Systems Theory in Soil Agroecology

by

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Dedication

To life-long learners and collective movements toward environmental justice extending beyond traditional university systems.

El aprendizaje a lo largo de la vida de estudiantes de los movimientos colectivos que buscan la justicia ambiental más allá de los sistemas universitarios tradicionales.

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Abstract

Systems theory is increasingly important for understanding the recently globalized Anthropocene period. Popular ideas from complex systems in physical and social sciences appear in some news media, including ‘fractals’ and ‘six degrees of separation’. One key example complex system is soil, which is both the namesake for humans (i.e., “humus”) and a fundamental basis for sustainable agriculture, yet also experiencing drastic erosion, degradation, and loss, and in need of conservation. Soils are a highly heterogeneous medium and habitat, which could be better understood by applying tools from the emerging field of complex systems, especially in human-centric contexts such as small-scale tropical and urban agriculture. The first study in this dissertation is a transdisciplinary synthesis of how soils can be studied using analytical tools from complexity theory, highlighting important phenomena to be explored, namely: memory or time lags; temporal and/or spatial oscillations highlighting relevance of reporting non-linear dynamics; and critical tipping points and hysteresis curves that may describe future longer-term changes in key soil properties such as soil organic matter, bulk density, and possibly microbial diversity, as well as robust scaling laws for soil aggregates, soil pores, microbial diversity, and/or regional soil carbon concentrations. The second study tests an analog to traditional island biogeography theory at the micro-scale of soil aggregates, and finds support for soil aggregates as reservoirs of soil biodiversity, which increases or scales following long-tailed distribution families observed in other complex physical and social systems. Soil aggregate-associated microbial community assembly also involved relatively more ecological drift, as communities in larger soil aggregates were more similar in composition to randomly simulated communities. This second study overall ties the fine-scale physical soil habitat with higher-level ecological processes and patterns. Expanding on higher-order ecological interactions, the third study links above-belowground interactions by examining the impact of an ant that nests in trees and soil properties, testing the extent near the tree. In this third study, we test the extent of food web cascades by keystone tropical species, and find evidence of more consistent accumulation of relatively larger soil aggregates, supported by consistent power function parameters that describe soil aggregate size-frequency distributions, as well as changes in soil aggregate size and variance under similar distribution families. Related results also show faster soil water infiltration under ant nests, and tendencies toward lower soil carbon and nitrogen stocks, depending on host tree species. Finally, the fourth study analyzes charred wood (biochar) as a potentially regenerative soil amendment in re-purposed urban agricultural soils, testing the mechanistic hypothesis about how biochar effects on soils are mediated by biochar particle size, as well as addressing practitioner-motivated questions about biochar application amount and comparison to current practices of separate- or co-additions with compost. We found detectable benefits, including lower soil compaction and increased soil organic matter along with bacterial diversity, but minor negative effects on fungal and invertebrate diversity. These studies together may broadly support more attention and a new sense of hope toward soil and ecosystem regeneration. Finally, this research supports the perspective that soil should be studied with a transdisciplinary approach, integrating multiple sub-disciplinary tools from microbial to mathematical ecology, as well as incorporating theories from entirely other disciplines like complex systems and non-linear dynamics, to test new hypotheses in ecology that are more general in scope.

Chapter 1 Introduction

In a more connected world known as the Anthropocene epoch, when soils and ecosystems face threats of severe loss, understanding how nature behaves and improving its stewardship requires whole systems-level approaches and integrative tools of analysis. Among many fields of study, from humanities to physical sciences, systems-level approaches that are useful for advancing ecology can be seen in the emerging field of complex systems (theory), which has been suggested (How Nature Works, Per Bak) to broadly argue, against common thought, that nature as a whole is not 'in balance' or relatively static. For some current researchers, as well as many indigenous peoples, this may already be somewhat intuitive; however, for many others, including both early and late life-long learners of rural and urban ecology, this is a relatively new and exciting way of improving how we understand and learn lessons from nature that can also apply to our daily lives. For example, ideas observed in nature that are highlighted by complex systems research such as 'fractals', 'tipping points', and 'six degrees of separation' have each appeared in popular media including books and news outlets, describing snowflake formation, irreversible climate change, and shortest connections within our social networks, respectively. Similarly, more specific concepts related to sub-topics of this research, namely 'nature's matrix' (by familiar authors), the 'soils will save us', and 'fantastic fungi' or the 'wood wide web', have also appeared in popular culture and enticed broad public interest. This dissertation, motivated by both a promise of more robust analytical frameworks being implemented in the field of complex systems, as well as the importance of soils as a fundamental basis for human livelihood via agriculture (and soil 'humus' as the namesake of our 'human' species), builds together a discussion and provides evidence leading to new ideas for future work about how soils can be more fundamentally understood and usefully studied using frameworks and tools from complex systems theory. Accordingly, this work draws holistic connections across many variables affecting soils across spatial scales, touching on topics from tiny soil particle aggregation into clumps, to human decision-making on urban land re-purposed and regenerated for agriculture.

