance in PyC content beyond OC alone. When OC was excluded from the multiple regression because it was used as a predictor in the PLSR to generate the estimated PyC data, many additional variables emerge as important predictors. However, overall these models did a poor job of explaining the variance in estimated PyC (R^2 = 0.03; Table 4.2).

Table 4.2. Multiple regression results for best 2 models from backwards selection based on AIC for measured (n = 94) and estimated (n = 397) PyC values. The first two models of measured PyC include OC while the remaining models exclude OC.

PyC Data Type	Model	AIC	multiple R^2	factor	p value
Measured	All predictors log10(PyC) ~ CEC + clay + OC	108.7	0.43	CEC Clay OC	0.08 0.05 < 0.0001
Measured	All predictors log10(PyC) ~ OC	108.8	0.41	OC	< 0.0001
Measured	Excluding OC log10(PyC) ~ CEC + clay + N + pH + sand + silt	119.1	0.40	CEC Clay N pH Sand Silt	0.01 0.09 0.002 0.008 0.11 0.12
Measured	Excluding OC log10(PyC) ~ CEC + clay + N + pH	119.3	0.38	CEC Clay N pH	0.014 0.02 0.0004 0.007
Estimated	Excluding OC log10(PyC) ~ N + sand	415.8	0.03	N Sand	0.06 0.001
Estimated	Excluding OC log10(PyC) ~ CEC + sand	416.2	0.03	CEC Sand	0.07 0.0007

We did not find a clear trend of PyC content with soil texture (Figure 4.4). Clay was a more important predictor of PyC content than sand or silt for the measured PyC data (Table 4.2). However, when OC was included in the model of measured PyC, the addition of clay only improved predictions by 2%, suggesting that OC is a more important factor for predicting PyC in soils than clay. Sand, rather than clay, emerged as an important predictor for estimated PyC, but these models did a poor job of explaining variance in estimated PyC (Table 4.2). When soil texture was considered as an integrated measure of sand, silt, and

clay, there were no significant differences in PyC content with soil textural class for the measured (p = 0.33; Figure 4.4a) and estimated (p=0.15; Figure 4.4b) PyC data.

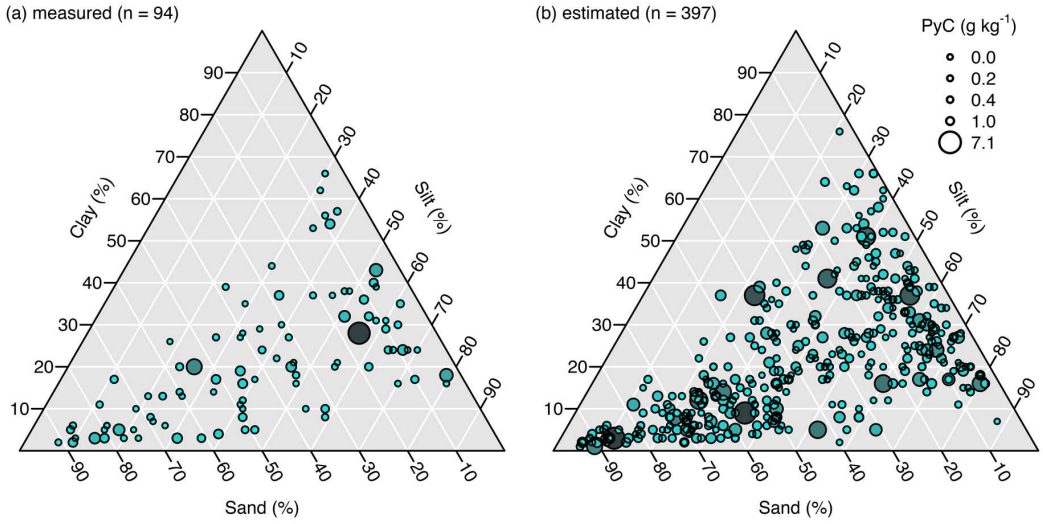

Figure 4.4. PyC (g kg^{-1}), estimated using the Glaser et al. (1998) conversion factor, as a function of soil texture for measured (a) and estimated (b) values. Larger, darker circles indicate higher PyC values.

We did not find any significant differences in PyC content (g kg^{-1}) between land cover types for measured (p = 0.12; Figure 4.5a) or estimated (p = 0.74; Figure 4.5b) values. Similarly, we found no differences in PyC as a proportion of SOC (g kg^{-1} SOC) between land cover types (p = 0.24; data not shown). There was a slight trend towards an increase in PyC in woodland soils, but the results were not statistically significant (Figure 4.5).

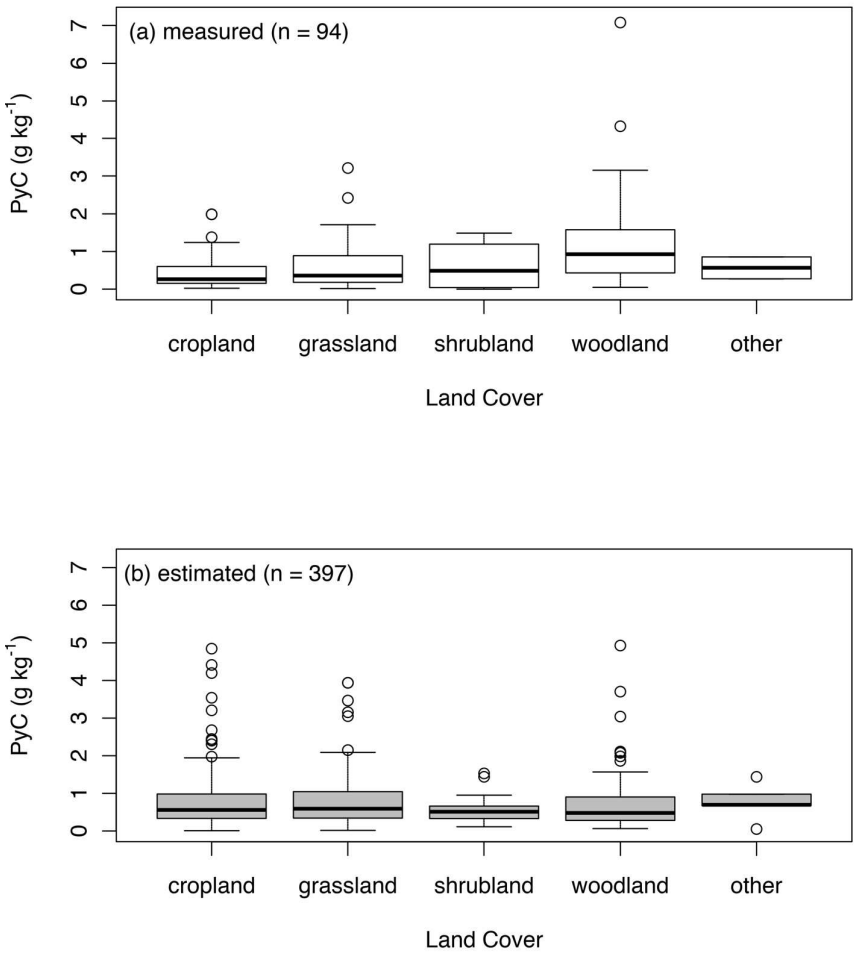

Figure 4.5. Boxplots of PyC (g kg^{-1}), estimated using the Glaser et al. (1998) conversion factor, across land cover types for measured (a) and estimated (b) values.

Discussion

We found that PyC comprised 2.7 - 5.4 % of SOC on average and up to 46.6% in some mineral soils. These estimates depend on which BPCA-C to PyC conversion factor is applied to the data. A more conservative conversion factor (Glaser et al., 1998) results in smaller range of PyC values (0-23.5%) whereas a less conservative conversion factor (Brodowski et al., 2005) would suggest that PyC can be anywhere from very little to up to half of SOC in some samples (0-46.6%). In either case, our average estimates (2.7% and 5.4%) are substantially lower than previous findings that suggest PyC comprises

13.7% of SOC on average (Reisser et al., 2016). The ranges of PyC values are similar between Reisser et al. (2016) (0-60%) and our results when using the Brodowski et al., (2005) conversion factor (0-46.6%). The estimate put forth by Reisser et al. (2016) is based on a literature synthesis and therefore retains the sampling bias from studies included in the synthesis. Researchers primarily measure PyC in the context of a single fire event or fire regime, and tend to sample at sites that have been recently burned or with a known fire history. Our findings confirm our expectation that PyC comprises a smaller proportion of SOC when measured in soils from sites over a large continental distribution that had not necessarily been recently burned. Our findings suggest that extrapolating PyC estimates derived from recently burned sites to soils at a global scale may overestimate the contribution of PyC to SOM.

Our average estimates of PyC also fall at the low end of estimates from marine and riverine sediments where PyC may comprise 5-15% of OC (Hockaday et al., 2007; Santin et al., 2016). This finding aligns with previous evidence that, after a wildfire, PyC is transported laterally from upland litter and surface soils to riverbank deposits and sediments rather than moving vertically deeper into the soil profile (Cotrufo et al., 2016). Given the low-density of PyC and its susceptibility to catchment-scale erosional processes after a fire (Cotrufo et al., 2016), it is not surprising that PyC constitutes a greater proportion of OC in sediments than in soils. Topographic patterns and erosional processes should be taken into account when estimating PyC in soils at regional, continental, and global scales.

Based on previous calibrations between BPCA-C and DRIFTS spectra in soils (Bornemann et al., 2008; Cotrufo et al., 2016), we expected a strong relationship between the two techniques that would allow us to expand our quantification of BPCA-C to a greater number of samples. Our PLSR calibration of BPCA-C and DRIFTS spectra was not as robust due to the high matrix heterogeneity in our dataset (Figure 4.2). Two previous studies that demonstrated a robust calibration between BPCA-C and DRIFTS spectra were derived from datasets where a large proportion of the soils has similar characteristics. The majority (75%) of the samples used in Bornemann et al. (2008) were derived from regions with typical Mollisols. Similarly, the soil samples in Cotrufo et al. (2016) were collected from two sites, with the majority (90%) originating from one forested site. As a result, most of the soils included in each dataset

likely had a relatively similar background matrix, even if the range of matrix characteristics was similar to our dataset (e.g. SOC: 0 - 200 g kg^{-1} in our study, 0-80 g kg^{-1} in Bornemann et al., (2008)). Consequently, the PyC signal in the DRIFTS spectra is likely easier to detect in datasets where the matrix heterogeneity is minimized.

Here, we measured PyC in soil samples from a continental scale geographic range that varies widely in soil texture and OC content (Figure 4.1). Our dataset maximized the variability in soil characteristics between samples resulting in highly variable background matrices between soil samples. This reduced our ability to detect a strong PyC signal from the DRIFTS spectra. To address the challenge of matrix heterogeneity in our samples, we included OC as a predictor in the BPCA-DRIFTS PLSR. In doing so, we were able to explain about 40% more variability in BPCA-C content than when just using DRIFTS spectra alone. The final PLSR model explained 62% of the variance in total BPCA-C content using OC and DRIFTS spectra (Figure 4.2). Despite these improvements, the predictive power of soil PyC estimates remains limited.

Unsurprisingly, OC emerged as the best predictor of PyC content in mineral soils. In the absence of OC, other soil properties including texture, pH, N, and CEC all emerged as important predictors of soil PyC content, but no one factor appeared to be greatly superior to the others. Further, these soil physicochemical variables together cannot predict PyC content any better than OC alone. Soil texture, pH, and CEC may help explain the variability in soil PyC content when considering global PyC as a % of SOC, as in Reisser et al. (2016), and at smaller, localized scales where the variability in OC content is minimal.

Contrary to our expectation and previous findings that soil texture is an important predictor of PyC content in soils, we did not find any evidence for differences in PyC with soil texture (Figure 4.4). We considered soil texture as an integrated measure of sand, silt, and clay and did not find any differences in PyC content with soil textural class. We did identify clay as a predictor of PyC content, but only when coupled with OC (Table 4.2). In fact, clay and CEC only marginally improved the prediction of PyC

beyond OC alone (Table 4.2). This aligns with previous findings from grassland soils where soil texture did not affect PyC accumulation (Glaser and Amelung, 2003). We conclude that soil texture alone is not a strong predictor of PyC content and cannot reliably be used to infer PyC content in soils.

As expected, we did not find any evidence to suggest that PyC content in soils varies with land cover (Figure 4.5). This result persists when considering both PyC content (g kg^{-1}) and PyC as a proportion of SOC (g kg^{-1} SOC). There was a slight trend towards woodland soils containing higher amounts of PyC than other land cover types, but this effect was not statistically significant (Figure 4.5). Our results differ from those of Reisser et al. (2016) who found the highest PyC content in agricultural soils both with and without slash and burn management practices, and lowest PyC content in forest soils. If PyC production were the major control of PyC distribution in soils in our dataset, we may expect that land cover types that are more prone to burning would have higher amounts of PyC. Our results suggest the opposite. Land cover is not a good indicator of soil PyC content, suggesting that PyC persistence, rather than production, is key to understanding the distribution of PyC in soils. However, land cover is unlikely to be a good proxy for PyC production because current land cover and land use may not reflect historical land cover in which the PyC was originally formed. Additional research is needed to parse the relative importance of PyC production and persistence in controlling PyC distribution in soils.

Our study revealed a more nuanced view of PyC distribution in soils by quantifying PyC across a continental distribution in mineral soils that vary widely in their physical and chemical properties. Even so, our analysis did not capture mineral soils with very sandy textures (Figure 4.4) or organic soils. Given that OC is the strongest driver of PyC content at this large geographic scale, our current model would suggest that organic soils with high OC contents will also have high PyC contents. However, very few PyC estimates exist for organic soils precluding our ability to test this hypothesis (Preston et al., 2006). Particularly as organic soils in tundra ecosystems are susceptible to increased fire frequencies and severities (Hu et al., 2015), studying PyC dynamics in organic soils is a necessary next step in furthering our understanding of the global PyC cycle.

Conclusions

Our study of PyC distribution in soils across Europe revealed three important conclusions. First, on average PyC may not be as large of a component of SOC as previously suggested when PyC is measured in soils in absence of a recent fire or known fire history. Even so, PyC content varies widely between soils and may contribute as little as 0% and up to 46% of SOC suggesting that it is still an important component of SOC at continental scales. Second, OC is the strongest predictor of PyC content in soils at the continental scale. While other soil physicochemical properties, such as texture and CEC, may be important, we cannot yet reliably use these properties to estimate PyC content. Third, using a calibration between BPCA and DRIFTS spectra to estimate PyC is only possible in soils with a strong PyC signal and minimal matrix heterogeneity. The BPCA-DRIFTS calibration is less robust when applied to any dataset with weak PyC signals and variable soils. Consequently, we cannot simply extrapolate currently available PyC estimates from soils to the global scale because of a sampling bias towards burned soils. To advance our understanding of the global PyC cycle, we need to better constrain our PyC estimates for pools and fluxes in terrestrial ecosystems.

Chapter 5: Coupled biochar amendment and limited irrigation strategies do not affect a degraded soil food web in a maize agroecosystem, compared to the native grassland[4]

Introduction

Arid and semi-arid regions are predicted to experience increased levels of drought (Seager et al., 2007) with increased temperatures and variability of rainfall associated with climate change (IPCC, 2014). Heightened drought will strain water resources in semi-arid agricultural systems, where water availability is a major limiting factor for crop productivity. Given that the agricultural sector uses approximately 80% of consumptive water in the United States (NASS, 2014), pressure to reduce overall agricultural water use in response to water scarcity is likely to increase. As a result, there is a critical need for semi-arid agriculture to find ways to manage water use in order to meet production demands for a growing population while simultaneously adapting to water scarcity.

Two such management strategies are amending soil with organic materials that increase the water holding capacity of the soil and limiting irrigation inputs. When applied at strategic time points, limited irrigation reduces water use (Fereres & Auxiliadora Soriano, 2007; DeJonge et al., 2011) while still maintaining equivalent crop yields in some systems (Schneekloth et al., 2009). Research on limited irrigation has gained popularity in the face of climate variability and more frequent drought (Schneekloth et al., 2009), but the majority of these studies have focused on crop responses and often neglected the response of belowground communities which mediate nutrient availability for plants. The few studies that have investigated the impacts of limited irrigation on soil biota are inconclusive but generally find a decrease in biomass and activity, although responses vary between different soil biota groups (Schnürer et al., 1986; Wang et al., 2008; Li et al., 2010).

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Biochar, the recalcitrant product of pyrolysis of biomass under minimal oxygen conditions (Lehmann & Joseph, 2015), is of particular interest as a soil amendment because it is a coproduct of cellulosic bioenergy production that slowly degrades in soils (Lehmann et al., 2006). Biochar has the potential to mitigate C emissions from bioenergy production through long-term belowground C storage (Lehmann et al., 2006). Biochar has also been shown to have a positive effect on water storage and crop yields in agricultural systems, thereby mitigating water challenges in semi-arid systems (Jeffery et al., 2011). Coupling biochar addition with limited irrigation strategies could therefore be a successful approach to reducing water consumption while sustaining crop productivity.

Previous research in temperate agricultural systems has focused on the effects of biochar on crop productivity (Jeffery et al., 2011; Crane-Droesch et al., 2013), while more recent investigations have highlighted the impacts of biochar amendment on soil biological communities (Liu et al., 2016), given their roles in regulating C and nutrient (e.g., nitrogen, phosphorous) cycling in soils (Paul, 2014). In general, agricultural conversion of native grasslands to cropland has shown detrimental impacts on belowground communities (Moore, 1994; Culman et al., 2010; DuPont et al., 2010). Such management-induced alterations to the structure of the soil food web can change the nature in which C and nutrients are processed in soils (Hendrix et al., 1986; Moore, 1994). For example, conventional agricultural management tends to support bacterially dominated soil food webs with increased C turnover, nutrient cycling rates, and losses, while less intensive practices create fungal dominated soil food webs with slower cycling rates resulting in greater C sequestration, nutrient use, and retention (Moore, 1994). Biochar addition may alter the soil environment through a number of different mechanisms that may favor fungal dominated soil food webs in agroecosystems: indirect effects on soil moisture and subsequent crop inputs (Atkinson et al., 2010; Spokas et al., 2012), changing bulk density and physical soil structure (Tryon, 1948; Atkinson et al., 2010; Laird et al., 2010; Abel et al., 2013), altering soil pH and nutrient dynamics (Cheng et al., 2008; Atkinson et al., 2010; Gaskin et al., 2010; Biederman & Harpole, 2013; Rogovska et al., 2014), and adding a recalcitrant C source that may or may not be utilized by the soil microbial community (Santos et al., 2012; Hammer et al., 2014; Jaafar et al., 2014; Gul et al.,

2015). Soil biota are sensitive to physical and chemical changes to the soil environment such as soil structure (Young et al., 1998; Beylich et al., 2010;), water dynamics (Schnürer et al., 1986; Williams, 2007; Wang et al., 2008), pH (Korthals et al., 1996; Pietri & Brookes, 2008; Rousk et al., 2010), and soil organic matter quantity and quality (Wardle, 1995). Given the complexity of biochar as a soil amendment, its potential utility in agriculture, and the multiple ways in which it can alter the soil environment, it is necessary to understand how biochar additions may change belowground functioning through direct and indirect effects on the soil biological community.

Few studies have investigated the impact of pyrolyzed materials including charcoal and ash on soil fauna (McCormack et al., 2013), and studies focused on the effects of human-made biochar on soil fauna are especially limited (e.g., Zhang et al., 2013; Marks et al., 2014; Domene et al., 2015; Soong et al., 2016). Fewer still have addressed the effects of biochar on the entire soil food web when applied in agricultural systems (McCormack et al., 2013). Many studies have considered the effect of biochar on the soil microbial community (bacteria and fungi) (Lehmann et al., 2011; Liu et al., 2016), but responses are variable depending on biochar addition rate, biochar production conditions, and soil conditions (Acea & Carballas, 1999; Gomez et al., 2014; Jiang et al., 2015). Given the importance of both soil microbial and faunal communities for agroecosystem functioning (Brussaard et al., 2007), there is need to investigate how the entire soil food web responds to coupled biochar amendment and limited irrigation to determine whether or not substantial positive or negative side effects on belowground functioning will occur as a result. This study is the first to explicitly evaluate the interactive effects of biochar addition and limited irrigation on soil biological communities in the field.

Here, we address how biochar amendment and limited irrigation strategies, separately as well as in interaction (1) influence soil micro- and meso-fauna biomass, and (2) alter structural and functional properties of the soil food web in an irrigated maize agroecosystem. We expect that the response of soil biota to biochar amendment and limited irrigation will vary between soil biota functional groups, given the differences in physiologies and water requirements of the different taxa. We hypothesize that limited irrigation will decrease the overall biomass and activity of soil biota, particularly those organisms that live

in water films (i.e., nematodes, protozoa). By contrast, we expect soil biota biomass and activity (C and N mineralization rates) to increase in biochar amended plots. We also hypothesize that biochar will favor fungi and their consumers relative to non-amended plots. Further, we hypothesize that biochar will mitigate the negative effects of limited irrigation by maintaining soil moisture. We expect a significant interaction between biochar and limited irrigation, resulting in greater benefits of biochar to the soil food web by increasing soil biota biomass and function under limited irrigation relative to full irrigation.

To address these hypotheses, we sampled a maize agroecosystem that was amended with biochar one year prior to sampling and subjected to limited irrigation for one growing season. From these samples, we extracted soil organisms to estimate biomass for all soil food web functional groups. We then modeled structural and functional properties of the soil food web (Moore & de Ruiter, 2012) in the maize agroecosystem under the different management treatments.

We then aimed to evaluate the short-term effects of a change in agricultural management (limited irrigation and biochar amendment) within the broader context of land-use conversion from native grassland to agricultural system at our site. To do so, we compared the structural and functional properties of the soil food web in the maize agroecosystem to those of a soil food web from a nearby native grassland soil (Andrés et al., 2016) used as an uncultivated reference.