The first study reviews analytical tools and frameworks from an interdisciplinary literature in complex systems to present and discuss how conceptual and mathematical models of soil processes can increase generality over precision, or be more widely applicable across environmental contexts/parameters. This perspective re-emphasizes modeling goals toward those of more basic soils research, rather than more specific future climate and carbon predictions, and can thus afford fewer yet more flexible parameters focusing on descriptive over predictive power. Currently, soil and Earth system models include very detailed equation sets for soil water, enzyme activity, gas diffusion, and other aspects, but to include such details, they also tend to rely on much data for estimating parameters (i.e., relevant fixed constant values). Instead, to more quickly generate and test new hypotheses about longer-term soil dynamics, which can be difficult to track consistently in the field, large equation sets could be more fundamentally interwoven, to achieve dimensional reductions which might miss fewer emergent or unexpected patterns, such as non-linear ones from time series. Model generality or flexibility can broadly stem from internal correlations among key soil (system) descriptors, such as soil organic matter, bulk density, and likely microbial diversity, representing internally compounding processes such as memory or time lags. Analogous to some features of recent large language network models and related artificial intelligence, accelerated internal dynamics in soils can lead to atypical patterns, namely peaks and valleys in temporal oscillations and effectively irreversible critical tipping points, such as permanent soil degradation or, ideally, regeneration, such as in active human-managed systems including urban agriculture. While patterns such as tipping points in the climate and economy have been understood as negative, they need not be and may imply strong potential benefits for cooperative land and soil stewardship, such as resulting from farming or environmental cooperatives practicing more local polycentric sovereign governance.

Tying large human scales to micro-levels, the second study analyzes how fine-scale spatial structure, made by hierarchical soil particle aggregation or clustering, affects bacteria and fungal communities' that self-organization or assembly into the consortia they tend to form in nature. Generally, scaling or power law distribution families, such as of soil aggregates, by definition represent compounding multiplicative iterations among components governed by just one exponent parameter, also referred to as self-organization *(Brown et al. 2002; Young and Crawford 2004)*, and can be generated by a preferential attachment process where 'rich get richer' *(Newman 2001)*. In soils, this process could explain observed scaling laws if, as soil aggregates get larger, they also increase their chance of assimilating nearby smaller aggregates. This additional analysis of microbial biodiversity layered atop fine-scale spatial structure was motivated by early island biogeography theory, which censused species on islands, including in the Caribbean, and explained why more biodiversity can be found on larger islands. These species-area curves are widely applicable in and beyond the field of ecology as scaling laws, since they describe a role for the fundamental facet of space, often universal. Results show that spatial complexity is likely a consistent positive contributing factor for microbiological complexity.

Another idea out of complex systems analysis is the importance of indirect interactions, potentially leading to system cascades. As ecosystem engineers, especially in the tropics, ants are clear examples of dynamic, collective, self-organized behavior. Using a well-studied interaction web of invertebrates that help maintain control of coffee pest diseases, this third study shows how the effects of a keystone arboreal ant species *Azteca sericeasur* extend from above-ground impacts on tree trunks and bushes to below-ground effects on soil micro-aggregate particle sizes and net formation rates, and affects soil water infiltration or movement, and potentially soil nutrient levels.

Finally, zooming back out to land management, and embracing the openness allowed by systems analyses, the fourth study shows that vacant urban land can be re-purposed for small-scale agriculture in only a few years with some active management, supporting the idea that soils, currently experiencing rapid erosion and degradation, could be restored in a non-linear fashion, offering hope for soil restoration. This specific study is in large part geared toward practitioners, with an academic perspective explaining how different viable farming practices can be, namely biochar and compost applications. Urban soils are likely to show quick responses to positive interventions such as biochar-compost co-amendments, but their magnitudes, underlying mechanisms, and potential trade-offs, especially among biological variables, remain under-studied, including in applied contexts. This takes place in nearby Detroit, whose history highlights both the socio-economic fragility of the auto industry and the ingenuity of local community organizing and ecological stewardship, and is now a leader in urban agriculture and environmental justice. Results suggest that biochar-compost co-applications can be useful for alleviating soil compaction initially, such as when re-purposing a vacant lot, with negative tradeoffs with fungal and ground invertebrate diversity. However, after structure is improved, the common local practice of adding compost alone, may be relatively better for improving available nutrients and microbial diversity, with likely additional support by cover crop polycultures.

Generally, this study might appreciably be seen as a case study studying complex soil systems that are influenced by socio-ecological dimensions that overlap somewhat with political ecology. Overall, this dissertation offers a basis for incorporating soil scaling and eventually network data into the field of complex systems and further disciplines, as well as vice versa.

Chapter 2 Developing Systems Theory in Soil Agroecology: Incorporating Heterogeneity and Dynamic Instability

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2.1 Abstract

Ecosystem management is integral to the future of soils, yet anthropogenic drivers represent a key source of uncertainty in ecosystem models. First- and new-generation soil models formulate many soil pools using first-order decomposition, which tends to generate simpler yet numerous parameters. Systems or complexity theory (here used interchangeably to facilitate transdisciplinarity), developed across various scientific and social fields, may help improve robustness of soil models, by offering consistent assumptions about system openness, potential dynamic instability and distance from commonly assumed stable equilibria, as well as new analytical tools for formulating more generalized model structures that reduce parameter space and yield a wider array of possible model outcomes, such as quickly shrinking carbon stocks with pulsing or lagged respiration. This paper builds on recent perspectives, typically siloed in nonlinear dynamics of mathematical ecology and geoscience, pointed toward soil modeling to ask how various soil functions can be better and more generally understood by applying a complex systems lens. We more broadly synthesized previous literature reviews with concepts from non-linear dynamical systems in theoretical ecology and soil sciences to identify areas for further study that may help improve the robustness of soil models under the uncertainty of human activities and management. Three broad dynamical concepts are highlighted: soil variable memory or state-dependence, oscillations, and tipping points and hysteresis. These themes represent less intuitive yet key dynamics that can emerge after assuming nuanced observations, such as reversibility of organo-mineral associations, dynamic aggregate- and pore hierarchies, persistent wet-dry cycles, higher-order microbial community and predator-prey interactions, cumulative legacy land use history, and social management interactions and/or cooperation. We discuss how these aspects may contribute useful analytical tools, metrics, and/or frameworks that can help integrate the uncertainties in future soil states, ranging from micro- to regional scales, including those indirectly affected by human activities and management decisions. Overall, this study highlights the potential benefits of incorporating spatial heterogeneity and dynamic instabilities into future model representations of whole soil processes. Additionally, it advocates for transdisciplinary collaborations between natural and social scientists, extending research into anthropedology and biogeosociochemistry, to better integrate and understand longer-term anthropogenic drivers of soil processes, potentially from soil structural dynamics to microbial community and food web ecology.