Materials and Methods

Site description & experimental design

The study site is an experimental maize field located at the Agricultural Research Development and Education Center (ARDEC), Colorado State University, Fort Collins, Colorado. Founded in 1993, the field at ARDEC have been continuously used for experimentation under conventional agricultural management with irrigation, fertilizer, and herbicide inputs varying with experiment. The region is semi-arid with an average high temperature of 16.7 °C and average low temperature of 1.1 °C, with 384 mm yearly average rainfall (Western Regional Climate Center, 2016). The soil is a Fort Collins Loam (Aridic

Haplustalfs in US Soil Taxonomy), with sandy clay loam texture (51% sand, 20% silt, 28% clay). The average soil bulk density is 1.3 g cm^{-3}, with a total carbon (C) content of 1.5% and a total nitrogen (N) content of 0.1% (Abulobaida, 2014). The pH of the soil is 8.7 (Foster et al., 2016). Prior to 2005, the agroecosystem was managed under an irrigated plow-based tillage approach with wheat, corn, and dry bean production (Abulobaida, 2014). In 2005, the field was converted to conservation tillage under full and reduced irrigation with alfalfa-corn and dryland wheat-corn rotations (Abulobaida, 2014).

For this study, the field was prepared for planting in September 2013 by deep tilling to 30 cm and disk tilling to 12 cm. In November of that year, biochar was surface applied at 30 Mg ha^{-1} and disc tilled to 15 cm. The biochar was produced from virgin pine wood by Confluence Energy, LLC, Kremmling, CO, pyrolyzed beginning at 400 °C and ramping up to a maximum of 700 °C with five minutes of reaction time. Chemical and physical properties of the biochar are as follows: 71.9% total organic C, 0.60% total N, 9.4 pH (wet), and 0.326 g cm-3 bulk density (Control Laboratories, Watsonville, CA). In early April 2014, fertilizer (200N-4P-1S-0.1Z g m^{-2}) was applied followed by additional tilling to 10 cm. Maize varieties P8954 and P9305 (DuPont Pioneer, Johnston, Iowa) were planted at 247 seeds km^{-2} in late May 2014 and standard herbicide application occurred in June 2014. The experimental maize field was organized in a split-plot design with four replicate blocks. Primary plots were full (F) and limited (L) irrigation treatments that were split into two 4.5 m x 4.5 m soil amendment treatment subplots, biochar (B) and non-amended control (C) (n = 16, averaged across the two corn varieties). Limited irrigation was based on maize phenology to coincide with noncritical ear development phases. Full irrigation was calculated from evapotranspiration and precipitation rates and ranged from 1.52 cm and 2.54 cm applied once weekly. Over the season, the full irrigation plots received 22 cm and the limited irrigation plots received 15 cm from May 3 to August 28, 2014. The limited irrigation plots did not receive irrigation from June 29 to July 28, 2014, resulting in approximately a 1/3 reduction in irrigation applied relative to the full irrigation treatment. Volumetric soil moisture (%) and gravimetric soil moisture (g water g dry $soil^{-1}$) were measured to determine the effect of limited irrigation and biochar amendment on soil

moisture content (see Foster et al. 2016 for gravimetric soil moisture data). To assess crop health status given the amendment and irrigation treatments described above, maize yield was determined at the end of the season as described in Foster et al. (2016).

We compared our agroecosystem soil food web data to that of a nearby native grassland that is considered an uncultivated reference. The grassland site is located at the Short Grass Steppe (SGS) Central Plains Experimental Range, just north of the agroecosystem site. Andrés et al. (2016) sampled soils from three sites within the SGS, each with one continuously grazed plot (30 x 30 m^2). Further details regarding climate, dominant vegetation, and experimental design at SGS can be found in Andrés et al. (2016).

Soil sampling

Soil sampling occurred on September 19, 2014 after the final maize harvest of the season. In each subplot, four soil cores (5.5 cm diameter) were taken to a depth of 10 cm; two between and two within maize rows. Each between-row core was combined with one within-row for a total of 2 bulked samples from each subplot. One of the final bulked samples remained intact for microarthropod extraction, while the other sample was used for all other biological assays. Soils were collected in sealed plastic bags, stored in a cooler in the field, and transported immediately to the lab for soil fauna extraction. Subsamples for nematode extraction were taken from well-mixed non-microarthropod samples before these samples were 2 mm sieved for further homogenization.

Soil fauna extractions

Bacteria and fungi

We estimated total bacterial and fungal biomass via direct counts using epiflourescent microscopy techniques (Bloem, 1995). For both the bacteria and fungi assays, a 2 mm sieved subsample (5 g) from each field sample was blended with sterile deionized water and aliquoted (10 µl) onto a 10-well slide.

Bacteria slides were stained with 5-(4,6 dichlorotriazin-2-yl) aminofluorescien (DTAF) and fungi slides were stained with calcoflour fluorescent brightener following Frey et al. (1999). All direct counts were conducted using an Olympus Photomicrographic Microscope System with reflected light fluorescence attachment at 490 nm for bacteria and 334-365 nm for fungi. Total fungal biomass was scaled to active fungal biomass (10%) as described in Ingham and Klein (1984).

Protozoan

We estimated total protozoan biomass using the most probable number (MPN) technique (Darbyshire et al., 1974). A 2 mm sieved subsample (10 g) in 90 ml of sterile deionized water was serially diluted with tenfold dilutions to 10^{-6} ml. After each dilution in the series, four 0.5 ml subsamples were pipetted into four consecutive wells of a standard 24-well tissue culture plate. As a food source for the protozoan during incubation, *Escherichia coli* suspended in growth media was added to each well (50 µl). The plates were incubated at 14°C for 5 days. Thereafter, we observed each well under an inverted compound microscope (100x magnification) and recorded presence and absence of amoebae, flagellates, and ciliates. Total protozoan biomass was estimated using the Most Probable Number estimate program of the US Environmental Protection Agency (U.S. EPA, 2013).

Nematodes and arthropods

We extracted nematodes from subsamples (20 g) of each sample using the Baermann funnel method (Baermann, 1917). Soil samples were left in the extraction apparatus for 3 days, after which nematode samples were collected and preserved in formalin for taxonomic identification. Nematodes were sorted into functional groups based on their feeding morphology (Yeates & Coleman, 1982). We heat-extracted microarthropods from bulked samples of intact cores as described in section 2.3 using the Tullgren funnel method (Moore et al., 2000). Microarthropods were sorted into taxonomic functional groups (Moore et al., 1988).

Soil food web modeling

We modeled structural and functional properties of the soil food webs for all the treatment combinations using a well-tested food web model (de Ruiter et al., 1994; Moore et al., 2005; Moore & de Ruiter, 2012;). The model structure was based on functional groups of soil biota sensu Moore et al. (1988). To evaluate changes in food web structure (Moore & de Ruiter, 2012) we calculated the following metrics for all four treatment combinations (Table 5.1): number of basal resources (Sr; number of resources at the base of the food web, in this case detritus and roots); number of soil biota functional groups (S); connectance (C; amount of possible food chain links that are realized in the observed food web); linkage density (LD; number of functional groups multiplied by connectance); maximum food chain length (FCLmax); mean food chain length (FCLmean).

Functional attributes of the food web were estimated using the model developed by Hunt et al. (1987) and Moore & de Ruiter (2012). Given the soil biota biomass estimates measured from soil samples, the model uses the untransformed field biomass estimates and published physiologies of the functional groups and the trophic interactions between them to estimate C and N mineralization rates through the web and their dynamic properties. Total biomass of all soil biota functional groups was determined based on our direct population counts and average estimates of organism size from Hunt et al. (1987), and was reported on a C basis, assuming 50% of biomass is C (Hunt et al., 1987). Bulk density was calculated from the intact cores used for microarthropod extraction and was used to convert biomass in mg C g dry soil^{-1} to mg C m^{-2}. The model incorporates the field estimates of biomass, known natural death rates, feeding preferences, assimilation efficiencies, production efficiencies, and C:N ratios. Then the model predicts feeding rates between functional groups and derives C and N mineralization rates for each functional group and for the entire soil food web (de Ruiter et al., 1994). We then compared these food web metrics and modeled C and N flux estimates to those of the soil food web at the Central Plains Experimental Range (Andrés et al., 2016; Hunt et al., 1987), a natural grassland used here as an uncultivated reference located just north of the agricultural site at ARDEC.

Statistical approach

Soil biota biomass estimates remained untransformed when serving as input for the food web model. Biomass estimates for all soil biota functional groups and modeled C and N mineralization rates were square root transformed prior to conducting further analysis. We fit a general linear mixed effects model with soil biota biomass, modeled C flux, or N mineralization rates as the response variable, with irrigation, amendment and functional group as the predictor variables, and block as a random effect prior to running ANOVA and Tukey adjusted pairwise comparisons in R (R Core Team, 2015). To determine differences in food web structure between the treatment combinations, we conducted ANOVA and Tukey adjusted pairwise comparisons on all standard soil food web metrics, and Fischer's Exact Test to assess whether the presence of soil biota functional groups differed between the irrigation and amendment treatment combinations. To compare food web metrics and modeled C and N mineralization rates between the agroecosystem and grassland site, we fit a general linear model with food web metrics or mineralization rates as the response variable and site (agroecosystem or grassland) as the predictor variable and conducted ANOVA and Tukey adjusted pairwise comparisons. It is important to note that the food web structure was identical for all three grassland sites because all soil organisms were present in all three food webs, thus resulting in the exact same food web metrics and standard errors of zero (Table 5.2).

Results

Soil and crop empirical observations

Extracted biomass of specific soil biota functional groups was not impacted by irrigation ($P = 0.94$), soil amendment ($P = 0.85$), or the interaction between irrigation and amendment ($P = 0.93$). Pairwise comparisons of soil amendment and irrigation treatments were not significant for any of the individual functional groups or total taxonomic groups (total microbial biomass, arthropods, nematodes, protozoa) (Table 5.1). Similarly, we observed no differences in biomass estimates of aggregates of functional

groups organized into different energetic pathways between the irrigation (P = 0.97), soil amendment (P = 0.88), nor their interaction (P = 0.95) (Figure 5.1).

Table 5.1. Biomass estimates (mg C m^{-2}) for all soil biota functional groups and taxonomic groups for all irrigation and amendment treatment combinations for the agroecosystem soils. Estimates are mean ± SE (n=4).

	Treatment			
	Full		**Limited**	
Functional Group	**Control**	**Biochar**	**Control**	**Biochar**
Total Microbial Biomass	2109.63 ± 134.15	1831.99 ± 226.14	1835.96 ± 195.56	1776.52 ± 238.26
Amoeba	1.08 ± 0.41	0.41 ± 0.13	1.55 ± 0.96	12.09 ± 11.68
Flagellates	0.54 ± 0.21	0.13 ± 0.07	0.52 ± 0.26	0.46 ± 0.40
Ciliates	0 ± 0	0.01 ± 0.01	0.05 ± 0.05	0.03 ± 0.03
Total Protozoa Biomass	1.14 ± 0.41	0.52 ± 0.21	1.68 ± 0.96	6.51 ± 6.11
Bacteriophagous Nematodes	3.00 ± 0.27	5.01 ± 2.91	4.67 ± 0.53	3.24 ± 0.29
Fungivorous Nematodes	4.64 ± 2.23	3.53 ± 1.28	4.18 ± 0.77	4.33 ± 1.71
Phytophagous Nematodes	4.72 ± 1.52	4.63 ± 1.43	2.53 ± 1.08	5.30 ± 3.30
Omnivorous Nematodes	18.56 ± 3.94	16.49 ± 8.75	25.71 ± 8.15	11.06 ± 1.40
Predaceous Nematodes	0 ± 0	0.30 ± 0.30	0 ± 0	0.16 ± 0.16
Total Nematode Biomass	19.22 ± 2.94	22.52 ± 11.03	24.51 ± 6.69	16.68 ± 4.70
Collembola	0.02 ± 0.02	0 ± 0	0.03 ± 0.03	0 ± 0
Cryptostigmatid Mites	0.89 ± 0.31	0.30 ± 0.22	0.64 ± 0.38	1.11 ± 0.63
Non-Cryptostigmatid Mites	2.50 ± 0.90	0.48 ± 0.18	1.11 ± 0.36	2.02 ± 1.12
Nematophagous Mites	0.91 ± 0.57	0.66 ± 0.30	0.72 ± 0.16	1.40 ± 0.41
Total Microarthropod Biomass	3.12 ± 0.89	1.10 ± 0.18	1.89 ± 0.41	2.90 ± 0.60

Across the entire season, full irrigation increased volumetric soil moisture by 22% relative to limited irrigation regardless of amendment (P < 0.0001) and a significant interaction between irrigation and soil

amendment was observed (P = 0.004) (Appendix 1). Additionally, biochar amendment increased volumetric soil moisture relative to the control under both limited (P < 0.0001) and full irrigation (P < 0.0001) across the entire season (Appendix 1). Two days prior to soil sampling (September 17, 2014), biochar amended soils maintained higher volumetric soil moisture under both limited (P = 0.005) and full irrigation (P = 0.02) (Appendix 1). Despite this increase in soil moisture, maize yield showed no response to irrigation treatment (P = 0.21) or biochar amendment (P = 0.88) at the end of the season (Foster et al., 2016).

Soil food web modeling

The irrigation treatment did not have a significant effect on food web connectance, the proportion of possible trophic links realized in a food web (P = 0.16) (Table 5.2), or the number of functional groups (P = 0.18) (Table 5.2). The soil amendment treatment only had a significant effect on connectance at the 0.10 level (P = 0.10) (Table 5.2) but did not significantly affect the number of functional groups (P = 0.11) (Table 5.2). Collembola were not present in biochar amended plots and predatory nematodes were not present in control plots, but these findings were not statistically significant under Fischer's Exact Test (P = 0.48, 1 respectively). Although no significant differences in food web structure were observed for the different amendment and irrigation treatment combinations at the agroecosystem, marked structural changes became clear when comparing these agroecosystem soil food webs to the native grassland soil food webs presented in Andrés et al. (2016). Regardless of amendment and irrigation treatment combination, the agricultural soil food webs contain substantially fewer soil biota functional groups (S; P < 0.0001) and possessed higher connectance (P = 0.0001) compared to their natural grassland counterpart (Table 5.2; Andrés et al., 2016). More specifically, bacterial soil food web energetic pathways dominate the agroecosystem (Figure 5.1). Linkage density was much greater in agroecosystem soil food webs than in the native grassland soil food web (Table 5.2; P < 0.0001). Also, the maximum food chain length was much reduced in the agroecosystem soil food webs relative to the native grassland food web (Table 5.2; P < 0.0001).

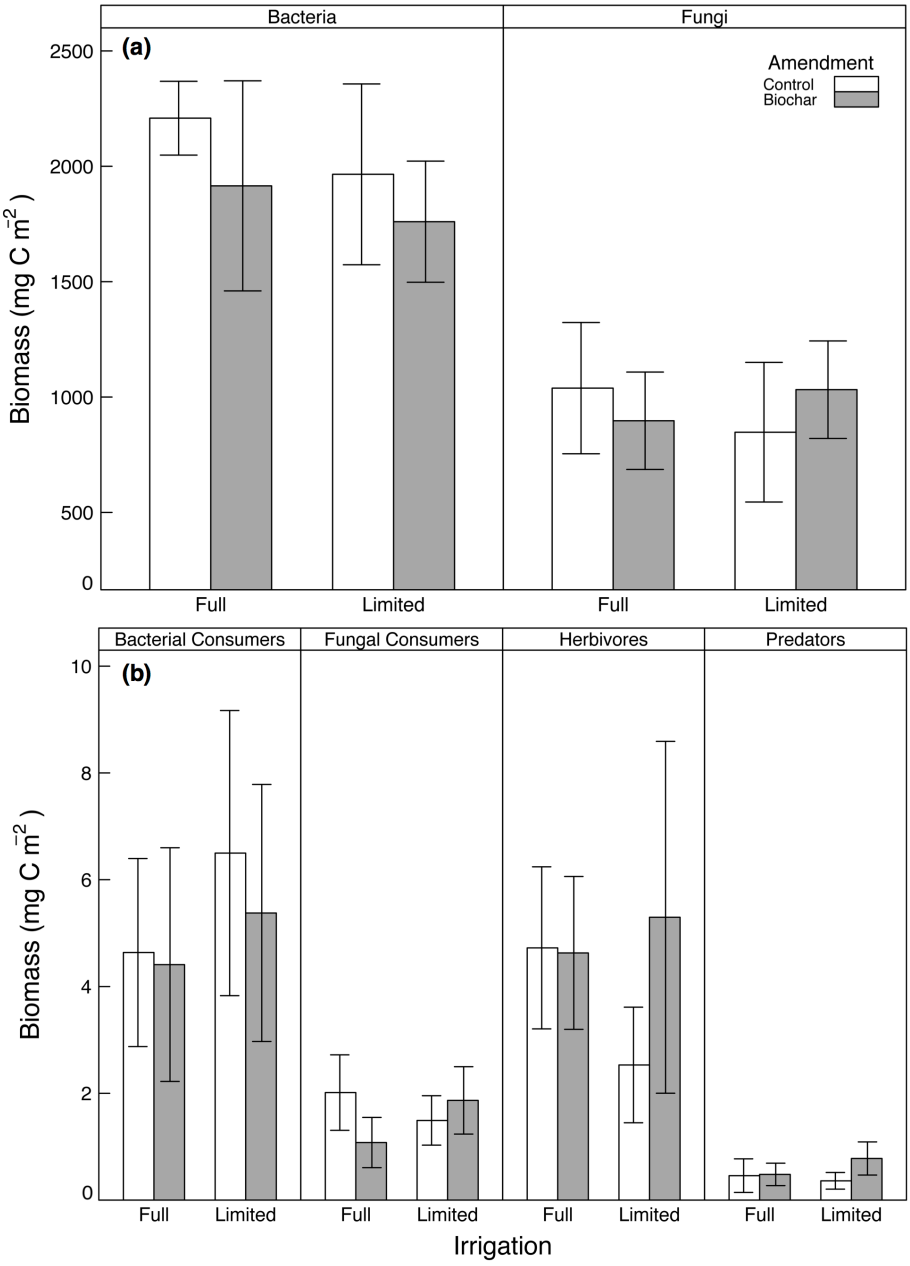


Figure 5.1. Biomass (mg C m^{-2}) of soil food web energetic pathways: primary consumers (a) and secondary and tertiary consumers (b) in control (white bars) and biochar (gray bars) amended plots under full and limited irrigation. Bars are mean ± SE of original (untransformed) data (n = 4).

Table 5.2. Summary of food web metrics for all irrigation and amendment treatment combinations for the agroecosystem and the native grassland (Andrés et al., 2016) soils. Number of basal resources (Sr), Number of Functional Groups (S), Connectance (C; proportion of possible trophic links realized), Linkage Density (LD), Maximum Food Chain Length (FCLmax), and Mean Food Chain Length (FCLmean) are mean of food webs ± SE (n = 8), except for the native grassland (n = 3).

Food Web	**Sr**	**S**	**C**	**LD**	**FCLmax**	**FCLmean**
Agroecosystem						
Full-Biochar	2 ± 0	11.38 ± 0.53	0.479 ± 0.04	5.30 ± 0.25	2.88 ± 0.13	2.16 ± 0.05
Limited-Biochar	2 ± 0	12.13 ± 0.30	0.406 ± 0.02	4.88 ± 0.13	3.13 ± 0.13	2.19 ± 0.08
Full-Control	2 ± 0	12.25 ± 0.31	0.398 ± 0.02	4.83 ± 0.14	3.00 ± 0.00	2.09 ± 0.03
Limited-Control	2 ± 0	12.63 ± 0.46	0.381 ± 0.03	4.70 ± 0.21	2.88 ± 0.13	2.15 ± 0.04
Native Grassland						
No Treatment	3 ± 0	18 ± 0	0.14 ± 0	2.52 ± 0	7 ± 0	-

We found no significant differences in modeled total soil respiration (irrigation: $P = 0.32$; amendment: $P= 0.27$) and total N mineralization (irrigation: $P = 0.71$; amendment: $P = 0.69$) between all irrigation and soil amendment treatment combinations (Table 5.3). However, in comparison to the native grassland soil food webs, modeled total soil respiration and N mineralization are lower in the agroecosystem soil food webs (Table 5.3). Modeled N mineralization is about four to eight times lower in the agroecosystem food webs than that of the grassland food webs (Table 5.3). Similarly, the agroecosystem food webs processed five to twelve times less C than the grassland food webs (Table 5.3). Both the total modeled N mineralization and C flux for the grassland food webs were significantly greater than for the agroecosystem food webs (N mineralization: $P < 0.0001$; C flux: $P = 0.0008$). When C flux was partitioned by soil biota functional groups within the food web, lower C processing capacity was observed in the agroecosystem soil food webs relative to the native grassland system (Figure 5.2). Carbon transferred from primary consumers (bacteria and fungi) to secondary consumers (bacterial consumers and fungal consumers) is much greater in the native grassland soil food webs (49 kg C ha^{-1} yr^{-1}), than in the agroecosystem soil food webs (6 kg C ha^{-1} yr^{-1}) (Figure 5.2). Figure 5.2a shows modeled C flux data

from the continuously grazed native grassland site B (GB in Andrés et al. 2016) because it has the lowest mineralization rates of all three sites (Table 5.3), and thus serves as a lower anchor for mineralization potential. Figure 5.2b displays modeled C flux data from the non-amended plot under full irrigation, which is the reference, "business as usual" management scenario in our maize agroecosystem. These data are not significantly different than any of the other soil amendment and irrigation treatment combinations (Table 5.3).

Table 5.3. Comparison of modeled total N mineralization (kg N ha^{-1} yr^{-1}) and CO_2 efflux (kg C ha^{-1} yr^{-1}) from soil food webs in all treatment combinations at the agroecosystem and grazed native grassland sites (Andrés et al., 2016). Agroecosystem estimates are mean ± SE (n = 4), average native grassland estimates are mean ± SE (n = 3).