2.2 Introduction

Soils represent the basis of recurring civilization *(Montgomery, 2007; Marris, 2022)*, and models depicting their structure and dynamics may help improve generalized understanding of their behavior and ecology. New generation models of soil nutrient cycles *(Sulman et al., 2018; Zhang et al., 2021)* certainly improve on older generation ones *(Coleman and Jenkinson, 1996; Powlson et al., 1996; Berardi et al., 2020)*, adding foci on organic matter stabilization by mineral association that considers the efficiency of microbial degradation *(Cotrufo et al., 2013)*, molecular and pool stochasticity *(Sierra et al., 2018; Waring et al., 2020; Azizi-Rad et al., 2021)*, and increasingly large datasets *(Todd-Brown et al., 2022)*. However, both handling large parameter spaces and making predictions across microbial to global scales remain difficult *(Wieder et al., 2015; Vereecken et al., 2016)*, explaining common decisions to make design tradeoffs between model generality, or qualitative diversity of dynamical output and potential case applications, with numerical precision and realism at a particular scale (e.g. global) *(Levins, 1966; Livingtson, 1985)*. Additionally, the increasing recognition of soil habitat structure for organic matter storage and stability *(Cotrufo et al., 2013; Kravchenko et al., 2019a; King, 2020)*, as well as for community assembly and biodiversity maintenance *(Erktan et al., 2017; Charlotte et al., 2022; Schweizer, 2022; Vogel et al., 2022)*, highlights the potential utility of re-conceptualizing how the soil environment is modeled and formulated. Given the breadth of services that soils offer, adopting modeling strategies that are transferable across soil ecology sub-disciplines *(Buchkowski et al., 2017)* indeed helps move toward addressing the most general of research goals in soil ecology, such as how soil biotic and abiotic spheres or networks interact over time to confer bulk soil properties. Fortunately, an old yet increasingly studied field of complex systems focuses on gaining generalized insights from large multi-component systems, from social and neural networks *(Marder and Calabrese, 1996)* to oscillating chemical reactions *(Epstein et al., 1983; Pacault et al., 1987; Epstein and Showalter, 1996)*, that offer potentially useful analytical perspectives and strategies for generalizing about how heterogeneous soil environments can behave. This synthesis builds on previous efforts to highlight how soil ecology may benefit from integrating tools and concepts from complex systems *(Baveye et al., 2000; Young and Crawford, 2004; Lavelle et al., 2016; Pachepsky and Hill, 2017; Bennett et al.,* *2019)*, elaborating on how specific modeling principles may help gain insight into a variety of soil processes in more generalized ways.

2.2.1 Systems perspective

Early studies of complex systems tended to include explicitly recognized spatial extent and/or separation among modules *(Turing, 1952; Levins, 1969; Mandelbrot, 1983)*, and others recognized explicit state-dependence or time lags *(Rutherford and Do, 1997)*. Mathematical roots of systems and complexity theory built upon nonlinear dynamics *(Lorenz, 1963; Winfree and Strogatz, 1984)* and chaos theory *(May, 1974; Li and Yorke, 1975; Rogers et al., 2022)*, which was facilitated by computational technologies allowing simulation. In soil science, early reports on fractal dimensions of particle size distributions also represent narratives working toward scale-invariant descriptions of the physical habitat *(Young and Crawford, 1991; Kravchenko et al., 1999)*, though fractal methods can also be useful in describing non-physical domains. Given how large and complicated some systems can be, analytical research questions have tended to switch focus from valuing the precision of model predictive ability to its generality *(Levins, 1966)*, or how consistent a result is across example systems. This strategy of finding intersecting truths across case studies is already somewhat familiar to research in the form of useful reviews, meta-analyses, and model comparison projects *(Sulman et al., 2018)*, but it can also take a more computational form. More useful analysis metrics for complex models with variable outputs tend to shift from distribution centrality (e.g., median, mean) to variance and/or key single exponents in cases of high skewness. Accordingly, research questions about similar complex systems also benefit from switching from precise future values to the probability of certain types of events occurring at any future time or location, depending on input data. Focusing on variance as output also promotes including realistic inherent variation or randomness as input, known as stochasticity, which has been a necessary part of explaining and reproducing natural time series of experimental populations *(Henson et al., 2001)*. Embracing and allowing for variability in time series also offers the potential to predict and observe temporal autocorrelation in anticipation of critical transitions or tipping points *(Scheffer et al., 2012)*, such as during accelerated soil degradation or restoration, or naturally unpredictable chaotic fluctuations with predictable bounds *(Schaffer and Kot, 1985; Hastings et al., 1993)*, such as in response to human drivers *(Berryman and Millstein, 1989)*.