Food Web	**N Mineralization (kg N ha^{-1} yr^{-1})**	**CO_2 Efflux (kg C ha^{-1} yr^{-1})**
Agroecosystem		
Full-Biochar	4.55 ± 1.26	41.86 ± 11.40
Limited-Biochar	4.34 ± 0.92	67.20 ± 12.75
Full-Control	4.38 ± 1.30	69.01 ± 9.02
Limited-Control	5.69 ± 0.73	86.41 ± 9.81
Native Grassland		
Site A	34.66	911.8
Site B	33.97	535.4
Site C	35.27	839.5
Average	34.633 ± 0.38	762.23 ± 115.32

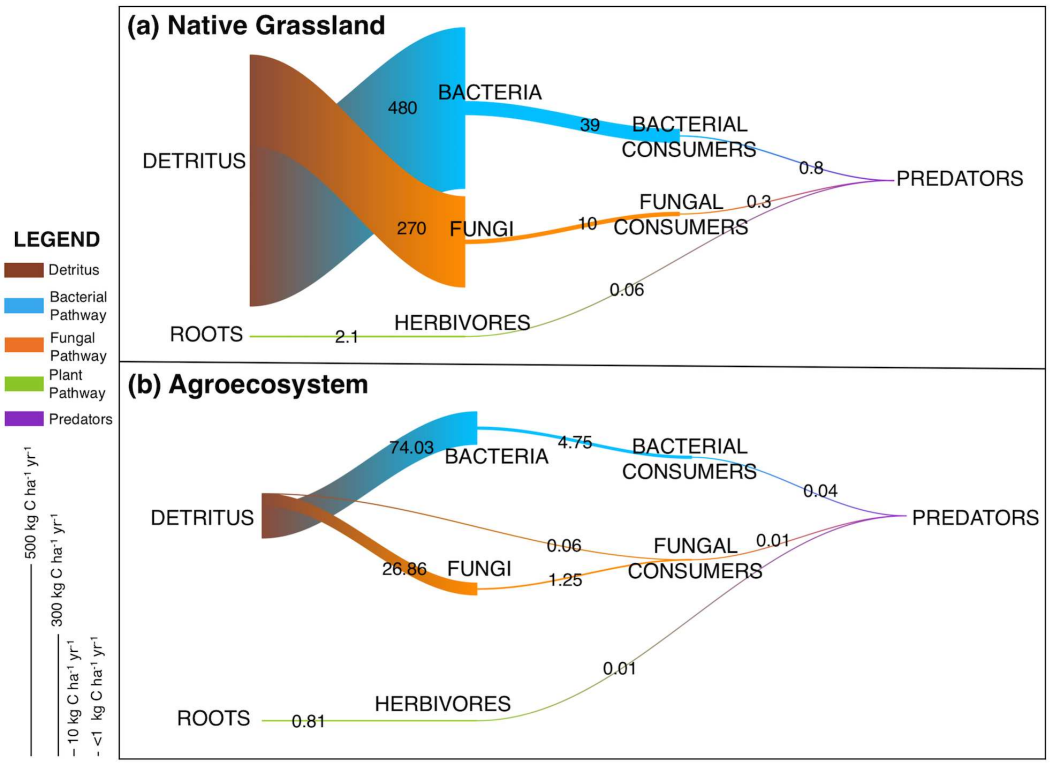


Figure 5.2. Modeled carbon flux (kg C ha^{-1} yr^{-1}) through the native grassland (site GB, Andrés et al., 2016) (a) and the agroecosystem (b) soil food webs. Vertical width of bars represent amount of carbon transferred between trophic levels. Colors represent energetic pathways as described in legend.

Discussion

As climate variability increases the strain of water resources on agriculture, management strategies that help mitigate these stressors have become especially important. Biochar amendment is of particular interest for its potential to increase water availability in soils (Atkinson et al., 2010), while also serving as a long term C management strategy in bioenergy production systems (Lehmann et al., 2006). However, the impacts of biochar on the soil biological community must first be critically evaluated before widespread application can be recommended. Overall, we did not see any effect of biochar amendment and irrigation treatment combinations on biomass of any soil biota functional groups, thus we reject our hypothesis that responses would differ among soil biota groups.

Counter to our hypothesis that limited irrigation would decrease nematode and protozoan biomass, we found no measurable effects of limited irrigation on any soil biota groups, regardless of biochar amendment (Figure 5.1). Likewise, our hypothesis that soil food webs would be dominated by different soil biota groups in biochar amended and non-amended plots was not supported, as we found no differences in either the biomass of soil biota (Table 5.1, Figure 5.1) or the structure and function of the soil food web (Table 5.2, 5.3). As hypothesized, biochar amendment did ameliorate the effect of limited irrigation on soil moisture by maintaining higher volumetric soil moisture relative to the control throughout the season and two days prior to sampling (Appendix 1). However, contrary to our expectation, the biochar-induced increase in soil moisture had no effect on the biomass and activity of the soil food web. Furthermore, the maize yield did not respond to either irrigation or amendment treatments (Foster et al., 2016).

The absence of significant effects of irrigation treatments on the soil biota and food webs is surprising considering that previous studies have shown positive responses of soil biota to increased soil moisture in irrigated agroecosystems (Schnürer et al., 1986; Wang et al., 2008; Li et al., 2010), although responses vary by functional group. Many natural and agricultural systems are water limited and soil biota (Hunt et al., 1987; Schnürer et al., 1986), enzymatic activity (Li et al., 2010), and microbial biomass (Li et al., 2010; Wang et al., 2008) often show differential responses to water additions. In a barley agroecosystem, soils that received just one water addition (limited irrigation) were compared to fully irrigated plots and had reduced fungal hyphal length, decreased bacterial numbers, and negligible changes in protozoan abundance (Schnürer et al., 1986). However, an increase in nematode abundance was observed in both limited and fully irrigated plots (Schnürer et al., 1986). This suggests that the response to irrigation treatments differs between soil biota groups, a trend we did not observe in our study likely because limited irrigation maintained adequate soil moisture for soil biota functioning. The fact that the crop showed no response to either treatment may explain our results given the tight coupling of soil biota to water and plant production in semi-arid regions (Collins et al., 2008).

Our results contribute to the mixed conclusions about the impacts of biochar on soil biota. While biochar has previously been observed to have substantial effects on soil biological community dynamics (Lehmann et al., 2011; Liu et al., 2016), numerous studies on the effects of biochar on soil microbial biomass have reported responses ranging from positive (Anderson et al., 2011; Domene et al., 2014; Gomez et al., 2014; Luo et al., 2013; Zhang et al., 2014b), to negative (Dempster et al., 2012), to neutral effects (Ameloot et al., 2014; Castaldi et al., 2011; Chen et al., 2013; Noyce et al., 2015; Rutigliano et al., 2014; Zhang et al., 2014a). Although not observed in our study, biochar has been shown to differentially affect primary consumers. Several studies suggest that pyrolyzed materials are preferentially consumed by bacteria, particularly gram positive bacteria (Gomez et al., 2013; Jiang et al., 2015; Santos et al., 2012; Soong et al., 2016), but biochar has also been observed to serve as suitable habitat for fungi (Hammer et al., 2014; Jaafar et al., 2014).

Studies that investigate the response of protozoa and soil fauna to biochar addition are particularly sparse (Lehmann et al., 2011). Our study is the first to explicitly examine the response of protozoan communities (amoebae, ciliates, and flagellates) to biochar addition. We did not observe an effect of biochar amendment on protozoan biomass or activity in this agroecosystem. Experimental investigations of microarthropod and nematode responses to biochar are limited and available results are contradicting. Microarthropods have been observed to both tolerate and avoid biochar in field and laboratory conditions (Bunting & Lundberg, 1987; Phillips et al., 2000; Salem et al., 2013; Domene et al., 2015). Although the absence of Collembolan in our biochar amended plots was not statistically significant, such biochar avoidance behavior has been observed (Domene et al., 2015). Our finding that soil nematode abundance and biomass did not respond to biochar addition after one year matches results from studies in a temperate grassland soil (Soong et al., 2016), a survey of forest soils (Matlack, 1999), and with the exception of increased fungivore abundance and decreased phytophagous nematode abundance, a report from a wheat agroecosystem (Zhang et al., 2013). While, caution must be taken when comparing man-made biochar with naturally occurring charcoal as the processes by which they are created can substantially alter the

way they function in soil and interact with soil biota (Lehmann & Joseph, 2015), the impacts of these materials on protozoan and soil invertebrates have largely been benign.

The soil food web model suggests little difference in food web structure (number of functional groups, connectance, and trophic links; Table 5.2) and no difference in function (C and N mineralization; Table 5.3) between biochar and irrigation treatments. Although connectance was slightly higher in the biochar-amended plots when averaging over irrigation treatment, this result was only significant at the $p = 0.10$ level. This effect is likely due to the lack of Collembolan in the biochar plots, resulting in lower number of functional groups, but this is not statistically significant (Table 5.2). Given the lack of significant differences in other structural and functional metrics of the soil food web, this marginally significant difference in Collembolan presence remains inconclusive. The similarity of modeled soil respiration levels between biochar-amended and non-amended plots suggests that biochar C was not mineralized to CO_2, indicating long-term C storage in these soils. Together, our results indicate that the soil biological community was not affected, structurally or functionally, by biochar amendment at a 30 Mg ha^{-1} application rate, temporal limited irrigation strategies, or their interaction after one year.

Soil food webs may be affected by biochar amendment through its indirect effect on soil pH (McCormack et al., 2013). In acidic soils, biochar may increase soil pH from very acidic (3-4) to slightly acidic (5-6) with potential consequences for soil food web structure and function (McCormack et al., 2013). However, the soil in our agricultural study site is basic (pH = 8.7) and no increase in pH was observed after biochar addition (Foster et al., 2016). The maintenance of soil pH after biochar addition may explain why we did not observe any effect of biochar amendment on soil biota biomass or food web structural and functional properties.

We propose two additional explanations for rejecting our initial hypotheses. First, our study duration (one year) and timing may have been too short to observe significant effects of coupled limited irrigation and biochar amendment. Our sampling occurred at the end of the growing season and may not have captured seasonal variability in responses of soil biota biomass to the treatments. Given the variability of

field conditions, climate, and soil biota biomass estimates, assessing soil biological response to biochar at one time point just one year after application may not have allowed for enough treatment-response time. For example, only after three years post biochar addition, in a temperate maize field in northern New York, Domene et al. (2014) found an increase in microbial abundance. However, in agreement with our results, no significant differences in mesofaunal activity were observed (Domene et al., 2014) suggesting that the stimulating effects of biochar addition were not transferred from microbial trophic levels through the rest of the soil food web even after three years. Moreover, field biomass estimates of soil biota were quite variable in our study (Table 5.1), resulting in greater difficultly detecting minor effect of the treatments at one time point.

Second, the biochar application rate used in our study (30 Mg ha^{-1}) may not have been enough to significantly alter directly the soil biota or the soil environment within which soil organisms interact and function. Our application rate was comparable to or greater than other field trials in temperate maize agroecosystems (Brantley et al., 2015; Domene et al., 2014; Jones et al., 2012), and larger application rates are not currently foreseen as feasible given high costs of biochar application (Field et al., 2013; Shackley et al., 2015).

Our results may be explained by the state of the agroecosystem food web before biochar and limited irrigation were applied. The agricultural field has been intensively managed with conventional techniques including frequent tillage and pesticide application, all of which have been shown to adversely affect the soil food web increasing soil organic matter decomposition with subsequent losses of soil fertility (Figure 5.2; Table 5.2, 5.3) (Moore et al., 1984; Hendrix et al., 1986; Moore, 1994; Neher, 1999; Kladivko, 2001). Additionally, agricultural conversion in general has been observed to negatively impact soil biota (Culman et al., 2010; DuPont et al., 2010). Therefore, we hypothesized that past agricultural practices may have degraded the soil food web to a point that biochar application and limited irrigation did not have significant effects. To examine this, we put our results in a larger agricultural context by comparing

the soil food web structural and functional properties from the agricultural site in this study to the soil food webs of an adjacent native grassland that served as an uncultivated reference (Andrés et al., 2016).

Clear structural differences were observed between the agroecosystem and native grassland soil food webs. The agroecosystem soil food webs had fewer functional groups present, and higher connectance values than that of the native grassland soil food webs (Table 5.3), suggesting a loss in functional diversity and trophic interactions among soil biota functional groups with the onset of agriculture. Several treatment combinations in the agroecosystem lacked ciliates, predatory nematodes and Collembola (Table 5.1) which were present in the grassland sites (Andrés et al., 2016), further confirming the loss of soil biota functional groups due to intensive agriculture. Additionally, the native grassland system supported greater food chain lengths, decreasing linkage density across the web (Table 5.2), suggesting greater food web stability than in the agroecosystem. The decrease in maximum food chain length in the agroecosystem relative to the native grassland food webs (Table 5.2) again points to a degradation of trophic structure during the agricultural conversion.

Functionally, the food webs within the agroecosystem also showed signs of being altered in all treatment combinations relative to those from the native grassland. Modeled total C and N mineralization are markedly reduced in the agroecosystem relative to the native grassland (Table 5.3). This suggests a loss of C and N cycling capacity in the agricultural soils, relative to their native grassland reference. In fact, the amount of C transferred from primary consumers (bacteria and fungi) to secondary consumers (bacterial and fungal consumers) is approximately 8 times greater in the native grassland than in the agroecosystem soil food webs (Figure 5.2), providing evidence that belowground C and N cycling has been altered as a result of agricultural conversion. We propose that, in this system and others (see Castracani et al., 2015), agricultural management impacts greatly outweigh any potential effects of biochar amendment and limited irrigation. For intensively managed agricultural systems, biochar addition and its possible effects on soil water and nutrient dynamics are not significant drivers of soil food web productivity and the ecosystem processes they regulate in the short term.

Our study is the first to take a comprehensive, field-based approach to assessing the impacts of coupled biochar amendment and limited irrigation on soil food web structure and function. Our findings suggest that, in the short term, biochar may not impact the entire soil food web in temperate agroecosystems, as has previously been observed for soil microorganisms alone (Lehmann et al., 2011). When considering the potential benefits of biochar as a C storage mechanism to close the C loop in bioenergy production, our results support continued research of biochar additions, as there were no negative effects on belowground food web structure and function. However, our comparison with the native grassland points to a bigger issue: the degradation of soil food web structure and function due to traditional agricultural management strategies. We contend that biochar amendment and limited irrigation likely did not have an effect on the soil food web because the soil biological community in our system is already degraded, lacks complexity, and functions at a reduced rate relative to its native grassland equivalent. In this setting, after one year, coupled limited irrigation and biochar amendment did not mitigate, nor further contribute to the negative effects of agricultural management in this semi-arid maize agroecosystem.

Given our findings and the short duration of our study, long-term studies are needed to gain insights on the ecological effects of biochar and limited irrigation on the soil food web. In addition to conventional agricultural settings, investigations of soil food web responses to biochar in alternative agricultural management systems, such as no till and organic, are important lines of inquiry. Examining how biochar may affect these soil food webs will lead to meaningful insights and management implications for key ecosystem services provided by soil biota.

Chapter 6: Teaching authentic soil and plant science in middle school classrooms with a biochar case study[5]

Introduction

Soils are the foundation of terrestrial ecosystems and produce food and fiber, filter freshwater, and regulate carbon cycling and therefore global climate (Wall et al., 2012). Demands for increased agricultural productivity, climate change, and impacts of drought (IPCC, 2014) put soils at the forefront of global environmental issues and provide unique opportunities for teaching real-world life science. Soils provide a great opportunity to study fundamental ecosystem processes in a local context because they are found everywhere! Most teachers and students have multiple ways to access soils - in their backyards, parks, local forests, or garden stores. Studying soils in classrooms is inexpensive, easy, and provides an ideal way to involve students in timely and authentic scientific inquiry.

We designed two classroom experiments focused on the use of biochar as a soil amendment to improve soil health. The activities are aligned with the Next Generation Science Standards at the middle school level but can be adapted to high school classrooms (NGSS Lead States, 2013). Biochar serves as an ideal case study for students learning fundamentals of soil and plant interactions because it is easily accessible, connected to global environmental issues, and is the focus of ongoing research. Studying biochar also supports students in engaging in authentic science by discussing data variability and interpreting contrasting research results.

Biochar is made by heating organic matter in the absence of oxygen (Figure 6.1). Indigenous people of the Amazon have long incorporated charcoal into soils to increase fertility and productivity in what would otherwise be infertile tropical soils. In South America, the treated soils are known as *Terra Preta*

[5] Accepted for publication in *The American Biology Teacher* with M. Hunter-Laszlo, S. Bucko, B.A. Covitt, S. Urban, C. Benton, M. Bartholomew, A.J. Morrison, E.J. Foster, S.D. Parker, M.F. Cotrufo, and J.C. Moore in 2018

de Indio soils, or Amazonian Dark Earths (Figure 6.2; Glaser et al., 2001). Scientists have found that biochar can increase soil moisture (Basso et al., 2013; Yu et al., 2013) and stimulate the growth and activity of soil microorganisms (e.g., bacteria and fungi) to enhance nutrient availability to plants (Biederman and Harpole, 2013; Liu et al., 2016). Current investigations focus on how biochar might enhance soil fertility and improve crop productivity (Lehmann, 2007).

Figure 6.1. Biochar is the carbon-rich product of biomass charring. The biochar pictured here is derived from palm fronds (A) and pine wood (B,C). It is highly porous (A), heterogeneous in structure (B), and is amended to agricultural soils to improve soil fertility (B). (Credit: image A is by N. Ziv; images B and C are by E.J. Foster)

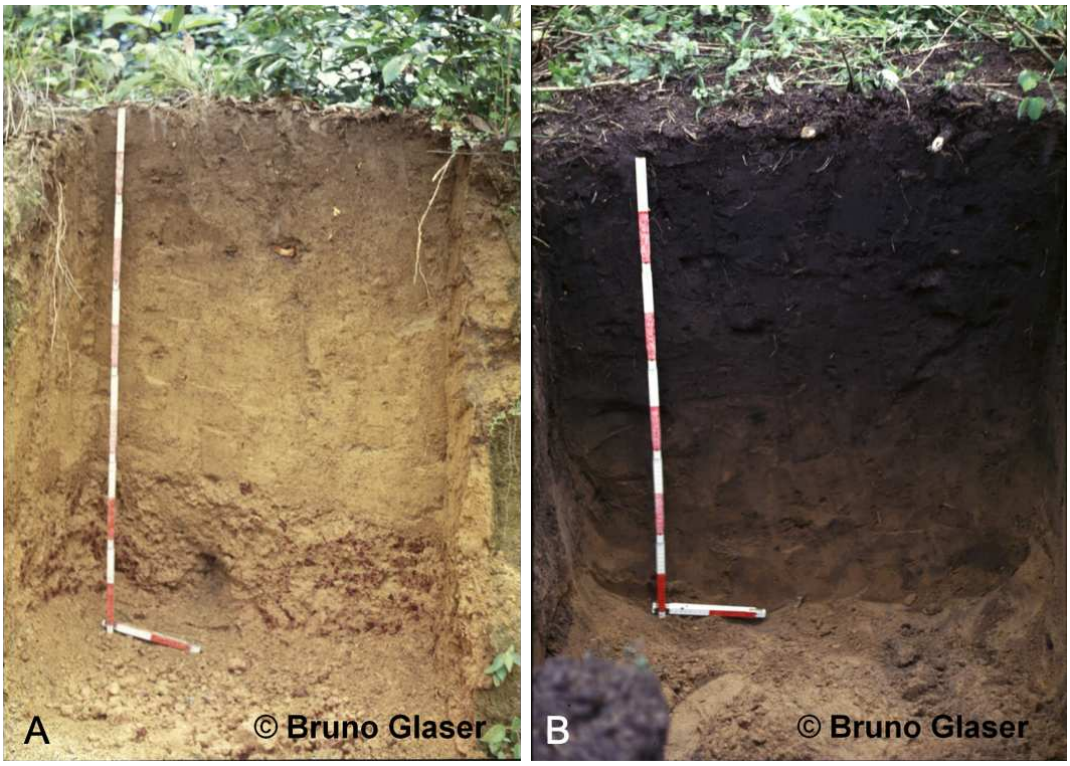


Figure 6.2. A typical tropical soil (A) compared to a *Terra Preta de Indio* soil (B). (Credit: images are by B. Glaser)

The impacts of biochar amendment depends on the type of biochar (i.e., from what and how it was produced) and the soil type (e.g., texture, organic matter content, nutrient availability, acidity; Lehmann and Joseph, 2015). These factors explain why many biochar experiments yield different results. So far, the greatest effects of biochar have been seen in tropical, arid, and acidic soils (Jeffery et al., 2011). Research determining which types of biochar improve soil moisture and nutrient availability is still ongoing (Zhang et al., 2016).

Aside from improving soils for plant growth, research suggests that the production and application of biochar may store carbon in soils for decades to millennia, reduce the net amount of carbon released to the atmosphere, and help mitigate rising atmospheric CO_2 concentrations responsible for climate change (Liu et al., 2016; Figure 6.3). The pyrolysis process to make biochar requires carbon and energy inputs. By using a local feedstock for the pyrolysis process or even using the solid waste product from biofuel production, biochar can sequester more carbon than is used in its production process (Field et al., 2012;

Woolf et al., 2010). Biochar alone is not a sufficient strategy to address climate change, but it can serve as one part of a multipronged approach to mitigating climate change.