Key examples across fields of study have become increasingly emblematic of complex systems theory. For example, in geophysics, measuring irregular structures in nature using relative units instead of absolute ones *(Mandelbrot, 1983)* has widely uncovered power law frequencies of system components sub-sets, famously used in geophysics to study regional earthquake magnitudes *(Bak et al., 2002; Christensen et al., 2002)*. This tool from the fractal geometry branch of mathematics was later applied to ecology, both implicitly *(Macarthur and Wilson, 1963)* and explicitly *(Harte, 1999; Ostling, 2000)*, as well as to soil structure, including aggregation and porosity, with implications for predator-prey (i.e. invertebrate-microbe) interactions *(Baveye et al., 2000)*. Although current applications of nonlinear dynamical systems principles to soils remain somewhat siloed in geophysics, potential applications to soil ecology remain understudied. Another exemplary analytical tool is re-framing focal metrics from distribution centrality to variance measures, as mentioned above. This shift has advanced understanding of consistent spatial and temporal fluctuations *(Taylor, 1961)* to focus on sub-module synchronization potential, notably formulated as coupled pendulum dynamics in physics *(Kuramoto, 1984)*, and has recently inspired various applications in ecology, including to predator-prey dynamics on interaction networks *(Vandermeer, 2021)*, dispersal shifts across urban agricultural landscapes *(Ong et al., 2020)*, and biodiversity collapse, more broadly. These analytical methods tend to uncover internal consistencies or rules among heterogeneous components that predict relatively new patterns at the whole system level, advancing the specific goal of using theory to advance hypothesis testing, compared to other assumed goals like precision forecasting.

2.2.2 Early treatment of complexity in soils research

In ecology generally, there appears to be a consensus that micro- and meso-habitats are complex, and indeed natural soil systems are regarded as the most complex habitats we know of. Yet formal modeling frameworks remain relatively simplified, in part due to common statistical limitations and disciplinary influences. For example, Michaelis-Menten kinetics generated a strong biochemical influence on representing soil organic carbon cycling and decomposition, which has over time been modified to be "reversed" to focus on total enzyme production rather than individual catalysis *(Schimel and Bennett, 2004)*, and has maintained wide use as a core model structure for fine-scale carbon and nutrient transformations. This was even applied to population ecology *(Volterra, 1928)*, where population growth equations analogous to enzyme kinetics have been well analyzed to yield technically unstable but noticeably consistent oscillatory dynamics, also observable in soil data empirically *(Reijneveld, 2013; Kuzyakov and Zamanian, 2019)* and modeled *(Wang et al., 2014)*. Other modeling approaches can also yield counter-intuitive results, which may contradict existing empirical data, or pre-date supporting data from future long-term studies. One case of this was the incorporation of adaptive or environmentally-responsive (i.e. non-linear) enzyme production, also interpretable as emphasizing a positive biological feedback based on phenotypic plasticity, and formulated as a flux- or interaction modification *(Ludington, 2022)*. Contrasting contemporary evidence supporting decomposition as primarily nitrogen-limited, this early modeling study *(Schimel, 2003)* predicted that nitrogen additions to soil would suppress rather than stimulate decomposition, a result that preceded future validating evidence of this from some longer-term nitrogen fertilization studies. In this case, a key multiplicative (i.e., non-linear) model term associating organic matter decay with both its current pool size (first-order decay) and also a dynamically-responsive enzyme pool, ultimately improved model generality, specifically across time scales from minutes (when proteins turnover) to multiple years and seasons. More broadly, mathematical ecology has provided very interesting insights based on linear stability analysis tools to focus analyses *(King and Schaffer, 1999)*, but recent pursuits have expanded to include what was otherwise considered unstable outcomes, like transient dynamics *(Hastings, 2004; Hastings et al., 2018)* with implications for introduced species establishment *(Armstrong and McGehee, 1976; McGehee and Armstrong, 1977; Wilson and Abrams, 2005; Xiao and Fussmann, 2013)*. These approaches indeed provided new insight into consistently observable patterns, based on the increasing use and acceptance of graphical analytical methods like cobwebbing, following the rise of personal computing and simulation power. Recently, comparable tools and concepts from community ecology have been synthetically presented to potentially help address questions in soil carbon cycling and soil community ecology *(Buchkowski et al., 2017)*.

Accordingly, this perspective now draws from theoretical ecology to further extend recent efforts to integrate complex systems principles into soil sciences *(Baveye et al., 2000; Young and Crawford, 2004; Sierra and Müller, 2015; Lavelle et al., 2016; Buchkowski et al., 2017; Pachepsky and Hill, 2017; Bennett et al., 2019)*, and thereby generalize our understanding of the soil habitat, from micro- to macro- scales, notably embracing persistent variation and heterogeneity as critical to fundamental soil processes *(O'Leary et al., 2018)*. Specifically, this paper includes non-exhaustive discussion of various aspects of soil research that may benefit from integrating perspectives from other complex systems research: including memory, oscillations, tipping points and hysteresis. Relevant supporting phenomena observed in soils include reversible organo-mineral associations, aggregate- and pore hierarchy and dynamics, persistent wet-dry cycles, multi-year gas fluxes (e.g., respiration) and nutrient availability, higher-order microbial community and predator-prey interactions, and cumulative legacy land use history. Overall, adopting tools from systems and complexity theory, offers ways of reducing model structural uncertainties *(Bradford et al., 2016)*, thereby also potentially facilitating model-data integration efforts, for example by reducing parameter space *(Bennett et al., 2019)*.