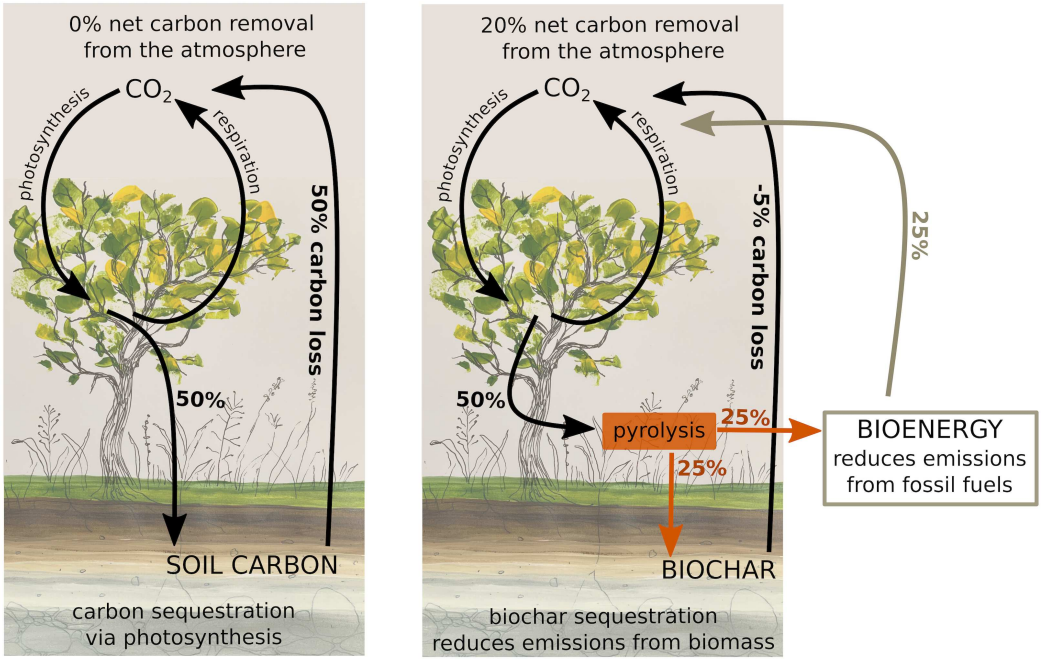


Figure 6.3. The natural carbon cycle (left) compared with the biochar carbon cycle (right). In the natural carbon cycle, carbon withdrawn from the atmosphere through photosynthesis is balanced by carbon released to the atmosphere through respiration. In the biochar carbon cycle, a portion of the carbon withdrawn via photosynthesis is used for bioenergy production, which results in a biochar co-product that can be sequestered in soils. (Credit: image redrawn and adapted from Lehmann (2007))

We present two classroom experiments that investigate the effect of biochar on soils and plants. We align these activities with Next Generation Science Standards (NGSS; NGSS Lead States, 2013) by integrating disciplinary core ideas, science practices, and crosscutting concepts. The activities' objectives are to: (1) Gain an appreciation for soils as an important natural resource, (2) explore issues in soils and agriculture, and examine biochar as a possible solution, (3) manipulate soil properties and biochar amendment to investigate impacts on soils and plants, and (4) conduct an experiment, collect and analyze data, and engage in scientific inquiry to interpret real-world data.

Properties of Biochar

Biochar is produced by breaking-down organic matter under high heat (250 - 700ºC) and limited oxygen (Lehmann and Joseph, 2015). Biochar is often a co-product of bioenergy derived from naturally renewable organic matter sources, including agricultural products, such as crop and animal waste, and non-agricultural products such as woody biomass and algae (Table 6.1). The physical and chemical properties of biochar vary depending on its source and how it is produced. Different sources and production temperatures yield biochars with different surface areas, pore sizes, pH values, and carbon contents. Biochar surface area is a function of pore size, with smaller pore sizes resulting in higher surface area biochars. Pore size, and thus surface area, is controlled by the temperature at which biochars are produced (Lehmann and Joseph, 2015). As a consequence of this variability, not all biochars interact with the environment in the same way. Students can draw on their understanding of the variable properties of biochar to explain their own experimental results.

Biochar is easy to obtain from garden stores or online (www.biochar-international.org). Teachers should read the material safety data sheet provided by biochar producers and handle biochar appropriately. Dust inhalation is an important but manageable safety concern. We recommend purchasing pelletized biochar products or products with > 3 mm particle size. To minimize dust, take care when opening a bag of biochar as dust may accumulate during transport. Wear facemasks and gloves when handling raw biochar. Spraying or adding small amounts of water to biochar can reduce dust. Students can observe large pellets of biochar under the microscope and handle the materials after the biochar has been added to soils.

Table 6.1. Properties of biochar from different feedstocks including surface area, total carbon and pH. Values are taken from research studies that incorporated different biochars into soils. Values vary depending on production conditions.

Biochar Feedstock		Production Temperature (°C)	Surface Area (m^2 g^{-1})	Total C (%)	pH
Agricultural crop	Corn stover[a]	550	12	74.3	9.89
	Wheat Straw[b]	200	2.53	38.7	5.43
	Wheat Straw[b]	350	3.48	59.8	8.69
	Wheat Straw[b]	500	33.2	62.9	10.2
Nonagricultural organic matter	Pine[c]	400-700	232.72	72	9.2
	Grass[b]	500	3.33	62.1	10.2
	Bamboo[d]	300	1.3	66.2	7.9
	Algae[e]	305	1.15	28.9	8.0
Animal Waste	Cow Manure[b]	500	21.9	43.7	10.2
	Pig Manure[b]	200	3.59	37.0	8.22
	Pig Manure[b]	350	4.26	39.1	9.65
	Pig Manure[b]	500	47.4	42.7	10.5

[a]Fuertes et al. (2010) [b]Zhao et al. (2013) [c]Foster et al. (2016) [d]Sun et al. (2014) [e]Bird et al. (2011)

Connections to Next Generation Science Standards

We developed two classroom experiments that mirror recent experiments conducted by scientists: effects of biochar on (1) plant growth and (2) soil respiration. Both experiments align with NGSS through addressing disciplinary core ideas, crosscutting concepts, and science practices (Table 6.2). The experiments follow the claim, evidence, reasoning (CER) instructional framework (McNeill and Krajcik, 2008), in which students construct a scientific argument based on experimental evidence from the students' experiments and from published studies. This integrated approach encourages students to use multiple lines of evidence, including their own observations, to support a claim with reasoning.

Table 6.2. NGSS Performance Expectations linked to plant and soil science classroom experiments.

NGSS Performance Expectation	**Associated Learning Goals for Plant Growth Experiment**	**Associated Learning Goals for Soil Respiration Experiment**
MS-LS1-5. Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms.	Explain how and why environmental factors including soil properties (e.g., texture, organic matter content, moisture, nutrients, pH, biochar) may impact plant growth metrics including height, aboveground biomass, and belowground biomass.	Explain how and why environmental factors including soil properties (e.g., texture, organic matter content, moisture, nutrients, pH, biochar) may impact soil biota respiration as measured by CO_2 flux.
MS-LS2-1. Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.	Collect and analyze data, identify patterns, and provide evidence concerning relationships among resource availability, biochar amendment, soil type, and plant growth metrics.	Collect and analyze data, identify patterns, and provide evidence concerning relationships among resource availability, biochar amendment, soil type, and soil biota respiration.
MS-ETSI-1. Define the criteria and constraints of a design problem with sufficient precision to ensure a successful solution, taking into account relevant scientific principles and potential impacts on people and the natural environment that may limit possible solutions.	Use knowledge and practice developed through the plant growth investigation to propose and evaluate whether/how biochar amendment would be appropriate to increase crop yield in different conditions - considering issues such as soil and crop type.	Use knowledge and practice developed through the soil respiration investigation to propose and evaluate whether/how biochar amendment would be an appropriate strategy for increasing soil carbon storage in different conditions - considering issues such as soil type.

Soil and Plant Science Classroom Investigations

We describe two classroom experiments that were co-developed by K-12 educators and university researchers and conducted in classroom settings. The data shown were collected by students. Prior to conducting the experiments, provide students with an introduction to biochar and its application in soils and agriculture. Ask students to observe biochar and different soil types under a microscope and discuss differences in properties such as porosity (Figure 6.1) and pH (Appendix 2A). Discuss how different properties of the biochar and soil they observed might influence plant growth and soil respiration.

Experiment 1: plant growth

Biochar is typically added to agricultural soils and scientists are interested in how biochar will affect crop productivity. Here, students conduct an analogous experiment in which they ask the question, "How does biochar affect plant growth in different soil types?". Prompt students to make a prediction about whether biochar will increase, decrease, or have no effect on plant growth and to explain their ideas about why (Appendix 2B). Ask them to list the dependent variables they would measure in order to answer this question (Appendix 2B). Variables may include plant height, plant biomass, stem diameter, and number of leaves, among others. Scientists typically assess crop productivity by measuring plant height and aboveground (shoot) and belowground (root) biomass, and compare the amount of biomass plants have allocated to roots in relation to total plant biomass (root mass ratio). Students could measure plant height and aboveground and belowground biomass, and other variables that emerge from your classroom discussions.

Supplies

- Pots with holes at the bottom (3 in2 (7.6 cm2) recommended)
- Window screen mesh
- Commercial potting soil (fill to 80% volume of the pot)

- Topsoil (collected locally; fill to 80% volume of the pot)
- Sand (fill to 80% volume of the pot)
- Biochar (10% by volume)
- Balance
- Forceps
- Foil
- Mung bean seeds
- pH paper or probes
- Deionized water
- Metric ruler
- Camera to document growth

Experimental design and protocols

The experiment is a comparative manipulative study with three soil types (potting soil, top soil, and sand) and two biochar amendments (no biochar [control] and 10% biochar). Prior to the experiment, germinate the Mung bean seeds by placing them between two damp paper towels for 24 hours. Prepare half the soil for biochar amendment by mixing in 10% biochar by volume; a concentration that is typically used in laboratory experiments and agricultural fields (Biederman and Harpole, 2013). Leave the other half of the soil unamended as the control. Clearly label the pots with soils with the appropriate six experimental treatments (potting soil control; potting soil biochar; top soil control; top soil biochar; sand control; sand biochar). Place germinated seeds on the soil surface and cover with 3-5 mm of soil. Place the potted plants under fluorescent grow lights (13-50 watt bulbs) and maintain them at room temperature. Each experimental treatment should be replicated at least three times to make it possible to investigate variability by calculating means and standard deviations. If necessary, use pipe cleaners or other wires to help keep plants upright during the experiment. Add more water immediately after planting and as needed.

Teacher considerations

We grew fast-growing and easy-to-maintain Mung beans in natural topsoil, commercial potting soil, and sand, but other plant species and soil types may be used. Have students work in groups to maintain one replicate of a soil type and biochar treatment combination (e.g., potting soil control and potting soil biochar).

Student activities

Plant growth

Prepare and label each pot with the appropriate experimental treatment for your group as described above. Record the date of planting and the date that the plants break through the soil surface. After each watering, record visual observations about the plant. On day 7, select the tallest plant and measure the height (cm) with a ruler. Place a toothpick near your selected plant to mark which plant you will measure each week and record the height of this plant after each watering (Appendix 2C). Take a photo of your plants each week. Plants are delicate and must be handled carefully each time you measure them to prevent them from breaking.

Plant biomass harvest

After at least three weeks, take a final height measurement and then harvest plant biomass. Gently remove the plants and soil from the pot. Using forceps and a small paintbrush, carefully remove soil from the roots. You can rinse the roots with water to remove remaining soil. Separate the roots (belowground biomass) from the stems and leaves (aboveground biomass). Place the plant material into foil labeled with the experimental treatment and aboveground or belowground biomass. Fold the aluminum foil to minimize losses during transport. Dry the plant material in an oven at 60 ºC for at least 24 hours. You can leave the plant material in the oven over the weekend for up to 72 hours. Weigh dried biomass and record (Appendix 2C).

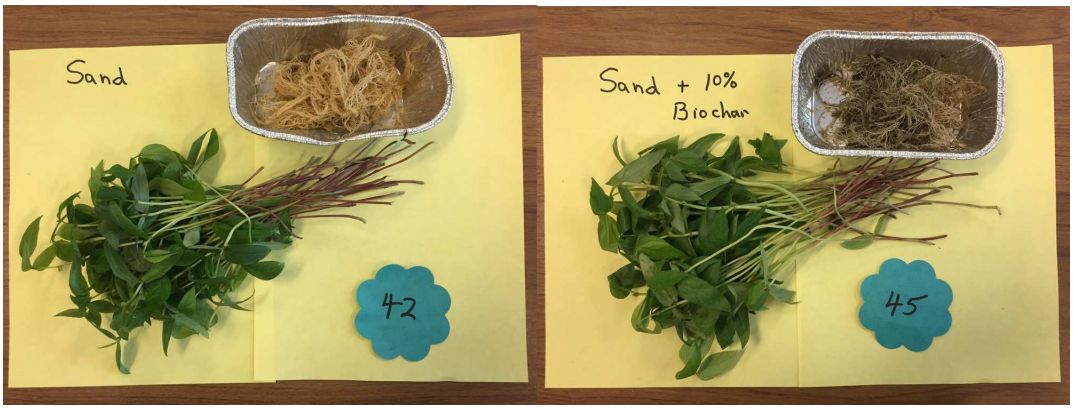


Figure 6.4. Example of aboveground and belowground biomass harvest from sand – control and sand - biochar treatments for plant growth classroom experiment. Aboveground biomass (shoots) and belowground biomass (roots) are separated, dried, and weighed. (Credit: Photos by M. Hunter-Laszlo)

Soil pH

Students can also investigate the impact of biochar on soil pH as an indicator of the chemical and biological environment of the soil (Appendix 2A). Soil pH is considered the "master variable" in determining which soil organisms are present in the soil and how they function (Fierer and Jackson, 2006; Wu et al., 2011). Protocols for measuring soil pH are detailed in Appendix 2A.

Data analysis

Students can graph plant growth over time using plant height data from each treatment over the experimental period (Figure 6.5). Plant biomass data can be plotted in a bar graph as shown in Figure 6.6. Students can then clearly see the differences between aboveground and belowground biomass production. Notice that belowground responses do not necessarily mimic aboveground responses to the same treatment. Plant and soil scientists commonly represent these data as ratios. Students can calculate and graph root mass ratios from their biomass data by dividing root biomass by the total plant biomass (Appendix 2C and Figure 6.7). Students can also calculate means and standard deviations and add these to the graphs (Figures 6.5, 6.6, and 6.7).

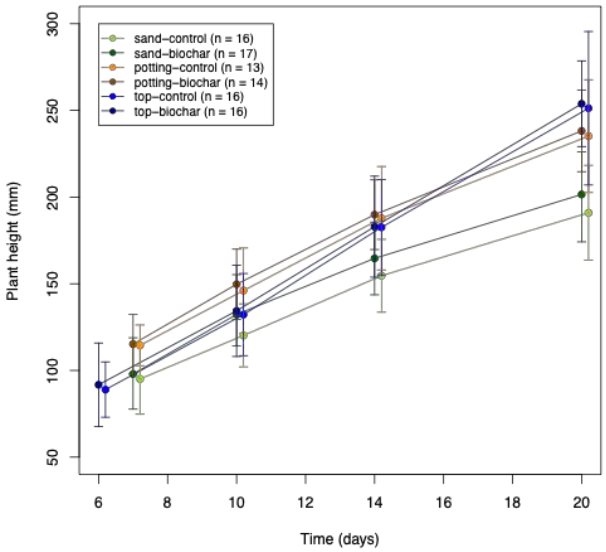


Figure 6.5. Plant height data collected by students during plant growth experiment. Points represent means. Error bars represent one standard deviation from the mean. Numbers in legend are number of replicates within each treatment.

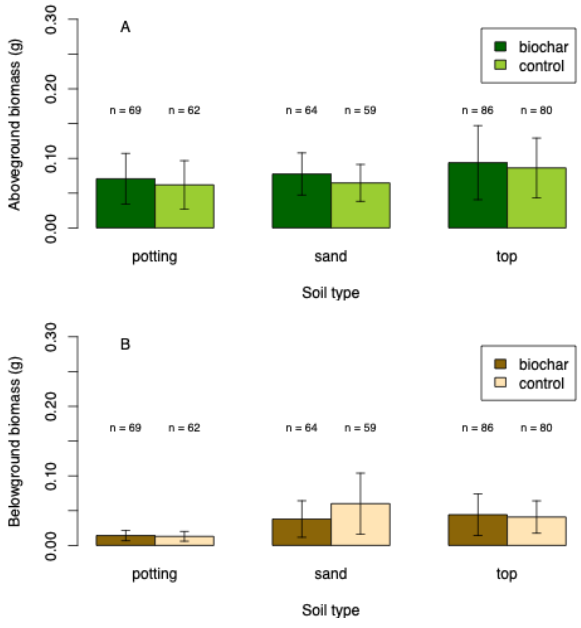


Figure 6.6. Aboveground (a) and belowground (b) biomass data collected by students during plant growth experiment. Bars represent means. Error bars represent one standard deviation from the mean. Sample sizes shown above bars are number of plant replicates in each treatment.

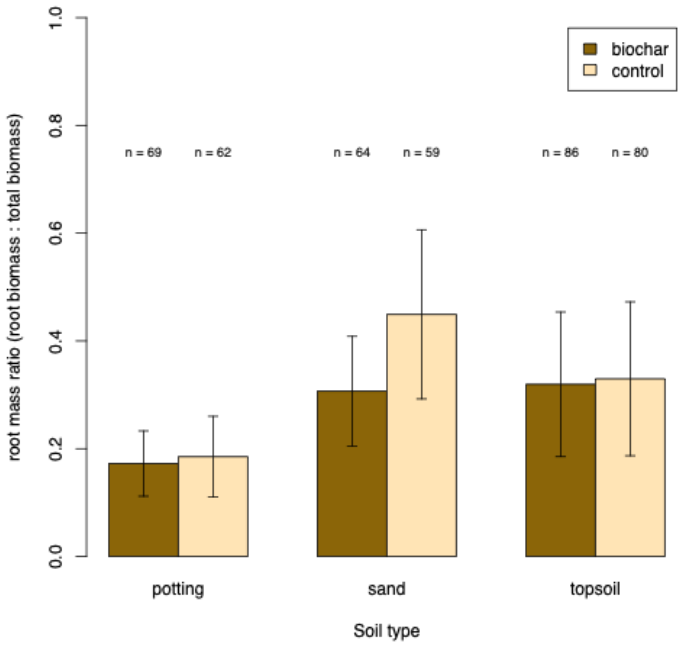


Figure 6.7. Ratios of root mass to total plant biomass from data collected by students during plant growth experiment. Bars represent means. Error bars represent one standard deviation from the mean. Sample sizes shown above bars are number of plant replicates in each treatment.

Experiment 2: soil respiration

Students conduct an experiment in which they add biochar to soil and compost and measure the response of soil respiration. Students ask, "How does adding biochar to soil and compost affect soil respiration?" Based on their observations of biochar, prompt your students to predict whether biochar will increase, decrease, or not affect soil respiration and to explain their ideas about why before they begin the experiment. Soil microbes metabolize organic and inorganic compounds via extracellular enzymes, respire carbon dioxide (CO_2) and excrete nitrogen as by-products of growth and reproduction (Schimel and Bennett, 2004; Paul, 2014). Scientists measure the flux of CO_2, or "soil respiration," to assess microbial activity and rates of CO_2 entering the atmosphere. Scientists are also interested in the nitrogen released by microbial activity because nitrogen is necessary for plant growth.

Supplies

- Garden soil
- Compost
- Biochar
- Buckets for soil collection
- 50 mL graduated cylinders
- CO_2 probe, sensor, or gas detecion tube
- Data collection device
- Sample container (200 mL minimum)
- Deionized water

Experimental design and protocols

We collected garden soil and compost, but other soil types may be used. Compost supports high microbial activity and respiration and serves as a useful comparison with natural soils. Use half of each soil type as the unamended control and prepare the other half with biochar amendment by adding 10% biochar by volume. Label the soil and compost with the four experimental treatments (compost control; compost biochar; garden soil control; garden soil biochar) and leave them open to air for a day before taking measurements to allow soils and microbial communities to re-equilibrate after the disturbance of set up. Prepare the soil or compost sample by filling the sample container to the 150 mL mark with your assigned soil. Label the bottle with your group name, soil type (compost or garden soil) and experimental treatment (control or biochar). Using a graduated cylinder, moisten the soil with 20 mL of water. Set up the experiment away from windows to maintain stable temperature conditions (ideally around 25-30 °C). Replicate each treatment at least three times to calculate means and standard deviations. Prepare the CO_2 measurement instrument for data collection. For probes or sensors, allow the instrument to warm up until the readings begin to stabilize. Calibrate the sensor before setting up the experiment. Set up the data collection device to take a measurement every 1 minute for 24 hours (1440 measurements). Place the CO_2

sensor into the sample container (Figure 6.8). For gas detection tubes, measure CO_2 concentration at least three times at the start, at 30 minutes, and at 24 hours. You may adjust intervals to fit your classroom and scheduling needs.

Teacher considerations

Organize students into groups of two to four to conduct the experiment on one of the four treatments. Treatment combinations and replicates can be spread across class periods and students can analyze the combined data from all classes. Test the CO_2 probes or sensors to ensure they are working properly before beginning the experiment with students. For CO_2 gas detection tubes, ensure that the CO_2 concentration of your soil and compost is within the detectable range of the instrument. Prior to setting up the experiment, determine the number of measurements you will make over the course of the experiment and the time between measurements. Consider using a larger container to avoid inhibiting respiration via oxygen depletion if you conduct the experiment over a longer period.

Student activities

Prepare and label each pot with the appropriate experimental treatment for your group and familiarize yourself with the CO_2 probes or sensors as described above. Start collecting data. Download the data after 24 hours. If you are using CO_2 gas detection tubes, keep the sample container closed until you are ready to make a measurement. Place the tube into the sample container and read the CO_2 concentration from the side of the tube. Repeat this at the time intervals you have chosen.

Figure 6.8. Example set up for one experimental treatment replicate for soil respiration classroom investigation using a CO_2 sensor and data collection device. (Credit: Photo by S. Bucko)

Data analysis

Students can graph the cumulative CO_2 concentration for each treatment over the 24-hour period (Figure 6.9). In our experiments, students observed two patterns: (1) CO_2 concentration increased over time and eventually leveled off in the compost treatments. This pattern could be due to a decline in oxygen concentration inhibiting respiration in the small chamber, a decrease in organic matter availability, or, in the case of the compost treatment, CO_2 concentration exceeding the detection limit of the sensor, (2) CO_2 concentration differed between compost and garden soil. The cumulative CO_2 concentration tends to be higher in compost with biochar than without it, but lower in garden soil with biochar than without it (Figure 6.9). Biochar may have impacted soil pH or moisture that positively affected microorganisms and thus cumulative CO_2 concentration in compost, but not in garden soil. However, cumulative CO_2 concentration was not statistically different between the control and biochar treatments for either soil type. Discuss these results with your students and ask them whether their results confirmed or conflicted with their predictions and why.