2.3 Memory

2.3.1 Iteration and hierarchy

The concept of memory represents a form of self-referential dynamics specifically based on an event occurring at least one-time step in the past, also referred to as time lags, which in some real systems can result in hierarchical structures. An example from the field of physics is nuclear fusion, in which heavier atoms with more protons are made not by the instant fusion of many lighter atoms, but by the simpler merging of a few medium atoms previously built, interestingly, also with an apparent critical drop in stability at higher atomic numbers after iron *(Pfützner et al., 2012)*. This shows not only a hierarchical building process resulting from the dependence of the existence of larger nuclei on past states (i.e. previously formed smaller nuclei), but also shows related consequences, namely a skewed distribution of nuclear stability across the spectrum of existing nucleus sizes, where the nuclei of smaller atoms are more tightly bound together than larger ones.

Highly skewed or long-tailed distributions, like the power law family, based on component or module attributes like cluster size, are increasingly recognized as (Gillespie, 2015) properties of complex systems (Clauset et al., 2009; Locey and Lennon, 2016). This contrasts the normal "bell curve" distribution, which is often assumed for classic statistical procedures, from simple averages to within-group variance during linear regression. As a result, observing power laws in data has served as initial support for hypothesizing hierarchical or self-organizing processes, offering a novel path to infer processes from patterns. Although various underlying processes can yield similar distributions (Bashkirov and Vityazev, 2000; Curado et al., 2018), observing non-normal distributions may at least help de-emphasize selection-like mechanisms that tend to produce bell curves.

Somewhat surprisingly, the nuclear fusion example parallels soil aggregation almost exactly, where larger aggregates tend to be weaker and less structurally stable than smaller aggregates. In this case, soil aggregation is in part affected by the amount of surface area allotted to binding agents, as well as the strengths of the various binding agents themselves, ranging from fine-scale organo-mineral associations to fungal mycelial networks (Tisdall and Oades, 1982). Ultimately, this can be tied back to early discussions of fractal dimension parameters of power law distributions observed in porous media and landscapes (Burrough, 1981), which simultaneously converged with empirical support for soil aggregation as a hierarchical process (Tisdall and Oades, 1982), and inspired later conceptual explorations in ecology (Nortcliff, 1984; Armstrong, 1986; Tyler and Wheatcraft, 1989; Perrier et al., 1996; Assouline et al., 1998). As a result, while perhaps controversial (Baveye et al., 2000; Pachepsky and Hill, 2017), one approach for theoreticians and modelers may be to further attempt the study of simple models (Vitousek et al., 2022) to explore ideas and test underlying hypotheses about the implications of hierarchical structural dynamics for soil properties *(Stamati et al., 2013)*. This may be especially important for processes that remain near impossible to measure empirically *(Bennett et al., 2019)*. Some studies seem to align with this process-based modeling focus *(Waring et al., 2020)*, while others tend to prioritize more output precision after long-term simulation *(Coleman and Jenkinson, 1996; Powlson et al., 1996; Cong et al., 2014)*. Additionally, there is also supporting evidence for various hierarchical processes in soils, including those underlying clay flocculation *(Brostow et al., 2007; Cuthbertson et al., 2018)*, aggregation of solids *(Tisdall and Oades, 1982)* (mentioned above) as a result of physical mixing *(Klaminder et al., 2013)* and fungal enmeshment *(Rillig and Mummey, 2006)* amplified by wet-dry cycles *(Denef et al., 2001)*, and for pore cluster networks *(Quigley and Kravchenko, 2022; Vogel et al., 2022)*, all of which represent interesting modules of the soil environment to explore with hypotheses about hierarchical structural dynamics and their implications. Specifically, hierarchical aggregation has been previously analyzed by binning all aggregates into either micro- or macroaggregate functional groups, a fractionation that has served as a basic structure for some models specific to solid aggregation *(Segoli et al., 2013; Stamati et al., 2013)*, though it may also be relevant for other soil models, such as ones specific to greenhouse gas production *(Kravchenko et al., 2019b; Wang et al., 2019)* or microbial diversity.

Overall, these hierarchical patterns emerging from system-wide memory (i.e. past state-dependence) tend to generally reflect natural solutions for coarser level system limitations. Endogenous solutions to system limits can, in some cases, be formulated and analyzed as an issue of optimizing information flow *(Czaplicka et al., 2013)*. If applied to modeling soil pore dynamics, for example, this general mechanism or principle could potentially help explain empirical soil pore structure data specifically as a single naturally-resulting geometric solution (stable or temporary/unstable) for a set of constraints. Biological constraints might be microbial cross-feeding rates fueled by metabolite-carrying water flowing through pore networks. Physical constraints could be spatial, such as topsoil depth, and/or temporal, such as growing season length, both of which would affect pore-forming processes including microbial secretions and gas exchange (micro-pores) and invertebrate burrowing activity (macro-pores). Importantly, real soils also have historical constraints, which help determine where old and new pores exist, and such legacy effects consider network structures of soil pores as ongoing solutions to regimes of environmental change. Similarly, hierarchical aggregation of solids may also amplify the effects of environmental changes enough to minimize compositional variance of microbial communities living in the smallest habitat pockets *(Rillig et al., 2017; Wilpiszeski et al., 2019)*. System memory, in the form of iterative structural dynamics, can also be environmentally adaptive, such as when system components are dynamic or continuously dismantled and re-assembled into new yet familiar structures, which range in scope from soil micro-habitats to landscape profiles over months and years. Fine-scale positive feedbacks may help explain the diversity of soil profiles and pedons across and within order-level taxonomic soil classifications *(Jenny, 1961; Phillips, 2017)*. In addition to hierarchies, soil systems' internal or temporal memory can also be a regular source of heterogeneity that increases uncertainty about how soil behaves. In soils, this hypothesis of increasing heterogeneity along hierarchical trajectories could, for example, predict greater variance in diversity or simply beta diversity among microbial communities observed in macro-aggregates compared to those among micro-aggregates. However, there remains little evidence addressing this topic, although technological advances may facilitate future studies *(Bailey et al., 2012)*.