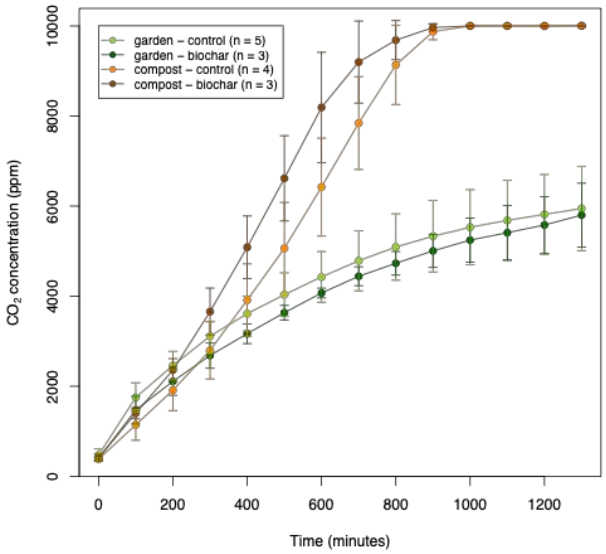


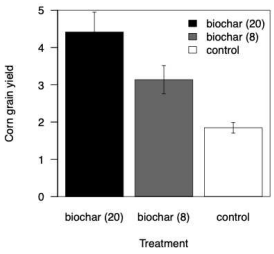
Figure 6.9. CO_2 concentration data collected by students during soil respiration classroom experiment. Points represent means. Error bars represent one standard deviation from the mean. Sample sizes shown in the legend are number of replicates in each treatment.

Data Interpretation

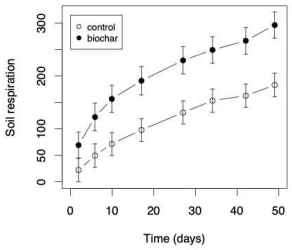
Students can compare their findings with other experimental results from working scientists (Figure 6.10). As is often the case, results from other experiments may be similar to or quite different from classroom findings. Given the variability in biochar and soil properties, it is likely that classroom findings will differ from findings of other experiments. Results in Figure 6.10 were taken from published field, greenhouse, and laboratory experiments to show variability in responses of plant growth (A & B) and soil respiration (C & D) to biochar addition. These previous experiments have shown positive, negative, and neutral results of biochar soil amendment depending on the soil type and the biochar properties. One reason for this variability is soil pH (Appendix 2A). Differences in pH between biochar and control treatments and soil types may help explain differences in plant growth and soil respiration. For example, biochar has a large effect on corn yield in an acidic soil (Figure 6.10A), whereas biochar has no

measurable effect on corn yield in a basic soil (Figure 6.10B). Students can measure soil pH and visualize differences between treatments by graphing dot charts (Appendix 2A).

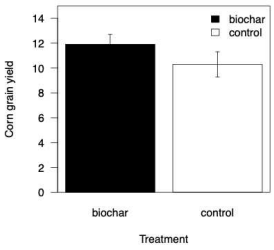
Comparing classroom experimental designs and results with previous experiments gives context to the students' work and allows for meaningful discussion about the nature of scientific investigation. For example, our classroom soil respiration experiment was conducted over a 24-hour period, while the laboratory (Figure 6.10C) and greenhouse (Figure 6.10D) soil respiration experiments were conducted over 50 days and 10 weeks, respectively. Ask your students why the soil respiration results over 10 weeks differ from those of their 24-hour experiment. In this case, the classroom experimental design resulted in a CO_2 concentration that reached the maximum detectable limit in the compost treatments (Figure 6.9), whereas the garden-soil CO_2 concentrations continued to increase similar to the results in Figure 6.10. Use these results as an opportunity for students to objectively critique the experimental design and suggest ways to improve it, rather than criticizing themselves for "doing it wrong." Teachers and students can refer to Figure 6.10 to discuss variability in experimental results and show that scientific experiments rarely come to one "right" answer or conclusion. Using the CER framework, task students to use multiple pieces of experimental evidence from classroom experiments and other experiments to develop a scientific argument (Appendix 2B).



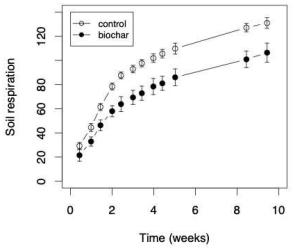
(A) Biochar increases corn yield (in tons per hectare) in a tropical savanna soil. Tropical soils are generally less fertile than temperate agricultural soils. Adding biochar to these soils has a greater effect on plant growth. Numbers next to biochar treatments indicate the amount of biochar in tons per hectare that was added to the soil. Data from Major et al. (2010).



(C) Biochar increases soil respiration (in microgram of C in CO_2 per gram of soil) in a temperate soil. Biochar can stimulate microbial activity in soils with already active microbial communities. Data from Smith, Collins, and Bailey (2010).



(B) Biochar has no meaningful effect on corn yield (in megagrams per hectare) in a temperate agricultural soil. Biochar can increase soil moisture, but this experiment occurred in a wet year, so biochar did not have a meaningful effect on plant growth. Data from Foster et al. (2016).



(D) Biochar decreases soil respiration (in milligrams of C in CO_2 per kg soil) in a coarse textured soil. This is an effect known as negative priming where biochar suppresses microbial activity when added to soil. This could be due to the chemical properties of the biochar (e.g. high pH). Data from Dempster et al. (2012).

Study	Plant Growth		Soil Respiration	
	A	B	C	D
Soil type	Clayey, tropical	Loam, temperate	Silt loam, temperate	Sandy, tropical
Soil pH	3.9	8.7	5.4	4.8
Type of biochar	Wood	Pine wood	Switchgrass	Jarrah wood
Amount of biochar	0, 8, 20 tons per hectare	0, 30 megagrams per hectare	0, 44.8 megagrams per hectare	0, 5 tons per hectare
Type of experiment	Field study	Field study	Lab study	Greenhouse study

Figure 6.10. Previous experiments show the variation in responses of plant growth (A, B) and soil respiration (C, D) to biochar addition. Details of the soil type, soil pH, type of biochar, amount of biochar and experimental designs are given in the table below the graphs.

Conclusion

The plant growth and soil respiration experiments align with NGSS and the CER framework to engage students in an authentic science experience. Students will develop soil and plant disciplinary knowledge and gain experience with science practices. Together, students will craft a scientific argument using data generated both by their class and by professional scientists. This integrated approach connects students to ongoing, relevant research in soil and plant science.

Chapter 7: Conclusion

Broadly, my dissertation aims to determine the consequences of fire disturbance and agricultural management for the ecology of soils. To do so, I considered a number of soil ecology disciplinary elements including soil microorganisms and fauna, soil food webs, and soil organic matter (SOM). Throughout the preceding chapters, I presented studies that investigated each of these elements in the context of single fire events, fire regimes, and agricultural management strategies using observational, experimental, synthesis, and modeling approaches. I ended each chapter by describing discipline-specific conclusions for each study. Here, I discuss two overarching themes that emerge when considering all the studies together. First, the responses of soil communities and SOM to disturbance are highly context dependent. Second, due to this context-dependency, hidden, unexpected, and sometimes contradictory patterns appear when considering the same ecological questions in a new context. To conclude, I reflect on how my experience completing my dissertation informs my non-linear approach to science.

Soil ecological responses to disturbance and management are context dependent

The answer to the central question of my dissertation — what are the consequences of disturbance and management for the ecology of soils? — is not a straightforward one. The unsatisfying and familiar ecological refrain, "it depends", may perhaps be the single best answer to this broad question. However, the research presented in my dissertation does provide insight to the natural follow-up question of, "depends on what?". In the meta-analysis in Chapter 2 that sought to determine how fire affects soil biota biomass, abundance, and diversity, I found that the responses of the soil community depended on the organism. I found that overall, fire has a strong negative effect on soil biota, but the effect depended on taxa. Soil microorganisms as whole, and particularly fungi are very sensitive to fire, while soil fauna are surprisingly resistant to fire. The fire return interval experiment presented in Chapter 3 told yet another

story. In this case, the response of soil community complexity and stability depended on fire frequency, primarily through an increase in soil pH with more frequent fires.

In Chapters 4 and 5, the consequences of disturbance for soils depended more on the spatial and temporal scales upon which we observed these phenomena. In Chapter 4, I took a closer look at the consequences of fire for SOM by seeking to understand the distribution of pyrogenic carbon (PyC) in soils across Europe. I found that PyC comprises a much lower proportion of soil organic carbon than previously suggested, and that matrix heterogeneity plays an important role in our ability to predict PyC content in soils. Together, these findings both illustrate the power of spatial scale in modulating our inference about a certain ecological pattern or process. In this case, our previous understanding of PyC in soils was based on studies that focused on smaller spatial scales with datasets that were able to minimize matrix heterogeneity. The work presented in Chapter 4 considered PyC in soils at the continental scale and, as a result, came to much different conclusions.

Similarly, the responses of soil food webs to agricultural management strategies presented in Chapter 5 depended on the temporal scale of our observations. Short-term management strategies, such as biochar amendment, did not have meaningful effects on the soil food web. However, when I compared the soil food webs from the conventional agricultural system with those of its native grassland counterpart, I found profound differences in their structure and function. I found that the long-term effects of historical management and land use conversion outweighed any effects of short-term agricultural practices. In Chapter 6, I illustrated the context-dependency of biochar amendment in analogous K-12 classroom experiments. I asked students to compare the results of their experiments to other findings in the scientific literature and consider why their results may suggest a different effect of biochar on plants and soils than the one they expected.

Context dependency reveals hidden, unexpected, and contradictory patterns

Given that the responses of soil to disturbance is context dependent, the studies presented in my dissertation revealed a number of previously hidden, unexpected, and even contradictory results. In

Chapter 2, I concluded that soil microorganisms are more sensitive and less resilient to fire than soil fauna. This finding was quite unexpected and counterintuitive given that soil microorganisms have shorter generation times and faster turnover rates than soil fauna. I expected soil microbial communities to be able to recover more quickly from fire than fauna, yet I do not have evidence to support this claim. I also found contradictory results across studies. Chapters 2 and 3, for example, come to opposite conclusions. In Chapter 2, I found that fire has profound and long-lasting negative effects on the biomass of soil microorganisms, whereas in Chapter 3, I found no evidence for differences in soil microbial biomass across the fire return interval treatments. Contradictions even emerged within the same study. In Chapter 3, I found that while fire had no measurable effect on soil biota biomass, soil food web structure, or belowground functioning, I did see an effect of fire frequency on soil food web stability.

These contradictions again illustrate the importance of approaching a scientific question from many different angles, because the results of one approach may not align with the results of another. Scientific progress is often made when attempting to reconcile such convergent results. It is tempting to explain away these contradictions and anomalies under the guise of context-dependencies, as I have throughout my dissertation. Here, however, I find myself pushing up against the bounds of "normal science" (sensu Kuhn, 1962). There is a fundamental human tendency to explain away the things we do not yet understand. Scientists often put their ideas before their data and explain away anomalies until they can no longer ignore the evidence that has been in front of them all along (Kuhn, 1962). Unexpected, contradictory, and null results are often the beginnings of a shift in our scientific understanding. In attempting to reconcile the fact that soil communities can be both highly sensitive and resistant to disturbance, the results of my dissertation hint at changing perspectives on the consequences of disturbance for soil communities.

Science is a non-linear process

I started my dissertation with an interest in soils, their inhabitants, and the people that study them. I now complete my dissertation with a deep appreciation for these, and a newly acquired connection to

writing and the nature of science that will inform my career as a soil ecologist for many years to come. I thought I knew what science was when I I started graduate school. I envisioned a linear progression of scientific understanding with an almost predictable sequence of events: generating hypotheses, conducting controlled experiments, drawing conclusions, asking new research questions, and developing further experiments. What I have learned in completing my doctoral degree is so much more profound that I could have ever imagined at the onset.

Completing my dissertation leaves me extremely humbled by the enormity of the endeavor of science. I have learned that science is not an individual pursuit but rather a culture of knowledge generation formed by many people, from different backgrounds, asking intertwined questions from diverse perspectives, with outcomes that are only meaningful in the context of the whole. The results from one individual study may not always change the way we view the world, but the insights that emerge from the aggregation of our scientific efforts define, and redefine, the world that we live in — an idea that I find deeply moving.

Scientific discovery is not a destination. Instead, it is a non-linear, iterative, and creative process. Completing my doctoral degree has been more than just accumulating data, compiling publications, and drawing conclusions. My graduate experience has been about developing a unique way of coming to understand the world. From here, I can apply what I have learned to all matter of topics within the field of ecology. I am completing my dissertation with more than just content knowledge and technical skills in the field of ecology, but also a deep appreciation for the epistemology of the field and the clear beginnings of my own unique scientific approach that I will continue to cultivate and refine throughout my career.

At the beginning of her memoir, *Coming to my Senses*, Alice Waters, the well-known founding chef of the restaurant Chez Panisse, describes her approach to cooking as a non-linear process. She wanders her local farmers market selecting ingredients that draw her in without a specific recipe in mind. It is not until Alice lays all the ingredients out on her kitchen counter that the idea for the forthcoming meal emerges. I see many similarities between Alice's approach to cooking and my own approach to science.

My goal to answer a central research question is akin to Alice' goal to create a delicious meal — both traveling towards a loosely defined destination and allowing the path to manifest as we work. Soil organisms, disturbances, and diverse inquiry approaches are my ingredients and completing my dissertation has been a journey of "coming to my senses" in soil ecology.

References

Abney, R.B., Berhe, A.A., 2018. Pyrogenic carbon erosion: implications for stock and persistence of pyrogenic carbon in soil. Frontiers in Earth Science 6, 26.

Acea, M.J., Carballas, T., 1996. Changes in physiological groups of microorganisms in soil following wildfire. Fems Microbiology Ecology 20, 33-39.

Ajwa, H.A., Dell, C.J., Rice, C.W., 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biology and Biochemistry 31, 769-777.

Akema, T., Nurhiftisni, I., Suciatmih, Simbolon, H., 2009. The impact of the 1998 forest fire on ectomycorrhizae of dipterocarp trees and their recovery in tropical rain forests of east Kalimantan, Indonesia. Jarq-Japan Agricultural Research Quarterly 43, 137-143.

Aleixo, A.P., Kaschuk, G., Alberton, O., 2014. Soil fungal and bacterial biomass determined by epifluorescence microscopy and mycorrhizal spore density in different sugarcane managements. Ciencia Rural 44, 588-594.

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105, 11512.

Allison, S.D., McGuire, K.L., Treseder, K.K., 2010. Resistance of microbial and soil properties to warming treatment seven years after boreal fire. Soil Biology and Biochemistry 42, 1872-1878.

Andersson, M., Michelsen, A., Jensen, M., Kjoller, A., 2004. Tropical savannah woodland: effects of experimental fire on soil microorganisms and soil emissions of carbon dioxide. Soil Biology and Biochemistry 36, 849-858.

Andersson, M., Michelsen, A., Jensen, M., Kjøller, A., 2004. Tropical savannah woodland: effects of experimental fire on soil microorganisms and soil emissions of carbon dioxide. Soil Biology and Biochemistry 36, 849-858.

Andrés, P., Moore, J.C., Simpson, R.T., Selby, G., Cotrufo, F., Denef, K., Haddix, M.L., Shaw, A.E., Tomasel, C.d., Molowny-Horas, R., Wall, D.H., 2016. Soil food web stability in response to grazing in a semi-arid prairie: The importance of soil textural heterogeneity. Soil Biology and Biochemistry 97, 131-143.

Baas, P., Bell, C., Mancini, L.M., Lee, M.N., Conant, R.T., Wallenstein, M.D., 2016. Phosphorus mobilizing consortium Mammoth P™ enhances plant growth. PeerJ 4, e2121.

Baath, E., Frostegard, A., Pennanen, T., Fritze, H., 1995. Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biology and Biochemistry 27, 229-240.

Baermann, G., 1917. Eine eifache methode zur auffindung con anklyostomum (nematoden) larvel in erdproben. Geneesk. Tijdschrift woor Nederlands Indie 57, 131-137.

Barcenas-Moreno, G., Garcia-Orenes, F., Mataix-Solera, J., Mataix-Beneyto, J., Baath, E., 2011. Soil microbial recolonisation after a fire in a Mediterranean forest. Biology and Fertility of Soils 47, 261-272.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505-511.

Bardgett, R.D., Wardle, D.A., 2010. Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford University Press, Oxford, U.K.

Barratt, B.I.P., Tozer, P.A., Wiedemer, R.L., Ferguson, C.M., Johnstone, P.D., 2006. Effect of fire on microarthropods in new zealand indigenous grassland. Rangeland Ecology and Management 59, 383-391.

Barreiro, A., Bååth, E., Díaz-Raviña, M., 2016. Bacterial and fungal growth in burnt acid soils amended with different high C/N mulch materials. Soil Biology and Biochemistry 97, 102-111.

Barreiro, A., Fonturbel, M.T., Lombao, A., Martin, A., Vega, J.A., Fernandez, C., Carballas, T., Diaz-Ravina, M., 2015. Using phospholipid fatty acid and community level physiological profiling techniques to characterize soil microbial communities following an experimental fire and different stabilization treatments. Catena 135, 419-429.

Barreiro, A., Martin, A., Carballas, T., Diaz-Ravina, M., 2010. Response of soil microbial communities to fire and fire-fighting chemicals. Science of the Total Environment 408, 6172-6178.

Barreiro, A., Martín, A., Carballas, T., Díaz-Raviña, M., 2016. Long-term response of soil microbial communities to fire and fire-fighting chemicals. Biology and Fertility of Soils 52, 963-975.

Barton, K., 2018. MuMIn: Multi-Model Inference. https://CRAN.R-project.org/package=MuMIn.

Basilio Azevedo, L.C., Morais, M., Lambais, M.R., 2015. Early changes in soil metabolic diversity and bacterial community structure in sugarcane under two harvest management systems. Revista Brasileira De Ciencia Do Solo 39, 701-713.

Basso, A.S., Miguez, F.E., Laird, D.A., Horton, R., Westgate, M., 2013. Assessing potential of biochar for increasing water-holding capacity of sandy soils. Global Change Biology Bioenergy 5, 132-143.

Beleites, C., Sergo, V., 2018. hyperSpec: a package to handle hyperspectral data sets in R. https://CRAN.R-project.org/package=hyperSpec.

Bellgard, S.E., Whelan, R.J., Muston, R.M., 1994. The impact of wildfire on vesicular-arbuscular mycorrhizal fungi and their potential to influence the reestablishment of postfire plant-communities. Mycorrhiza 4, 139-146.

Berch, S.M., Battigelli, J.P., Hope, G.D., 2007. Responses of soil mesofauna communities and oribatid mite species to site preparation treatments in high-elevation cutblocks in southern British Columbia. Pedobiologia 51, 23-32.

Berhe, A.A., Barnes, R.T., Six, J., Marín-Spiotta, E., 2018. Role of Soil Erosion in Biogeochemical Cycling of Essential Elements: Carbon, Nitrogen, and Phosphorus. Annual Review of Earth and Planetary Sciences 46, 521-548.

Beringer, J., Hutley, L.B., Tapper, N.J., Coutts, A., Kerley, A., O'Grady, A.P., 2003. Fire impacts on surface heat, moisture and carbon fluxes from a tropical savanna in northern Australia. International Journal of Wildland Fire 12, 333-340.

Beyer, S., Kinnear, A., Hutley, L.B., McGuinness, K., Gibb, K., 2011. Assessing the relationship between fire and grazing on soil characteristics and mite communities in a semi-arid savanna of northern Australia. Pedobiologia 54, 195-200.

Biederman, L.A., Harpole, W.S., 2013. Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. Global Change Biology Bioenergy 5, 202-214.

Binkley, D., Fisher, R., 2012. Ecology and management of forest soils. John Wiley & Sons.

Bird, M.I., Wurster, C.M., Silva, P.H.d.P., Bass, A.M., de Nys, R., 2011. Algal biochar - production and properties. Bioresource Technology 102, 1886-1891.

Bird, M.I., Wynn, J.G., Saiz, G., Wurster, C.M., McBeath, A., 2015. The pyrogenic carbon cycle. Annual Review of Earth and Planetary Sciences 43, 273-298.

Bloem, J., 1995. Fluorescent staining of microbes for total direct counts. In, Molecular microbial ecology manual. Springer, pp. 367-378.

Bogorodskaya, A.V., Kukavskaya, E.A., Ivanova, G.A., 2014. Transformation of microbial cenoses in soils of light coniferous forests caused by cuttings and fires in the Lower Angara River basin. Eurasian Soil Science 47, 194-202.

Bond, W.J., Keeley, J.E., 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. Trends in Ecology and Evolution 20, 387-394.

Boot, C.M., Haddix, M., Paustian, K., Cotrufo, M.F., 2015. Distribution of black carbon in ponderosa pine forest floor and soils following the High Park wildfire. Biogeosciences 12, 3029-3039.

Bornemann, L., Welp, G., Brodowski, S., Rodionov, A., Amelung, W., 2008. Rapid assessment of black carbon in soil organic matter using mid-infrared spectroscopy. Organic Geochemistry 39, 1537-1544.

Bowman David, M.J.S., Balch, J., Artaxo, P., Bond William, J., Cochrane Mark, A., D'Antonio Carla, M., DeFries, R., Johnston Fay, H., Keeley Jon, E., Krawchuk Meg, A., Kull Christian, A., Mack, M., Moritz Max, A., Pyne, S., Roos Christopher, I., Scott Andrew, C., Sodhi Navjot, S., Swetnam Thomas, W., 2011. The human dimension of fire regimes on Earth. Journal of Biogeography 38, 2223-2236.

Brady, N.C., Weil, R.R., 2010. Elements of the nature and properties of soils. In. Pearson Educational International Upper Saddle River, NJ.

Brennan, K.E.C., Christie, F.J., York, A., 2009. Global climate change and litter decomposition: more frequent fire slows decomposition and increases the functional importance of invertebrates. Global Change Biology 15, 2958-2971.

Brodowski, S., Rodionov, A., Haumaier, L., Glaser, B., Amelung, W., 2005. Revised black carbon assessment using benzene polycarboxylic acids. Organic Geochemistry 36, 1299-1310.

Brown, S.P., Callaham, M.A., Oliver, A.K., Jumpponen, A., 2013. Deep Ion Torrent sequencing identifies soil fungal community shifts after frequent prescribed fires in a southeastern US forest ecosystem. Fems Microbiology Ecology 86, 557-566.

Broza, M., Izhaki, I., 1997. Post-fire arthropod assemblages in Mediterranean forest soils in Israel. International Journal of Wildland Fire 7, 317-325.

Brussaard, L., 1998. Soil fauna, guilds, functional groups and ecosystem processes. Applied Soil Ecology 9, 123-135.

Butenko, K.O., Gongalsky, K.B., Korobushkin, D.I., Ekschmitt, K., Zaitsev, A.S., 2017. Forest fires alter the trophic structure of soil nematode communities. Soil Biology and Biochemistry 109, 107-117.

Camann, M.A., Gillette, N.E., Lamoncha, K.L., Mori, S.R., 2008. Response of forest soil Acari to prescribed fire following stand structure manipulation in the southern Cascade Range. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 38, 956-968.

Caruso, T., Migliorini, M., 2006. Micro-arthropod communities under human disturbance: is taxonomic aggregation a valuable tool for detecting multivariate change? Evidence from Mediterranean soil oribatid coenoses. Acta Oecologica-International Journal of Ecology 30, 46-53.

Carvalho, T.S., Jesus, E.d.C., Barlow, J., Gardner, T.A., Soares, I.C., Tiedje, J.M., Moreira, F.M.d.S., 2016. Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. Ecology 97, 2760-2771.

Celik, I., Barut, Z.B., Ortas, I., Gok, M., Demirbas, A., Tulun, Y., Akpinar, C., 2011. Impacts of different tillage practices on some soil microbiological properties and crop yield under semi-arid Mediterranean conditions. International Journal of Plant Production 5, 237-254.

Cerevkova, A., Renco, M., 2009. Soil nematode community changes associated with windfall and wildfire in forest soil at the High Tatras National Park, Slovak Republic. Helminthologia 46, 123-130.

Certini, G., 2005. Effects of fire on properties of forest soils: a review. Oecologia 143, 1-10.

Certini, G., 2014. Fire as a Soil-Forming Factor. AMBIO 43, 191-195.

Cilliers, C.D., Botha, A., Esler, K.J., Boucher, C., 2005. Effects of alien plant management, fire and soil chemistry on selected soil microbial populations in the Table Mountain National Park, South Africa. South African Journal of Botany 71, 211-220.

Cobb, A.B., Wilson, G.W.T., 2018. Influence of smallholder farm practices on the abundance of arbuscular mycorrhizal fungi in rural Zambia. Pedobiologia 69, 11-16.

Conway, J., Kosemen, C.M., Naish, D., Hartman, S., 2013. All yesterdays: unique and speculative views of dinosaurs and other prehistoric animals. Irregular books.

Coolon, J.D., Jones, K.L., Todd, T.C., Blair, J.M., Herman, M.A., 2013. Long-term nitrogen amendment alters the diversity and assemblage of soil bacterial communities in tallgrass prairie. Plos One 8, e67884.

Cotrufo, M.F., Boot, C., Abiven, S., Foster, E.J., Haddix, M., Reisser, M., Wurster, C.M., Bird, M.I., Schmidt, M.W.I., 2016. Quantification of pyrogenic carbon in the environment: An integration of analytical approaches. Organic Geochemistry 100, 42-50.

Cotrufo, M.F., Boot Claudia, M., Kampf, S., Nelson Peter, A., Brogan Daniel, J., Covino, T., Haddix Michelle, L., MacDonald Lee, H., Rathburn, S., Ryan-Bukett, S., Schmeer, S., Hall, E., 2016. Redistribution of pyrogenic carbon from hillslopes to stream corridors following a large montane wildfire. Global Biogeochemical Cycles 30, 1348-1355.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 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.

Cowan, A.D., Smith, J.E., Fitzgerald, S.A., 2016. Recovering lost ground: Effects of soil burn intensity on nutrients and ectomycorrhiza communities of ponderosa pine seedlings. Forest Ecology and Management 378, 160-172.

Coyle, D.R., Nagendra, U.J., Taylor, M.K., 2017. Soil fauna responses to natural disturbances, invasive species, and global climate change: Current state of the science and a call to action. Soil Biology and Biochemistry 110, 116-133.

Cuchta, P., Miklisova, D., Kovac, L., 2012. Changes within collembolan communities in windthrown European montane spruce forests 2 years after disturbance by fire. Annals of Forest Science 69, 81-92.

Cuchta, P., Miklisova, D., Kovac, L.u., 2013. The impact of disturbance and ensuing forestry practices on Collembola in monitored stands of windthrown forest in the Tatra National Park (Slovakia). Environmental Monitoring and Assessment 185, 5085-5098.

Czimczik, C.I., Masiello, C.A., 2007. Controls on black carbon storage in soils. Global Biogeochemical Cycles 21.

Czimczik, C.I., Schmidt, M.W.I., Schulze, E.D., 2004. Effects of increasing fire frequency on black carbon and organic matter in Podzols of Siberian Scots pine forests. European Journal of Soil Science 56, 417-428.

D'Ascoli, R., Rutigliano, F.A., De Pascale, R.A., Gentile, A., De Santo, A.V., 2005. Functional diversity of the microbial community in Mediterranean maquis soils as affected by fires. International Journal of Wildland Fire 14, 355-363.

Dahlberg, A., Schimmel, J., Taylor, A.F.S., Johannesson, H., 2001. Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. Biological Conservation 100, 151-161.

Dai, X., Boutton, T.W., Glaser, B., Ansley, R.J., Zech, W., 2005. Black carbon in a temperate mixed-grass savanna. Soil Biology and Biochemistry 37, 1879-1881.

Dannenmann, M., Willibald, G., Sippel, S., Butterbach-Bahl, K., 2011. Nitrogen dynamics at undisturbed and burned Mediterranean shrublands of Salento Peninsula, Southern Italy. Plant and Soil 343, 5-15.

Darbyshire, J.F., Wheatley, R.E., Greaves, M.P., Inkson, R.H.E., 1974. Rapid micromethod for estimating bacterial and protozoan populations in soil. Revue D Ecologie Et De Biologie Du Sol 11, 465-475.

de Azevedo, L.C.B., Sturmer, S.L., Lambais, M.R., 2014. Early changes in arbuscular mycorrhiza development in sugarcane under two harvest management systems. Brazilian Journal of Microbiology 45, 995-1005.

DeAngelis, K.M., 2016. Chemical communication connects soil food webs. Soil Biology and Biochemistry 102, 48-51.

DeBano, L.F., 2000. The role of fire and soil heating on water repellency in wildland environments: a review. Journal of Hydrology 231-232, 195-206.

Dechene, A.D., Buddle, C.M., 2009. Effects of experimental forest harvesting on oribatid mite biodiversity. Forest Ecology and Management 258, 1331-1341.

DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil Science Society of America Journal 70, 448-453.

Dempster, D.N., Gleeson, D.B., Solaiman, Z.M., Jones, D.L., Murphy, D.V., 2012. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. Plant and Soil 354, 311-324.

Dhillion, S.S., Anderson, R.C., 1993. Growth dynamics and associated mycorrhizal fungi of little bluestem grass schizachyrium-scoparium (michx) nash on burned and unburned sand prairies. New Phytologist 123, 77-91.

Dhillion, S.S., Anderson, R.C., 1994. Root-growth, and microorganisms associated with the rhizoplane and root-zone soil of a native c-4 grass on burned and unburned sand prairies. Biology and Fertility of Soils 17, 115-120.

Doamba, S., Savadogo, P., Nacro, H.B., 2014. Effects of burning on soil macrofauna in a savanna-woodland under different experimental fuel load treatments. Applied Soil Ecology 81, 37-44.

Docherty, K.M., Balser, T.C., Bohannan, B.J.M., Gutknecht, J.L.M., 2012. Soil microbial responses to fire and interacting global change factors in a California annual grassland. Biogeochemistry 109, 63-83.

Doerr, S.H., Shakesby, R.A., Walsh, R.P.D., 2000. Soil water repellency: its causes, characteristics and hydro-geomorphological significance. Earth-Science Reviews 51, 33-65.

Dooley, S.R., Treseder, K.K., 2012. The effect of fire on microbial biomass: a meta-analysis of field studies. Biogeochemistry 109, 49-61.

Dove, N.C., Hart, S.C., 2017. Fire reduces fungal species richness and in situ mycorrhizal colonization: a meta-analysis. Fire Ecology 13, 37-65.

Dress, W.J., Boerner, R.E.J., 2004. Patterns of microarthropod abundance in oak-hickory forest ecosystems in relation to prescribed fire and landscape position. Pedobiologia 48, 1-8.

Dumontet, S., Dinel, H., Scopa, A., Mazzatura, A., Saracino, A., 1996. Post-fire soil microbial biomass and nutrient content of a pine forest soil from a dunal Mediterranean environment. Soil Biology and Biochemistry 28, 1467-1475.

Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629.
Eisenhauer, N., 2010. The action of an animal ecosystem engineer: Identification of the main mechanisms of earthworm impacts on soil microarthropods. Pedobiologia 53, 343-352.

Esquilin, A.E.J., Stromberger, M.E., Massman, W.J., Frank, J.M., Shepperd, W.D., 2007. Microbial community structure and activity in a Colorado Rocky Mountain forest soil scarred by slash pile burning. Soil Biology and Biochemistry 39, 1111-1120.

Esquilin, A.E.J., Stromberger, M.E., Shepperd, W.D., 2008. Soil scarification and wildfire interactions and effects on microbial communities and carbon. Soil Science Society of America Journal 72, 111-118.

Feltrin, R.P., Will, R.E., Meek, C.R., Masters, R.E., Waymire, J., Wilson, D.S., 2016. Relationship between photosynthetically active radiation and understory productivity across a forest-savanna continuum. Forest Ecology and Management 374, 51-60.

Field, J.L., Keske, C.M.H., Birch, G.L., DeFoort, M.W., Cotrufo, M.F., 2012. Distributed biochar and bioenergy coproduction: a regionally specific case study of environmental benefits and economic impacts. Global Change Biology Bioenergy 5, 177-191.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences. 103, 626-631.

Flannigan, M., Cantin, A.S., Groot, W.J., Wotton, M., Newbery, A., Gowman, L.M., 2013. Global wildland fire season severity in the 21st century. Forest Ecology and Management 294, 54-61.

Flannigan, M.D., Krawchuk, M.A., de Groot, W.J., Wotton, B.M., Gowman, L.M., 2009. Implications of changing climate for global wildland fire. International Journal of Wildland Fire 18, 483-507.

Fonturbel, M.T., Barreiro, A., Vega, J.A., Martin, A., Jimenez, E., Carballas, T., Fernandez, C., Diaz-Ravina, M., 2012. Effects of an experimental fire and post-fire stabilization treatments on soil microbial communities. Geoderma 191, 51-60.

Fontúrbel, M.T., Fernández, C., Vega, J.A., 2016. Prescribed burning versus mechanical treatments as shrubland management options in NW Spain: Mid-term soil microbial response. Applied Soil Ecology 107, 334-346.

Foster, E.J., Hansen, N., Wallenstein, M., Cotrufo, M.F., 2016. Biochar and manure amendments impact soil nutrients and microbial enzymatic activities in a semi-arid irrigated maize cropping system. Agriculture Ecosystems and Environment 233, 404-414.

Frey, S.D., Elliott, E.T., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology and Biochemistry 31, 573-585.

Fritze, H., Smolander, A., Levula, T., Kitunen, V., Malkonen, E., 1994. Wood-ash fertilization and fire treatments in a scots pine forest stand - effects on the organic layer, microbial biomass, and microbial activity. Biology and Fertility of Soils 17, 57-63.

Fuertes, A.B., Arbestain, M.C., Sevilla, M., Macia-Agullo, J.A., Fiol, S., Lopez, R., Smernik, R.J., Aitkenhead, W.P., Arce, F., Macias, F., 2010. Chemical and structural properties of carbonaceous products obtained by pyrolysis and hydrothermal carbonisation of corn stover. Australian Journal of Soil Research 48, 618-626.

Fultz, L.M., Moore-Kucera, J., Dathe, J., Davinic, M., Perry, G., Wester, D., Schwilk, D.W., Rideout-Hanzak, S., 2016. Forest wildfire and grassland prescribed fire effects on soil biogeochemical processes and microbial communities: Two case studies in the semi-arid Southwest. Applied Soil Ecology 99, 118-128.

Garcia-Oliva, F., Sanford, R.L., Kelly, E., 1999. Effect of burning of tropical deciduous forest soil in Mexico on the microbial degradation of organic matter. Plant and Soil 206, 29-36.

Glaser, B., Amelung, W., 2003. Pyrogenic carbon in native grassland soils along a climosequence in North America. Global Biogeochemical Cycles 17.

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Organic Geochemistry 29, 811-819.

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 2001. The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. Naturwissenschaften 88, 37-41.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – a review. Biology and Fertility of Soils 35, 219-230.

Gongalsky, K.B., 2006. Forest fires as a factor of formation of soil animal communities. Zhurnal Obshchei Biologii 67, 127-138.

Gongalsky, K.B., Malmstrom, A., Zaitsev, A.S., Shakhab, S.V., Bengtsson, J., Persson, T., 2012. Do burned areas recover from inside? An experiment with soil fauna in a heterogeneous landscape. Applied Soil Ecology 59, 73-86.

Gongalsky, K.B., Persson, T., 2013. Recovery of soil macrofauna after wildfires in boreal forests. Soil Biology and Biochemistry 57, 182-191.

Gongalsky, K.B., Zaitsev, A.S., 2016. The role of spatial heterogeneity of the environment in soil fauna recovery after fires. Doklady Earth Sciences 471, 1265-1268.

Gongalsky, K.B., Zaitsev, A.S., Korobushkin, D.I., Saifutdinov, R.A., Yazrikova, T.E., Benediktova, A.I., Gorbunova, A.Y., Gorshkova, I.A., Butenko, K.O., Kosina, N.V., Lapygina, E.V., Kuznetsova, D.M., Rakhleeva, A.A., Shakhab, S.V., 2016. Diversity of the soil biota in burned areas of southern taiga forests (Tver oblast). Eurasian Soil Science 49, 358-366.

González-Pérez, J.A., González-Vila, F.J., Almendros, G., Knicker, H., 2004. The effect of fire on soil organic matter—a review. Environment International 30, 855-870.

Grabczynska, O., Olejniczak, I., Predecka, A., Russel, S., 2009. Short-term effects of prescribed forest fire on soil mites (acari). Polish Journal of Ecology 57, 805-809.

Grady, K.C., Hart, S.C., 2006. Influences of thinning, prescribed burning, and wildfire on soil processes and properties in southwestern ponderosa pine forests: A retrospective study. Forest Ecology and Management 234, 123-135.

Gundale, M.J., DeLuca, T.H., Fiedler, C.E., Ramsey, P.W., Harrington, M.G., Gannon, J.E., 2005. Restoration treatments in a Montana ponderosa pine forest: Effects on soil physical, chemical and biological properties. Forest Ecology and Management 213, 25-38.

Gunderson, L.H., 2000. Ecological resilience - in theory and application. Annual Review of Ecology and Systematics 31, 425-439.

Guo, J., Chen, G., Xie, J., Yang, Z., Yang, Y., 2015. Effect of heat-disturbance on microbial biomass carbon and microbial respiration in Chinese fir (Cunninghamia lanceolata) forest soils. Journal of Forestry Research 26, 933-939.

Haimi, J., Fritze, H., Moilanen, P., 2000. Responses of soil decomposer animals to wood-ash fertilisation and burning in a coniferous forest stand. Forest Ecology and Management 129, 53-61.

Halvorson, J.J., Bolton, H., Smith, J.L., 1997. The pattern of soil variables related to Artemisia tridentata in a burned shrub-steppe site. Soil Science Society of America Journal 61, 287-294.

Hamman, S.T., Burke, I.C., Stromberger, M.E., 2007. Relationships between microbial community structure and soil environmental conditions in a recently burned system. Soil Biology and Biochemistry 39, 1703-1711.

Hammes, K., Schmidt, M.W.I., Smernik, R.J., Currie, L.A., Ball, W.P., Nguyen, T.H., Louchouarn, P., Houel, S., Gustafsson, Ö., Elmquist, M., Cornelissen, G., Skjemstad, J.O., Masiello, C.A., Song, J., Peng, P.a., Mitra, S., Dunn, J.C., Hatcher, P.G., Hockaday, W.C., Smith, D.M., Hartkopf-Fröder, C., Böhmer, A., Lüer, B., Huebert, B.J., Amelung, W., Brodowski, S., Huang, L., Zhang, W., Gschwend, P.M., Flores-Cervantes, D.X., Largeau, C., Rouzaud, J.-N., Rumpel, C., Guggenberger, G., Kaiser, K., Rodionov, A., Gonzalez-Vila, F.J., Gonzalez-Perez, J.A., de la Rosa, J.M., Manning, D.A.C., López-Capél, E., Ding, L., 2007. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. Global Biogeochemical Cycles 21.

Hanan, E.J., D'Antonio, C.M., Roberts, D.A., Schimel, J.P., 2016. Factors regulating nitrogen retention during the early stages of recovery from fire in coastal chaparral ecosystems. Ecosystems 19, 910-926.

Harris, W.N., Boutton, T.W., Ansley, R.J., 2008. Plant community and soil microbial carbon and nitrogen responses to fire and clipping in a southern mixed grassland. Rangeland Ecology and Management 61, 580-587.

Hart, S.C., DeLuca, T.H., Newman, G.S., MacKenzie, M.D., Boyle, S.I., 2005. Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils. Forest Ecology and Management 220, 166-184.

Hartnett, D.C., Wilson, G.W.T., 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. In: Smith, S.E., Smith, F.A. (Eds.), Diversity and Integration in Mycorrhizas: Proceedings of the 3rd International Conference on Mycorrhizas (ICOM3) Adelaide, Australia, 8–13 July 2001. Springer Netherlands, Dordrecht, pp. 319-331.

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

Hedlund, K., Griffiths, B., Christensen, S., Scheu, S., Setälä, H., Tscharntke, T., Verhoef, H., 2004. Trophic interactions in changing landscapes: responses of soil food webs. Basic and Applied Ecology 5, 495-503.

Henry, H.A.L., Chiariello, N.R., Vitousek, P.M., Mooney, H.A., Field, C.B., 2006. Interactive effects of fire, elevated carbon dioxide, nitrogen deposition, and precipitation on a California annual grassland. Ecosystems 9, 1066-1075.

Heydari, M., Faramarzi, M., Pothier, D., 2016. Post-fire recovery of herbaceous species composition and diversity, and soil quality indicators one year after wildfire in a semi-arid oak woodland. Ecological Engineering 94, 688-697.

Hockaday, W.C., Grannas, A.M., Kim, S., Hatcher, P.G., 2007. The transformation and mobility of charcoal in a fire-impacted watershed. Geochimica et Cosmochimica Acta 71, 3432-3445.

Holling, C.S., 1973. Resilience and Stability of Ecological Systems. Annual Review of Ecology and Systematics 4, 1-23.

Holling, C.S., Gunderson, L.H., 2002. Panarchy: understanding transformations in human and natural systems. Island Press, Washington D.C.

Hu, F.S., Higuera, P.E., Duffy, P., Chipman, M.L., Rocha, A.V., Young, A.M., Kelly, R., Dietze, M.C., 2015. Arctic tundra fires: natural variability and responses to climate change. Frontiers in Ecology and the Environment 13, 369-377.

Hudson Lawrence, N., Emerson, R., Jenkins Gareth, B., Layer, K., Ledger Mark, E., Pichler Doris, E., Thompson Murray, S.A., O'Gorman Eoin, J., Woodward, G., Reuman Daniel, C., 2012. Cheddar: analysis and visualisation of ecological communities in R. Methods in Ecology and Evolution 4, 99-104.

Huebner, K., Lindo, Z., Lechowicz, M.J., 2012. Post-fire succession of collembolan communities in a northern hardwood forest. European Journal of Soil Biology 48, 59-65.

Hunt, H.W., Coleman, D.C., Ingham, E.R., Ingham, R.E., Elliott, E.T., Moore, J.C., Rose, S.L., Reid, C.P.P., Morley, C.R., 1987. The detrital food web in a shortgrass prairie. Biology and Fertility of Soils 3, 57-68.

Ingham, E.R., Klein, D.A., 1984. Soil fungi - relationships between hyphal activity and staining with fluorescein diacetate. Soil Biology and Biochemistry 16, 273-278.

IPCC, 2014. Climate Change 2014 Synthesis Report. Contribution of Working Groups I, II, and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In, pp. 1-151.

Jangid, K., Williams, M.A., Franzluebbers, A.J., Blair, J.M., Coleman, D.C., Whitman, W.B., 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biology and Biochemistry 42, 302-312.

Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture Ecosystems and Environment 144, 175-187.

Jensen, M., Michelsen, A., Gashaw, M., 2001. Responses in plant, soil inorganic and microbial nutrient pools to experimental fire, ash and biomass addition in a woodland savanna. Oecologia 128, 85-93.

Jiang, R., Sun, L., Hu, H.-q., 2012. The seasonal dynamics of soil microbial biomass of a Larix gmelinii forest after wildfire. African Journal of Microbiology Research 6, 2329-2337.

Jin, Y., Randerson James, T., Goetz Scott, J., Beck Pieter, S.A., Loranty Michael, M., Goulden Michael, L., 2012. The influence of burn severity on postfire vegetation recovery and albedo change during early succession in North American boreal forests. Journal of Geophysical Research: Biogeosciences 117.

Jin, Y., Randerson, J.T., Goulden, M.L., Goetz, S.J., 2012. Post-fire changes in net shortwave radiation along a latitudinal gradient in boreal North America. Geophysical Research Letters 39.

Jin, Y., Roy, D.P., 2005. Fire-induced albedo change and its radiative forcing at the surface in northern Australia. Geophysical Research Letters 32.

Johnson, C.N., 1995. Interactions between fire, mycophagous mammals, and dispersal of ectromycorrhizal fungi in eucalyptus forests. Oecologia 104, 467-475.