2.3.2 Soil depth and history

Studying subsoils and soil depth also represents an axis that integrates soil profile memory and time lags. Often only topsoils (e.g. to 10 or 20 cm depth) are studied due to their high nutrient concentrations, yet subsoil horizons store more total carbon *(Hicks Pries et al., 2017)* and can influence topsoil microbial activity, ultimately highlighting their relevance to whole profile soil functions. Pedological studies have long recognized that land use history, in addition to the classic five state factors of soils, affects current soil function *(Turley et al., 2020)*, especially tillage and fertilization via changes in soil structure and soil fertility *(Weitzman et al., 2022)*. Additionally, microbes may decompose stable organic matter reserves when new labile organic matter is added, known as soil priming *(Kuzyakov, 2006; Bastida et al., 2019; Liu et al., 2020)*, to which subsoils may be more sensitive *(Li et al., 2022)*. Thus, overall priming may offset any expected new carbon storage in topsoils. Similarly, a multi-year whole profile warming experiment recently showed that soil overall lost carbon mostly from subsoil even though topsoil accumulated carbon *(Soong et al., 2021)*, suggesting that opposite patterns in subsoils may require re-shaping fundamental understanding of soil systems at the profile and pedon levels. In contrast, subsoils at over one meter depth in agricultural systems may be better posed to accumulate carbon in the long-term compared to their topsoils, due to existing degradation from deeper tillage and the potential for added fertility from added root inputs by perennial plants with deep roots *(Button et al., 2022)* and other biological subsoiling methods *(Ning et al., 2022)*. Since soil pedogenesis is now understood to follow complex trajectories *(Phillips, 2017)*, future studies of subsoils should help develop explanations for underlying processes simultaneously affecting different soil horizons.

Ideas of memory also relate to group-level patterns and processes which are increasingly reported, alongside individual component-level processes *(Kerr and Godfrey-Smith, 2002;*

Traulsen and Nowak, 2006), and can have important implications for overall soil processes. Cooperation often manifests itself as synchrony among individuals, as in early examples of tree seed masting *(Ostfeld et al., 2006; Victor et al., 2016)* as well as disease transmission *(Ostfeld et al., 2005)*, with similar principles extended to apply to forests *(Filotas et al., 2014)* and soil rhizospheres to describe nutrient exchange *(Simard et al., 1997)* along with mycorrhizal symbioses *(van der Heijden and Horton, 2009; Simard et al., 2012)* (with ongoing discussions). Similarly, population quorum sensing by soil bacteria has been shown to affect antibiotic production *(Li et al., 2021)* and to provide other benefits at critical population sizes *(Heilmann et al., 2015)*, likely with weak time delays in reciprocity *(Alfaro and Sanjuan, 2022)*. Quorum sensing has also been hypothesized to affect enzyme production with implications for nitrogen cycling *(DeAngelis et al., 2008; Wang et al., 2014)*. Time delays, also affecting interactions between partners changing at different rates or temporal scales, could affect reciprocity of symbioses in soil, such as an example of a time delay between monthly root turnover and exudation, which could affect momentary microbial gene expression, as well as predatory grazing below- and aboveground. Ultimately, the combinations of spatial and temporal variation generate the fluctuations that make treating dynamic instability as a somewhat unavoidable and thus inherent property of natural soil systems.

2.4 Oscillations

2.4.1 Focus on variance

Diel cycles in soil temperature and respiration are commonly observed, yet few studies analyze the implications of natural cycles for modeling soil responses to environmental changes, which in some cases can lead to hysteretic irreversibility *(Phillips et al., 2011)*. Complexity and systems theory offers generalized tools and perspectives to better incorporate variance (i.e. by soil depth) into more generalized models of soil processes. The tendency of a systems perspective to shift analysis toward variance is ultimately more inclusive of a diversity of model outcomes, such as regular oscillations or constrained chaotic fluctuations, and more generally validates informative model outputs that are not precise single-point solutions and are otherwise considered unstable by linear stability analysis. One method of incorporating variances has been re-formulating dynamical systems using trigonometric functions, which are unique for producing repeated symmetrical curves, from bounded measures of relative distance from a fixed line segment *(Coolidge, 1952)*. These first principles already reflect modern principles now understood about many complex systems, like relativity and symmetry with modification, which likely increase the generality and applicability of modeled output especially when processes and questions are newly framed and formulated in tractable ways.