Jones, M.D., Twieg, B.D., Ward, V., Barker, J., Durall, D.M., Simard, S.W., 2010. Functional complementarity of Douglas-fir ectomycorrhizas for extracellular enzyme activity after wildfire or clearcut logging. Functional Ecology 24, 1139-1151.

Jonsson, L., Dahlberg, A., Nilsson, M.C., Zackrisson, O., Karen, O., 1999. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Molecular Ecology 8, 205-215.

Kara, O., Bolat, I., 2009. Short-term effects of wildfire on microbial biomass and abundance in black pine plantation soils in Turkey. Ecological Indicators 9, 1151-1155.

Kardol, P., Wardle, D.A., 2010. How understanding aboveground–belowground linkages can assist restoration ecology. Trends in Ecology and Evolution 25, 670-679.

Keen, Y.C., Jalloh, M.B., Ahmed, O.H., Sudin, M., Besar, N.A., 2011. Soil organic matter and related soil properties in forest, grassland and cultivated land use types. International Journal of Physical Sciences 6, 7410-7415.

Kennedy, N., Egger, K.N., 2010. Impact of wildfire intensity and logging on fungal and nitrogen-cycling bacterial communities in British Columbia forest soils. Forest Ecology and Management 260, 787-794.

Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biology and Biochemistry 43, 385-392.

Kim, O.S., Yoo, J.J., Lee, D.H., Ahn, T.S., Song, H.G., 2004. Monitoring of bacterial community in a coniferous forest soil after a wildfire. Journal of Microbiology 42, 278-284.

Kitchen, D.J., Blair, J.M., Callaham, M.A., 2009. Annual fire and mowing alter biomass, depth distribution, and C and N content of roots and soil in tallgrass prairie. Plant and Soil 323, 235-247.

Knapp Alan, K., Conard Shawn, L., Blair John, M., 1998. Determinants of soil CO_2 flux from a sub-humid grassland: effect of fire and fire history. Ecological Applications 8, 760-770.

Knicker, H., 2007. How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85, 91-118.

Knicker, H., 2011. Pyrogenic organic matter in soil: Its origin and occurrence, its chemistry and survival in soil environments. Quaternary International 243, 251-263.

Koerner, S.E., Collins, S.L., 2014. Interactive effects of grazing, drought, and fire on grassland plant communities in North America and South Africa. Ecology 95, 98-109.

Koricheva, J., Gurevitch, J., Mengersen, K., 2013. Handbook of meta-analysis in ecology and evolution. Princeton University Press, Princeton, N.J.

Köster, K., Berninger, F., Heinonsalo, J., Lindén, A., Köster, E., Ilvesniemi, H., Pumpanen, J., 2016. The long-term impact of low-intensity surface fires on litter decomposition and enzyme activities in boreal coniferous forests. International Journal of Wildland Fire 25, 213-223.

Kuhn, T., 1962. The structure of scientific revolutions. Chicago University Press, Chicago, IL.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biology and Biochemistry 41, 210-219.

Lehmann, J., 2007. A handful of carbon. Nature 447, 143-144.

Lehmann, J., Joseph, S., 2015. Biochar for environmental management: science, technology and implementation. Routledge.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60-68.

Lehmann, J., Skjemstad, J., Sohi, S., Carter, J., Barson, M., Falloon, P., Coleman, K., Woodbury, P., Krull, E., 2008. Australian climate–carbon cycle feedback reduced by soil black carbon. Nature Geoscience 1, 832-835.

Lehmitz, R., Russell, D., Hohberg, K., Christian, A., Xylander, W.E.R., 2012. Active dispersal of oribatid mites into young soils. Applied Soil Ecology 55, 10-19.

Levine, J.S., Cofer, W.R., Cahoon, D.R., Winstead, E.L., 1995. A driver for global change. Environmental Science and Technology 29, 120A-125A.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black Carbon Increases Cation Exchange Capacity in Soils. Soil Science Society of America Journal 70, 1719-1730.

Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., Jin, Y., Zou, J., 2016. Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis. Global Change Biology Bioenergy 8, 392-406.

Liu, W., Xu, W., Han, Y., Wang, C., Wan, S., 2007. Responses of microbial biomass and respiration of soil to topography, burning, and nitrogen fertilization in a temperate steppe. Biology and Fertility of Soils 44, 259-268.

Liu, W., Xu, W., Hong, J., Wan, S., 2010. Interannual variability of soil microbial biomass and respiration in responses to topography, annual burning and N addition in a semiarid temperate steppe. Geoderma 158, 259-267.

Liu, X., Chen, C., Wang, W., Hughes, J.M., Lewis, T., Hou, E., Shen, J., 2015. Vertical Distribution of Soil Denitrifying Communities in a Wet Sclerophyll Forest under Long-Term Repeated Burning. Microbial Ecology 70, 993-1003.

Lombao, A., Barreiro, A., Carballas, T., Fonturbel, M.T., Martin, A., Vega, J.A., Fernandez, C., Diaz-Ravina, M., 2015. Changes in soil properties after a wildfire in Fragas do Eume Natural Park (Galicia, NW Spain). Catena 135, 409-418.

Longo, S., Nouhra, E., Goto, B.T., Berbara, R.L., Urcelay, C., 2014. Effects of fire on arbuscular mycorrhizal fungi in the Mountain Chaco Forest. Forest Ecology and Management 315, 86-94.

Luizao, R.C.C., Bonde, T.A., Rosswall, T., 1992. Seasonal-variation of soil microbial biomass - the effects of clearfelling a tropical rain-forest and establishment of pasture in the central amazon. Soil Biology and Biochemistry 24, 805-813.

Mabuhay, J.A., Nakagoshi, N., Isagi, Y., 2006. Soil microbial biomass, abundance, and diversity in a Japanese red pine forest: first year after fire. Journal of Forest Research 11, 165-173.

MacDonald, L.H., Huffman, E.L., 2004. Post-fire Soil Water Repellency. Soil Science Society of America Journal 68, 1729-1734.

Macías, F., Arbestain, M.C., 2010. Soil carbon sequestration in a changing global environment. Mitigation and Adaptation Strategies for Global Change 15, 511-529.

Mack, M.C., Bret-Harte, M.S., Hollingsworth, T.N., Jandt, R.R., Schuur, E.A.G., Shaver, G.R., Verbyla, D.L., 2011. Carbon loss from an unprecedented Arctic tundra wildfire. Nature 475, 489-492.

Mah, K., Tackaberry, L.E., Egger, K.B., Massicotte, H.B., 2001. The impacts of broadcast burning after clear-cutting on the diversity of ectomycorrhizal fungi associated with hybrid spruce seedlings in central British Columbia. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 31, 224-235.

Major, J., Rondon, M., Molina, D., Riha, S.J., Lehmann, J., 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant and Soil 333, 117-128.

Malmstrom, A., 2008. Temperature tolerance in soil microarthropods: Simulation of forest-fire heating in the laboratory. Pedobiologia 51, 419-426.

Malmstrom, A., 2010. The importance of measuring fire severity-Evidence from microarthropod studies. Forest Ecology and Management 260, 62-70.

Malmstrom, A., 2012. Life-history traits predict recovery patterns in Collembola species after fire: A 10 year study. Applied Soil Ecology 56, 35-42.

Malmstrom, A., Persson, T., Ahlstrom, K., 2008. Effects of fire intensity on survival and recovery of soil microarthropods after a clearcut burning. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 38, 2465-2475.

Malmstrom, A., Persson, T., Ahlstrom, K., Gongalsky, K.B., Bengtsson, J., 2009. Dynamics of soil meso- and macrofauna during a 5-year period after clear-cut burning in a boreal forest. Applied Soil Ecology 43, 61-74.

Maraun, M., Scheu, S., 2000. The structure of oribatid mite communities (Acari, Oribatida): patterns, mechanisms and implications for future research. Ecography 23, 374-383.

Marlon, J.R., Bartlein, P.J., Carcaillet, C., Gavin, D.G., Harrison, S.P., Higuera, P.E., Joos, F., Power, M.J., Prentice, I.C., 2008. Climate and human influences on global biomass burning over the past two millennia. Nature Geoscience 1, 697-702.

Marozas, V., Armolaitis, K., Aleinikoviene, J., 2013. Changes of ground vegetation, soil chemical properties and microbiota following the surface fires in scots pine forests. Journal of Environmental Engineering and Landscape Management 21, 67-75.

Martin-Pinto, P., Vaquerizo, W., Penalver, F., Olaizola, J., Oria-de-Rueda, J.A., 2006. Early effects of a wildfire on the diversity and production of fungal communities in Mediterranean vegetation types dominated by Cistus ladanifer and Pinus pinaster in Spain. Forest Ecology and Management 225, 296-305.

Masaphy, S., Zabari, L., 2013. Observations on post-fire black morel ascocarp development in an Israeli burnt forest site and their preferred micro-sites. Fungal Ecology 6, 316-318.

Masiello, C.A., 2004. New directions in black carbon organic geochemistry. Marine Chemistry 92, 201-213.

Masiello, C.A., Druffel, E.R.M., 1998. Black Carbon in Deep-Sea Sediments. Science 280, 1911-1913.

Mataix-Solera, J., Cerda, A., Arcenegui, V., Jordan, A., Zavala, L.M., 2011. Fire effects on soil aggregation: A review. Earth-Science Reviews 109, 44-60.

McNeill, K.L., Krajcik, J., 2008. Inquiry and scientific explanations: Helping students use evidence and reasoning. Science as inquiry in the secondary setting, 121-134.

McSorley, R., 1993. Short-term effect of fire on the nematode community in a pine forest. Pedobiologia 37, 39-48.

Mevik, B.-H., Wehrens, R., Liland, K.H., 2016. pls: Partial Least Squares and Principal Component Regression. https://CRAN.R-project.org/package=pls

Moore, J.C., 1988. The influence of microarthropods on symbiotic and non-symbiotic mutualism in detrital-based below-ground food webs. Agriculture, Ecosystems and Environment 24, 147-159.

Moore, J.C., DeRuiter, P.C., 2012. Energetic Food Webs: An Analysis of Real and Model Ecosystems. In, Energetic Food Webs: An Analysis of Real and Model Ecosystems. Oxford University Press, New York, pp. 1-333.

Moore, J.C., Tripp, B.B., Simpson, R.T., Coleman, D.C., 2000. Springtails in the Classroom. The American Biology Teacher 62, 512-519.

Moore, J.C., Walter, D.E., Hunt, H.W., 1988. Arthropod regulation of microbiota and mesobiota in belowground detrital food webs. Annual Review of Entomology 33, 419-439.

Moreira, M., Baretta, D., Tsai, S.M., Bran Nogueira Cardoso, E.J., 2006. Spore density and root colonization by arbuscular mycorrhizal fungi in preserved or disturbed Araucaria angustifolia (Bert.) O. Ktze. ecosystems. Scientia Agricola 63, 380-385.

Moritz, M.A., Parisien, M.-A., Batllori, E., Krawchuk, M.A., Van Dorn, J., Ganz, D.J., Hayhoe, K., 2012. Climate change and disruptions to global fire activity. Ecosphere 3, art49.

Motiejunaite, J., Adamonyte, G., Irsenaite, R., Juzenas, S., Kasparavicius, J., Kutorga, E., Markovskaja, S., 2014. Early fungal community succession following crown fire in Pinus mugo stands and surface fire in Pinus sylvestris stands. European Journal of Forest Research 133, 745-756.

Nasim, G., 2011. Crop straw burning practice-a threat to arbuscular mycorrhizal biodiversity. Pakistan Journal of Botany 43, 2961-2966.

Neary, D.G., Klopatek, C.C., DeBano, L.F., Ffolliott, P.F., 1999. Fire effects on belowground sustainability: a review and synthesis. Forest Ecology and Management 122, 51-71.

Nemani, R.R., Keeling, C.D., Hashimoto, H., Jolly, W.M., Piper, S.C., Tucker, C.J., Myneni, R.B., Running, S.W., 2003. Climate-Driven Increases in Global Terrestrial Net Primary Production from 1982 to 1999. Science 300, 1560.

Oliver, A.K., Callaham, M.A., Jr., Jumpponen, A., 2015. Soil fungal communities respond compositionally to recurring frequent prescribed burning in a managed southeastern US forest ecosystem. Forest Ecology and Management 345, 1-9.

Olsson, P.A., Bååth, E., Jakobsen, I., Söderström, B., 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycological Research 99, 623-629.

Orgiazzi, A., Ballabio, C., Panagos, P., Jones, A., Fernández-Ugalde, O., 2018. LUCAS Soil, the largest expandable soil dataset for Europe: a review. European Journal of Soil Science 69, 140-153.

Overby, S.T., Owen, S.M., Hart, S.C., Neary, D.G., Johnson, N.C., 2015. Soil microbial community resilience with tree thinning in a 40-year-old experimental ponderosa pine forest. Applied Soil Ecology 93, 1-10.

Palese, A.M., Giovannini, G., Lucchesi, S., Dumontet, S., Perucci, P., 2004. Effect of fire on soil C, N and microbial biomass. Agronomie 24, 47-53.

Paul, E.A., 2014. Soil microbiology, ecology and biochemistry. Academic Press, Cambridge, MA.

Pellegrini, A.F.A., Ahlström, A., Hobbie, S.E., Reich, P.B., Nieradzik, L.P., Staver, A.C., Scharenbroch, B.C., Jumpponen, A., Anderegg, W.R.L., Randerson, J.T., Jackson, R.B., 2018. Fire frequency drives decadal changes in soil carbon and nitrogen and ecosystem productivity. Nature 553, 194-198.

Pen-Mouratov, S., Ginzburg, O., Whitford, W.G., Steinberger, Y., 2012. Forest fire modifies soil free-living nematode communities in the biriya woodland of northern israel. Zoological Studies 51, 1018-1026.

Pinto Alexandre de, S., Bustamante Mercedes, M.C., Kisselle, K., Burke, R., Zepp, R., Viana Laura, T., Varella Renato, F., Molina, M., 2002. Soil emissions of N_2O, NO, and CO_2 in Brazilian Savannas: Effects of vegetation type, seasonality, and prescribed fires. Journal of Geophysical Research: Atmospheres 107.

Ponder, F., Tadros, M., Loewenstein, E.F., 2009. Microbial properties and litter and soil nutrients after two prescribed fires in developing savannas in an upland Missouri Ozark Forest. Forest Ecology and Management 257, 755-763.

Pourreza, M., Hosseini, S.M., Sinegani, A.A.S., Matinizadeh, M., Dick, W.A., 2014. Soil microbial activity in response to fire severity in Zagros oak (Quercus brantii Lindl.) forests, Iran, after one year. Geoderma 213, 95-102.

Pressler, Y., Moore, J.C., Cotrufo, M.F., 2018. Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos 00, 1-19.

Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397-420.

Prieto-Fernandez, A., Acea, M.J., Carballas, T., 1998. Soil microbial and extractable C and N after wildfire. Biology and Fertility of Soils 27, 132-142.

R Core Team, 2017. R: A language and environment for statistical computing. In. R Foundation for Statistical Computing, Vienna, Austria.

Rahkonen, J., Pietikainen, J., Jokela, H., 1999. The effects of flame weeding on soil microbial biomass. Biological Agriculture and Horticulture 16, 363-368.

Rashid, A., Ahmed, T., Ayub, N., Khan, A.G., 1997. Effect of forest fire on number, viability and post-fire re-establishment of arbuscular mycorrhizae. Mycorrhiza 7, 217-220.

Räty, M., Huhta, V., 2003. Earthworms and pH affect communities of nematodes and enchytraeids in forest soil. Biology and Fertility of Soils 38, 52-58.

Rau, B.M., Blank, R.R., Morgan, T., 2008. Microsite and time since prescribed fire's influence on soil microbiology in a pinyon woodland. In: Kitchen, Stanley G.; Pendleton, Rosemary L.; Monaco, Thomas A.; Vernon, Jason, comps. 2008. Proceedings-Shrublands under fire: disturbance and recovery in a changing world; 2006 June 6-8; Cedar City, UT. Proc. RMRS-P-52. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 175-178.

Read, D.J., Leake, J.R., Perez-Moreno, J., 2004. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Canadian Journal of Botany 82, 1243-1263.

Reisser, M., Purves, R.S., Schmidt, M.W.I., Abiven, S., 2016. Pyrogenic Carbon in Soils: A Literature-Based Inventory and a Global Estimation of Its Content in Soil Organic Carbon and Stocks. Frontiers in Earth Science 4, 80.

Renco, M., Cerevkova, A., 2015. Long-term effects of a wildfire on the soil nematode communities in the spruce forest ecosystem of High Tatra National Park. International Journal of Wildland Fire 24, 702-711.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F., Torn, M.S., 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant and Soil 233, 167-177.

Romps, D.M., Seeley, J.T., Vollaro, D., Molinari, J., 2014. Projected increase in lightning strikes in the United States due to global warming. Science 346, 851-854.

Rossi, J.P., Celini, L., Mora, P., Mathieu, J., Lapied, E., Nahmani, J., Ponge, J.F., Lavelle, P., 2010. Decreasing fallow duration in tropical slash-and-burn agriculture alters soil macro invertebrate diversity: A case study in southern French Guiana. Agriculture Ecosystems and Environment 135, 148-154.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4, 1340-1351.

Rumpel, C., Ba, A., Darboux, F., Chaplot, V., Planchon, O., 2009. Erosion budget and process selectivity of black carbon at meter scale. Geoderma 154, 131-137.

Rutigliano, F.A., D'Ascoli, R., De Marco, A., De Santo, A.V., 2002. Soil microbial community as influenced by experimental fires of different intensities. In: Trabaud, L. and Prodon, R. (eds), Fire and biological processes. Backhuyss, pp. 137–150.

Rutigliano, F.A., De Marco, A., D'Ascoli, R., Castaldi, S., Gentile, A., De Santo, A.V., 2007. Impact of fire on fungal abundance and microbial efficiency in C assimilation and mineralisation in a Mediterranean maquis soil. Biology and Fertility of Soils 44, 377-381.

Rutigliano, F.A., Migliorini, M., Maggi, O., D'Ascoli, R., Fanciulli, P.P., Persiani, A.M., 2013. Dynamics of fungi and fungivorous microarthropods in a Mediterranean maquis soil affected by experimental fire. European Journal of Soil Biology 56, 33-43.

San Emeterio, L., Múgica, L., Ugarte, M.D., Goicoa, T., Canals, R.M., 2016. Sustainability of traditional pastoral fires in highlands under global change: Effects on soil function and nutrient cycling. Agriculture, Ecosystems and Environment 235, 155-163.

Santín, C., Doerr, S.H., Kane, E.S., Masiello, C.A., Ohlson, M., de la Rosa, J.M., Preston, C.M., Dittmar, T., 2016. Towards a global assessment of pyrogenic carbon from vegetation fires. Global Change Biology 22, 76-91.

Santos, F., Torn, M.S., Bird, J.A., 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biology and Biochemistry 51, 115-124.

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

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods 9, 671-675.

Schneider, K., Migge, S., Norton, R.A., Scheu, S., Langel, R., Reineking, A., Maraun, M., 2004. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): evidence from stable isotope ratios (15N/14N). Soil Biology and Biochemistry 36, 1769-1774.

Seastedt, T.R., 1984. Microarthropods of burned and unburned tallgrass prairie. Journal of the Kansas Entomological Society 57, 468-476.

Setälä, H., Berg, M.P., Jones, T.H., 2005. Trophic structure and functional redundancy in soil communities. In: Bardgett, R., Usher, M., Hopkins, D. (eds) Biological diversity and function in soils pp. 236-249.

Shade, A., Peter, H., Allison, S., Baho, D., Berga, M., Buergmann, H., Huber, D., Langenheder, S., Lennon, J., Martiny, J., Matulich, K., Schmidt, T., Handelsman, J., 2012. Fundamentals of Microbial Community Resistance and Resilience. Frontiers in Microbiology 3, 417.

Shakesby, R.A., 2011. Post-wildfire soil erosion in the Mediterranean: Review and future research directions. Earth-Science Reviews 105, 71-100.

Shaw, P.J.A., 1997. Post-fire successions of Collembola in lowland heaths in South-Eastern UK. Pedobiologia 41, 80-87.

Shen, J.-p., Chen, C.R., Lewis, T., 2016. Long term repeated fire disturbance alters soil bacterial diversity but not the abundance in an Australian wet sclerophyll forest. Scientific Reports 6, 19639.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biology and Biochemistry 42, 2345-2347.

Smith, N.R., Kishchuk, B.E., Mohn, W.W., 2008. Effects of wildfire and harvest disturbances on forest soil bacterial communities. Applied and Environmental Microbiology 74, 216-224.

Smith, P., Cotrufo, M.F., Rumpel, C., Paustian, K., Kuikman, P.J., Elliott, J.A., McDowell, R., Griffiths, R.I., Asakawa, S., Bustamante, M., House, J.I., Sobocká, J., Harper, R., Pan, G., West, P.C., Gerber, J.S., Clark, J.M., Adhya, T., Scholes, R.J., Scholes, M.C., 2015. Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. SOIL 1, 665-685.

Song, H.G., Kim, O.S., Yoo, J.J., Jeon, S.O., Hong, S.H., Lee, D.H., Ahn, T.S., 2004. Monitoring of soil bacterial community and some inoculated bacteria after prescribed fire in microcosm. Journal of Microbiology 42, 285-291.

Soong, J.L., Cotrufo, M.F., 2015. Annual burning of a tallgrass prairie inhibits C and N cycling in soil, increasing recalcitrant pyrogenic organic matter storage while reducing N availability. Global change biology 21, 2321-2333.

Staddon, W.J., Duchesne, L.C., Trevors, J.T., 1997. Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilization patterns. Microbial Ecology 34, 125-130.

NGSS Lead States, 2013. Next Generation Science Standards. National Academy Press, Washington, D.C.

Stendell, E.R., Horton, T.R., Bruns, T.D., 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycological Research 103, 1353-1359.

Sugihara, N.G., Van Wagtendonk, J.W., Fites-Kaufman, J., 2006. Fire as an ecological process. Fire in California's ecosystems, 58-74.

Sul, W.J., Asuming-Brempong, S., Wang, Q., Tourlousse, D.M., Penton, C.R., Deng, Y., Rodrigues, J.L.M., Adiku, S.G.K., Jones, J.W., Zhou, J., Cole, J.R., Tiedje, J.M., 2013. Tropical agricultural land management influences on soil microbial communities through its effect on soil organic carbon. Soil Biology and Biochemistry 65, 33-38.

Sun, H., Santalahti, M., Pumpanen, J., Köster, K., Berninger, F., Raffaello, T., Asiegbu, F.O., Heinonsalo, J., 2016. Bacterial community structure and function shift across a northern boreal forest fire chronosequence. Scientific Reports 6, 32411.

Sun, Y., Gao, B., Yao, Y., Fang, J., Zhang, M., Zhou, Y., Chen, H., Yang, L., 2014. Effects of feedstock type, production method, and pyrolysis temperature on biochar and hydrochar properties. Chemical Engineering Journal 240, 574-578.

Sun, Y., Wu, J., Shao, Y., Zhou, L., Mai, B., Lin, Y., Fu, S., 2011. Responses of soil microbial communities to prescribed burning in two paired vegetation sites in southern China. Ecological Research 26, 669-677.

Swallow, M., Quideau, S.A., MacKenzie, M.D., Kishchuk, B.E., 2009. Microbial community structure and function: The effect of silvicultural burning and topographic variability in northern Alberta. Soil Biology and Biochemistry 41, 770-777.

Tateishi, T., Horikoshi, T., Tsubota, H., Takahashi, F., 1989. Application of the chloroform fumigation-incubation method to the estimation of soil microbial biomass in burned and unburned japanese red pine forests. Fems Microbiology Ecology 62, 163-172.

Tester, J.R., 1989. Effects of Fire Frequency on Oak Savanna in East-Central Minnesota. Bulletin of the Torrey Botanical Club 116, 134-144.

Tillé, Y., Matei, A., 2016. sampling: Survey Sampling. https://CRAN.R-project.org/package=sampling

Treseder Kathleen, K., Mack Michelle, C., Cross, A., 2004. Relationships among fires, fungi, and soil dynamics in alaskan boreal forests. Ecological Applications 14, 1826-1838.

Treseder, K.K., Mack, M.C., Cross, A., 2004. Relationships among fires, fungi, and soil dynamics in Alaskan Boreal Forests. Ecological Applications 14, 1826-1838.

Tuininga, A.R., Dighton, J., 2004. Changes in ectomycorrhizal communities and nutrient availability following prescribed burns in two upland pine-oak forests in the New Jersey pine barrens. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 34, 1755-1765.

U.S. EPA, 2013. U.S. EPA Most Probable Number (MPN) Calculator Version 2.0. In, User and System Installation and Administration Manual. U.S. Environmental Protection Agency, Washington D.C.

Úbeda, X., Outeiro, L., 2009. Physical and chemical effects of fire on soil. In: Cerda, A., and Robichaud, P.R. (eds) Fire effects on soils and restoration strategies. CRC Press, Boca Raton, FL, pp. 105-132.

Ulery, A.L., Graham, R.C., Bowen, L.H., 1996. Forest Fire Effects on Soil Phyllosilicates in California. Soil Science Society of America Journal 60, 309-315.

Valentine, J.C., Pigott, T.D., Rothstein, H.R., 2010. How Many Studies Do You Need?: A Primer on Statistical Power for Meta-Analysis. Journal of Educational and Behavioral Statistics 35, 215-247.

Van Straalen, N.M., Verhoef, H.A., 1997. The development of a bioindicator system for soil acidity based on arthropod pH preferences. Journal of Applied Ecology, 217-232.

Viechtbauer, W., 2010. Conducting Meta-Analyses in R with the metafor Package. Journal of Statistical Software 36, 1-48.

Vilarino, A., Arines, J., 1991. Numbers and viability of vesicular-arbuscular fungal propagules in field soil samples after wildfire. Soil Biology and Biochemistry 23, 1083-1087.

Violi, H.A., Barrientos-Priego, A.F., Wright, S.F., Escamilla-Prado, E., Morton, J.B., Menge, J.A., Lovatt, C.J., 2008. Disturbance changes arbuscular mycorrhizal fungal phenology and soil glomalin concentrations but not fungal spore composition in montane rainforests in Veracruz and Chiapas, Mexico. Forest Ecology and Management 254, 276-290.

Waldrop, M.P., Harden, J.W., 2008. Interactive effects of wildfire and permafrost on microbial communities and soil processes in an Alaskan black spruce forest. Global Change Biology 14, 2591-2602.

Wall, D.H., Bardgett, R.D., Behan-Pelletier, V., Jones, T.H., Herrick, J.E., 2012. Soil ecology and ecosystem services. Oxford University Press, Oxford, U.K.

Wall, D.H., Bradford, M.A., John, M.G., Trofymow, J.A., Behan-Pelletier, V., Bignell, D.E., Dangerfield, M.J., Pon, W.J., Rusek, J., Voigt, W., Wolters, V., Gardel, H., Ayuke, F.O., Shford, R., Beljakova, O.I., Bohlen, P.J., Brauman, A., Flemming, S., Henschel, J.R., Johnson, D.L., Jones, H.T., Kovarova, M., Kranabetter, J.M.Y., Kutny, L.E.S., Lin, K.C., Maryati, M., Dominique, S.E., Pokarzhevskii, A., Rahman, H., SarÁ, M.G., Salamon, J.A., Swift, M.J., Varela, A., Vasconcelos, H.L., White, D.O.N., Zou, X., 2008. Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. Global Change Biology 14, 2661-2677.

Wall, D.H., Moore, J.C., 1999. Interactions Underground: Soil biodiversity, mutualism, and ecosystem processes. Bioscience 49, 109-117.

Wang, C., Wang, G., Wang, Y., Rafique, R., Ma, L., Hu, L., Luo, Y., 2016. Fire alters vegetation and soil microbial community in alpine meadow. Land Degradation and Development 27, 1379-1390.

Wang, Y., Xu, J., Shen, J., Luo, Y., Scheu, S., Ke, X., 2010. Tillage, residue burning and crop rotation alter soil fungal community and water-stable aggregation in arable fields. Soil and Tillage Research 107, 71-79.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Putten, W.H.v.d., Wall, D.H., 2004. Ecological Linkages Between Aboveground and Belowground Biota. Science 304, 1629-1633.

Waters, J.R., McKelvey, K.S., Zabel, C.J., Oliver, W.W., 1994. The effects of thinning and broadcast burning on sporocarp production of hypogeous fungi. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 24, 1516-1522.

Westerling, A.L., Hidalgo, H.G., Cayan, D.R., Swetnam, T.W., 2006. Warming and earlier spring increase western U.S. Forest wildfire activity. Science 313, 940-943.

Whitford, W.G., Steinberger, Y., 2012. Effects of seasonal grazing, drought, fire, and carbon enrichment on soil microarthropods in a desert grassland. Journal of Arid Environments 83, 10-14.

Wiedemeier, D.B., Hilf, M.D., Smittenberg, R.H., Haberle, S.G., Schmidt, M.W.I., 2013. Improved assessment of pyrogenic carbon quantity and quality in environmental samples by high-performance liquid chromatography. Journal of Chromatography A 1304, 246-250.

Williams, R.J., Hallgren, S.W., Wilson, G.W.T., 2012. Frequency of prescribed burning in an upland oak forest determines soil and litter properties and alters the soil microbial community. Forest Ecology and Management 265, 241-247.

Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J., Joseph, S., 2010. Sustainable biochar to mitigate global climate change. Nature Communications 1, 56.

Wu, T., Ayres, E., Bardgett, R.D., Wall, D.H., Garey, J.R., 2011. Molecular study of worldwide distribution and diversity of soil animals. Proceedings of the National Academy of Sciences 108, 17720-17725.

Xiang, X., Gibbons, S.M., Yang, J., Kong, J., Sun, R., Chu, H., 2015. Arbuscular mycorrhizal fungal communities show low resistance and high resilience to wildfire disturbance. Plant and Soil 397, 347-356.

Yeates, G.W., Coleman, D.C., 1982. Role of nematodes in decomposition. In: Freckman, D.W. (Ed.), Nematodes in Soil Ecosystems. University of Texas Press, Austin, TX, pp. 55-80.

Yusiharni, E., Gilkes, R.J., 2012. Changes in the mineralogy and chemistry of a lateritic soil due to a bushfire at Wundowie, Darling Range, Western Australia. Geoderma 191, 140-150.

Zaitsev, A.S., Gongalsky, K.B., Malmström, A., Persson, T., Bengtsson, J., 2016. Why are forest fires generally neglected in soil fauna research? A mini-review. Applied Soil Ecology 98, 261-271.

Zaitsev, A.S., Gongalsky, K.B., Persson, T., Bengtsson, J., 2014. Connectivity of litter islands remaining after a fire and unburnt forest determines the recovery of soil fauna. Applied Soil Ecology 83, 101-108.

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.

Zhang, D., Yan, M., Niu, Y., Liu, X., van Zwieten, L., Chen, D., Bian, R., Cheng, K., Li, L., Joseph, S., Zheng, J., Zhang, X., Zheng, J., Crowley, D., Filley, T.R., Pan, G., 2016. Is current biochar research addressing global soil constraints for sustainable agriculture? Agriculture Ecosystems and Environment 226, 25-32.

Zhang, N.L., Xu, W.H., Yu, X.J., Lin, D., Wan, S.Q., Ma, K.P., 2013. Impact of Topography, Annual Burning, and Nitrogen Addition on Soil Microbial Communities in a Semiarid Grassland. Soil Science Society of America Journal 77, 1214-1224.

Zhao, L., Cao, X.D., Masek, O., Zimmerman, A., 2013. Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. J. Hazard. Mater. 256, 1-9.

Ziolkowski, L.A., Chamberlin, A.R., Greaves, J., Druffel, E.R.M., 2011. Quantification of black carbon in marine systems using the benzene polycarboxylic acid method: a mechanistic and yield study. Limnology and Oceanography: Methods 9, 140-140.

Appendix 1: Supplementary Information for Chapter 5

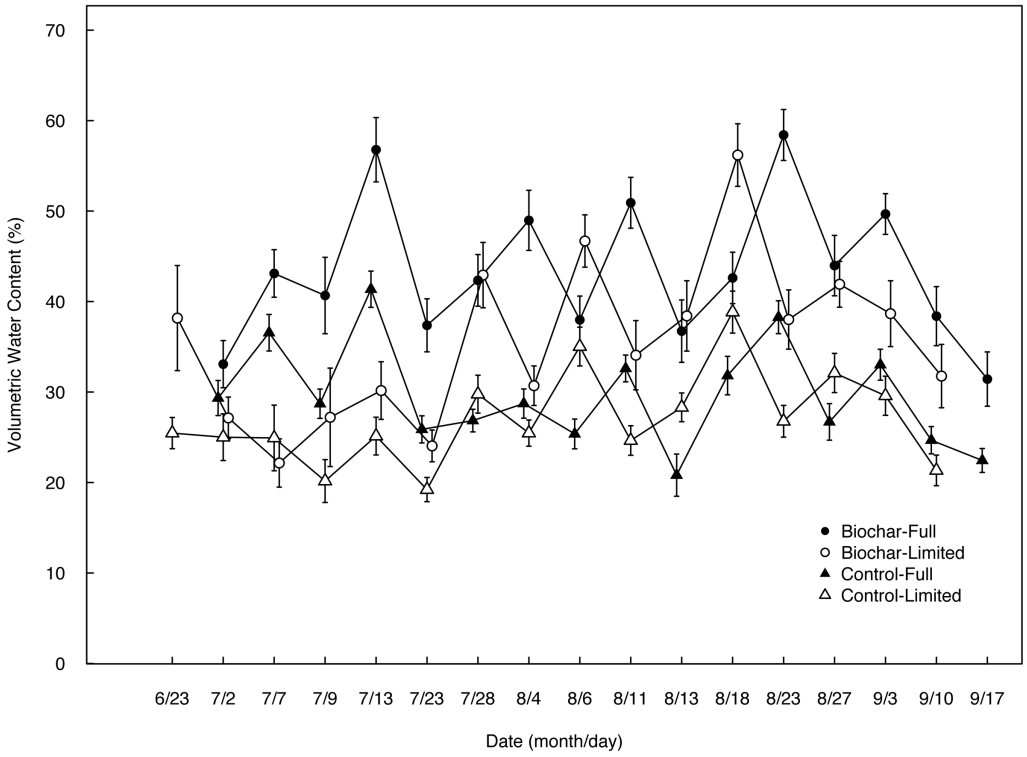


Figure A1. Volumetric water content (%) of soils for control and biochar plots under full and limited irrigation in across the 2014 growing season. Circles represent biochar plots; triangles represent control plots; filled in symbols are under full irrigation; open symbols are under limited irrigation. Points represent mean ± SE (n = 16).

Appendix 2: Supplementary Information for Chapter 6

Appendix 2A. Protocol for measuring soil and biochar pH

Why measure soil and biochar pH?

pH is a good metric of the chemical environment of the soil. It is often considered a "master variable" in determining soil microbial community composition and activity (Fierer and Jackson, 2006; Wu et al., 2011). As part of the pre-experiment observation, ask your students to measure and compare the pH of different soil types and biochar. You may also have students measure soil pH at the beginning and end of the plant growth experiment (example data shown below) or soil respiration experiments. Both plants and microorganisms acidify the soil environment through biological processes such as respiration (Paul, 2014). We would expect to see a decrease in soil pH over the experimental period. However, the pH of the biochar itself will alter the pH of the biochar-soil mixture when added. Biochar is typically basic and often increases the pH of the soil, but results are variable (Lehmann and Joseph, 2015). The effect of biochar on soil pH is often pointed to as a mechanism for observed plant and soil responses to biochar addition. Use the pH results to help your students interpret the results of the plant growth and soil respiration experiments. Consider the question, "how does biochar change soil pH, and how might this affect plant growth and soil respiration?"

Measuring pH

Using pH paper or a pH probe, measure the pH of 20 mL of deionized water. Record the pH of the water. Combine 10 g of soil or biochar that has been dried at room temperature with the 20 mL of deionized water. Stir or shake the water and soil or biochar solution. Let the solution settle for 10 minutes before measuring pH. If using a probe, place the probe in the solution without letting it touch the settled

soil or biochar. Compare the pH of the water to the pH of the soil or biochar solution. Determine whether the soil or biochar increased, decreased, or did not affect the pH of the deionized water.

Example pH results

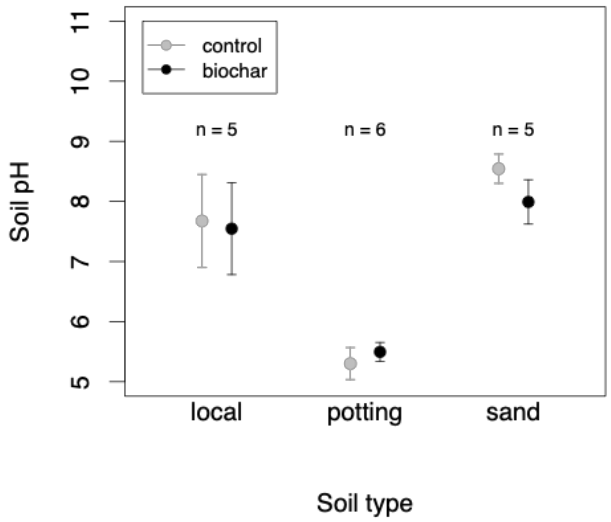


Figure A2. Soil pH data collected by students during plant growth classroom experiments. Points represent means. Error bars represent one standard deviation from the mean. Numbers above the points indicate sample size. Here, biochar only meaningfully decreases soil pH in the sand.

Appendix 2B. Plant Growth Experiment Lab Activity

The following lab activity example follows the CER framework and is designed to guide students through the plant growth experiment. The lab can be modified for the soil respiration experiment. Example student answers are given in italics. These examples are not the only possible responses but rather goal responses that have been crafted on the basis of real middle school student responses.

Modifications for the high school level may include asking students to first write a hypothesis, then a prediction, and discuss the variability in the data (standard deviations).

Scenario: You are a farmer. A salesperson knocks on your door selling a new "miracle" soil additive that she claims will both increase your crop yield and help the environment. After your discussion with the salesperson, you learn that the substance is biochar. Biochar is made from organic waste such as wood chips or agricultural byproducts that are burned in the presence of little or no oxygen.

The salesperson leaves you a small sample of the solid biochar so you can "see for yourself" the huge, amazing gains your crops will make with biochar. After hearing the saleperson's pitch, you decide to try this new "miracle substance" in a small plot before you use it on your entire farm. You decide to conduct an experiment to test the effectiveness of the addition of biochar to your mung bean crop.

Question to investigate: How does biochar affect plant growth in different soil types?

Prediction: Make a prediction about what will happen to the growth of your mung bean plants once biochar is added to the soil.

If biochar is added to the ____*top soil*_____ (topsoil, potting soil, sand), then the mung beans will *grow bigger than the mung beans in the soil without biochar.*

Initial Explanation for Prediction:

I think this will happen because

biochar increases how much water and nutrients is held by the soil, which helps plants grow.

Independent and dependent variables:

Independent variable: _____*time, soil type, and amount of biochar*_____

Dependent variable: __*plant growth (plant height, plant weight)*_____

Results:

Create a line graph that plots the average height of plants over time. The independent variable (x-axis) is the day of growth. The dependent variable (y-axis) is the plant height in mm. Include error bars that represent one standard deviation from the mean.

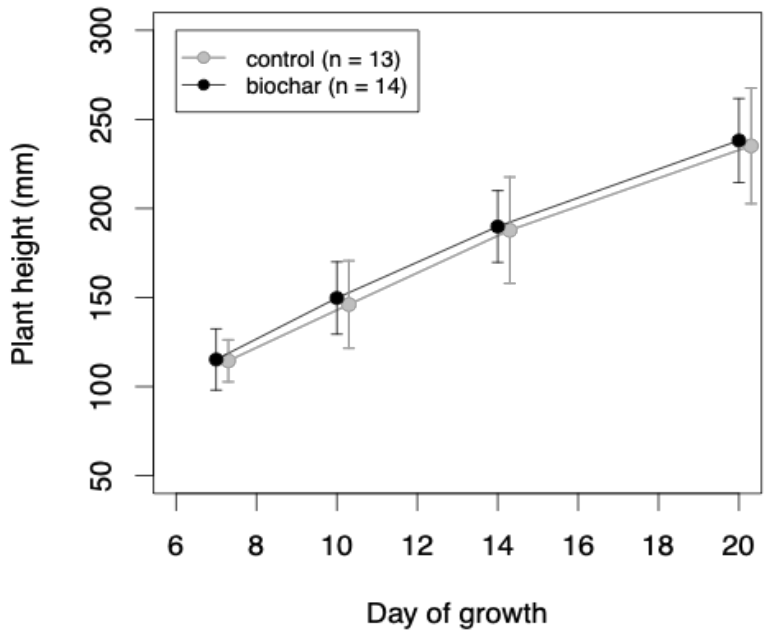


Figure A3. Plant height data for potting soil treatment collected by students during plant growth experiment. Points represent means. Errors bars represent one standard deviation from the mean. Numbers in legend are number of student replicates within each treatment.

Discussion & Explanation:

Offer an explanation for the class results and explain why it is either consistent or inconsistent with your original prediction. What did you find out after completing your experiment? Would you recommend adding biochar to fields of mung plants? Use data and evidence from the class experiment and from the results of other experiments by other scientists to support your reasoning.

Claim- What is your claim about biochar? Under what conditions (e.g., in what types of soils) is biochar an effective soil additive to increase plant biomass?

Biochar is not a good way to increase plant growth in topsoil. The mung beans did not grow larger when they were in topsoil with biochar compared with topsoil without biochar.

Evidence- What evidence (data) do you have to support your claim about biochar? Provide evidence from your class data in the form of a graph or table.

The plants were shorter when grown with biochar compared to without biochar in all the different soil types. The amounts of shoots and roots was almost the same in soils with biochar and without biochar.

Reasoning- Provide a biochar recommendation (whether to use it or not) to your neighbor who also farms mung beans. The neighbor asks, "Why do you make this claim about biochar?" Explain the reasons why you made this suggestion to your neighbor. Why is biochar effective in the conditions you identified and less effective in other conditions?

Based on my experiment, I would not recommend adding biochar to your soil because it did not make the plants grow any bigger. In the soils we tested, biochar did not increase plant growth. I think that the soils we used are already fertile and support good plant growth, so adding biochar didn't help. We learned that biochar can help keep soils moist. If your soils are dry, then adding biochar might be useful for keeping water in the soil so the plant can get the water it needs. We also learned that biochar doesn't affect all plants the same way. If you are growing a different plant than mung beans, biochar might help plant growth more.

<u>Reflection</u>:

- What variables that you did not test or control for might have influenced your results?

  We did not test the fertility and amount of nutrients in the soil types.

- Describe two things you would do differently if you repeated the experiment?

  I would try to measure or control some things about the soil types, like the amount of nutrients in the different soil types.

- What other experiments would you need to conduct before you make a decision about the use of biochar? How would they help you make a better-informed decision?

  I would need to do the experiment with different plants and soils. I would also need to try the same experiment on dry soils because other experiments show that biochar can keep soils moist in dry conditions.

- Provide two new questions you have since completing the experiment and analyzing the class data?

  Why didn't the biochar increase plant growth in the sand? Sand isn't very fertile and I thought the biochar would help add nutrients that would make the plants grow better. Why do plants grow more roots, but not more plants, in sand than in potting soil? If the sand is less fertile and the plants need more roots, how are they able to still grow the same amount of leaves?

Appendix 2C. Example data table worksheet for plant growth experiment.

Data tables will differ for the soil respiration experiment depending on data collection device.

Student names: _____

Date of planting: _____

Date plants broke soil surface: _____

Soil Type	Treatment	Day 7 Height (cm)	Day 10 Height (cm)	… Day X Height (cm)	Final Root Mass (g)	Final Shoot Mass (g)	Root : Shoot
Sand	Control						
Sand	Biochar						
Potting	Control						
Potting	Biochar						
Top	Control						
Top	Biochar						