2.4.2 Soil cycles

Oscillations have been predicted by novel and widely cited model structures in agroecology *(Vandermeer and Perfecto, 2017)* and soil ecology *(Baveye et al., 2018)*, and supported by empirical data across fine and coarse scales. At fine scales, soil carbon molecular turnover has been recently proposed to depend on functional group complexity that also hypothesizes spatial modularity or hotspots in activity, which could produce oscillations of broader soil properties over time *(Lehmann et al., 2020)*. Spatial structuring also strengthens positive feedbacks in decomposition between exo-enzyme activity and assembly or production, which has influenced some early soil models toward predicting consistent variance, in the form of limit cycles, in soil nutrient availability *(Schimel, 2003; Wang et al., 2014)*. Wet-dry cycle frequency has also been cited as affecting soil aggregate stability and as a determinant of patterns in microbial activity *(Evans et al., 2022)*. Various types of limit cycles have also been a classic prediction of predator-prey models, but are rarely applied to describe soil faunal grazer food webs *(Baveye et al., 2000; Buchkowski et al., 2017; Erktan et al., 2020; McCary et al., 2021)* especially involving viruses and their traits *(Emerson et al., 2018; Trubl et al., 2018)*. When these low-dimensional ecological models are explicitly extended in space, for example as metacommunities, a wide variety of mosaic landscapes can be generated with some sensitivity to model formulation or structure *(Vandermeer and Yitbarek, 2012; Vandermeer, 2013; Yitbarek and Vandermeer, 2017)*, suggesting that flexible model structures are likely important for a robust understanding of causes and consequences of soil heterogeneity. Spatial explicitness also emphasizes studying dispersal processes among soil modular populations more than internal community dynamics shaped by particular parameterizations, which have been increasingly studied *(Chaudhary et al., 2020; Hajian-Forooshani and Vandermeer, 2020)*. Spatial or temporal separation of populations has also inspired other model structures proposing competitive hierarchies resulting in intransitive loops *(Vandermeer, 2013; Vandermeer and Jackson, 2018)*, which is already supported by strong evidence *(Kerr et al., 2002; Lozano et al., 2019; Vandermeer and Perfecto, 2023)*. Again, this is a case where new and diverse model predictions precede empirical supporting evidence, which ultimately highlights the potential value of general models with a wide range of output, over precise ones, specifically for more basic than applied research. At coarser scales, soil respiration also experienced regular variance in magnitude over multiple years, explained by regime shifts among various dominant stages of community-level decomposer activity *(Sihi et al., 2016; Melillo et al., 2017)*. Various soil nutrients have also shown regular oscillations over decades *(Reijneveld, 2013)*, although common statistical analyses remain linear, which is an analytical limitation. Some nutrient oscillations are expected from direct harvests, but if nutrient cycles are coupled (i.e. by microbial metabolisms) while oscillating, unintended and unintuitive synergies or conflicts may emerge, as oscillations align either in or out of phase *(Vandermeer, 2006)*, leading to either robust, or more likely quickly degrading, soil nutrient availability. The appearance and prediction of oscillations among a range of soil variables from both advanced first principles and several empirical studies justifies further study of non-linear models of soil behavior and ecology, with example approaches listed in Table 2.1.

Table 2.1: Example cases where employing modeling strategies based on systems theory could be useful for making generalizations about soil ecology and agroecology.

Goal	**Limitation**	**Systems concept**	**Model approach**	**Pro**	**Con**	**Related Refs**
Forecast soil C for general agricultural or climate change management decisions	Reversible organo-mineral associations (e.g. priming); Enzyme production plasticity	Oscillatory dynamics; strong positive feedbacks	Simpler implicitly oscillatory pools, with different winding frequencies	Fewer pool-specific parameters; Computational efficiency allows more stochastic simulations for system accuracy	Less precise final SOM pool sizes	Schimel & Weintraub 2003; Wang et al 2014 Biogeosci; Sulman et al 2018; Kuramoto 1975
Estimate soil biodiversity for conservation and sustainable development	Large nested food webs, with trophic cascades	Indirect / higher-order interactions	Collection of coupled oscillator predator-prey pairs and/or synchronous community clusters	Fewer taxon-specific parameters; Computational efficiency allows more taxon pairs	Less precise species population sizes	Potapov 2022; Buchkowski et al 2017; Vandermeer et al 2021
Assess persistent pathogen risk from soil, compost, or municipal sludge	High heterogeneity in microbial pathogen population sizes	Chaos, bifurcations, period-doubling	Meta-population with chaotic (high) discrete growth and/or dispersal rates	Higher statistical confidence compared to plate colony count estimates	Output states more parameter dependent	Levins 1969; May 1974; Feigenbaum 1978

2.5 Tipping points and hysteresis

Hysteresis can be framed as a specific kind of non-linear transition and tipping point occurring in soils. Hysteresis is the directional dependency of strong changes in a response variable, in severe cases producing ‘tipping points’, and is revealed when model stability analyses include outcomes across range of parameters, and underlying component processes also change pace at different speeds *(Ong and Vandermeer, 2018)*. In soils, this occurs with overall water content and its matric pressure, or availability; this is because water always moves through wide pores first regardless of whether the process is drying or wetting, and wide pores have a weaker relationship between water content and matric pressure than in narrow pores, which always mediate water movement second. Here the phenomenon is explained by temporal consistency in process across spatial heterogeneity, which can be another cause of dynamical asymmetry more generally. For example, the relationship between soil temperature and total soil respiration is also hysteretic in that soil respiration responds only after considerable changes in temperature, making respiration stay low as soil warms, yet stay high while it cools, even across the same middle temperatures *(Riveros-Iregui et al., 2007; Phillips et al., 2011; Zhang et al., 2015)*, which can also be described as state-dependence or short-term memory for a system. A widely recognized implication of hysteresis is the practical and sometimes permanent *(Ong and Vandermeer, 2018)* irreversibility of past ecological states, like permanent wilting points for roots under drought, or more internally regulated predator or pest populations.

Soil carbon saturation is a related concept supported by field experiments and models, and is inspiring useful model structures for describing and understanding non-linear processes in soil. Appropriately, the concept of soil carbon saturation has already been formulated and incorporated into new-generation soil models like MEMS *(Zhang et al., 2021)*. An early proposed model *(Stewart et al., 2007)* simulated decadal field data by mixing two pools with different local equilibria: a stable mineral-bound carbon pool that saturates, and a labile pool that mostly decomposes but with no growth limit. This mixed structure produced flexible output – slow linear increases in soil carbon at high concentrations, and quicker but saturating increases at low concentrations – making it more widely applicable and representing a more generalized understanding of long-term soil carbon dynamics.

In contrast, older-generation soil models like CENTURY *(Powlson et al., 1996)* and RothC *(Coleman and Jenkinson, 1996)* do not incorporate pool saturation, but instead, offered a simpler initial model with widespread practical use. The relative simplicity of the model structure is broadly observed in the overall linear successive flow from one decomposition pool to the next. Furthermore, each individual pool was formulated to observe first-order decomposition kinetics. While first-order decomposition represents exponential decay, which is curvi-linear over time, the non-linearities and complex dynamics under investigation by systems theory instead stem from multiplicative associations or interactions among dynamic variables. In many cases, multi-pool soil systems tend to be represented with an attempted thoroughness, resulting in a list of coupled equations that influence each other by additive (or subtraction) terms, which usually consist of a key variable multiplied by a corresponding abiotic parameter, which is static relative to carbon or the nutrient of interest *(Zhang et al., 2021)*. This style of formulation acknowledges that soils are complicated habitats, but leaves room to lean into the complexities of the habitat with various nonlinear additions.

Fundamentally, because parameters and dynamic variables in an ordinary differential equation often represent concepts formulated to be operating at relatively distinct time scales, the re-formulation to incorporate multiplicative variable associations can also be interpreted as a change in descriptive timescale. For example, a representative modeling study may test the hypothesis that particulate organic matter and mineral-associated organic matter, even as distinct pools, might each observe transfer rates that could be affected by both its own and the other's size, within a model time step, rather than between them. More specifically, rather than implicitly modeling the primed loss of existing organic matter in the form of a saturating stable pool, soil priming could be formulated as an interaction modification that implicates the more stable pool's size back into its incoming transfer rate from the labile pool. Generally, this style of formulation may offer a new class of hypothesis testing, especially for soil processes that are currently difficult to test empirically *(Bennett et al., 2019)*, representing a wider array of model outcomes with just a few key soil descriptor variables, in line with recent qualitative syntheses *(Phillips, 2017; Kuzyakov and Zamanian, 2019)*. Modeling soil systems using tools from complexity theory appears to offer an antidote to the increasingly-large soil simulations that have become more popular alongside increasing computing power in cloud systems. The approach using complexity theory can improve conceptual efficiency by reformulating soil models into fewer modular components with more inter-linked process rates *(Lehmann et al., 2020)*. In this way, soil modeling studies may emerge as useful analyses not only for understanding soils themselves, but also for aiding other academic disciplines studying complex systems more generally.

Additional recent studies improved the generality of understanding by explicitly modeling biological *(Craig et al., 2021)* and microbial *(Wang et al., 2014)* processes underlying transformation rates, including with saturating enzyme activity *(Buchkowski et al., 2017)*. Even more general understanding can be added by expanding similar model structures to describe effects of soil fauna and invertebrates on soil micro-habitats including predatory and dispersive influences on microbial communities *(Grandy et al., 2016; Creamer et al., 2022)*. However, integrating models of short-term microbial processes with long-term carbon dynamics remains incomplete *(Todd-Brown et al., 2013)*, in part because they operate on very different timescales. Each model class improves understanding of soil, but for generalized predictions, integrating or coupling models with the fewest explicit dimensions that yield the most diverse qualitative output *(Levins, 1966; Lane, 2018)* may help at least bound possible outcomes, such as for soil carbon exchange and net storage, or other nutrient cycles *(Manzoni and Porporato, 2009)*. In addition, there are many aspects of soil ecology and functioning, such as biodiversity maintenance or specific soil pathogen suppression, that warrant continued understanding through modeling the soil environment.

2.5.1 Restorative agropedogenesis

Several non-linear dynamics or functions among soil variables may then combine to reveal critical transitions (*Fig 2.1*). A recent synthesis *(Kuzyakov and Zamanian, 2019),* revealed many non-linear relationships among key soil variables across several decades, such as between soil bulk density and organic carbon. Their analysis showed distinct phases of pedogenesis, or soil development, under human management, which they termed agro-pedogenesis. Ultimately, they presented a convergence of these processes into an attractor, which they considered to span a narrower range of values for key soil descriptors than would be found under natural pedogenesis. However, underlying non-linearities between the same key soil descriptors, such as bulk density and soil organic carbon, suggest potential future attractor instability and more potential divergence among local pedons, especially under human management. Relatedly, another recent review *(Phillips, 2017)* highlighted how natural pedogenesis can be described with concepts from non-linear dynamics and complexity theory, notably revealing how pedogenesis can show unstable trajectories, or many possible alternative end states. These studies suggest that while globally, recent anthropedogenesis has converged mostly toward degradation, locally, future anthropedogenesis could still diverge into soil with varying fertilities depending on regional management strategies. This inference that helps maintain agro-pedogenesis as a potentially regenerative force for soil fertility, rather than inherently degrading, especially when distinguishing between industrial vs. small-scale agricultural land management. Accordingly, a recent global synthesis of soil microbial biomass carbon showed evidence of higher variance in percent change where existing stocks were lower, also showing relative increases in some tropical regions such as the Caribbean (near southeast Ayiti) *(Patoine et al., 2022)*. In this region and others, including southern Africa and central Asia, even where soil microbial biomass carbon was lost due to climate change, land-use change effects on soil microbial biomass carbon were often much closer to positive. This higher variance in soil microbial biomass carbon, in part driven by positive land-use effects, together with global cropland analyses *(Padarian et al., 2022)*, points to a potential for land management to increase soil carbon, rather than necessarily degrade it *(Dynarski et al., 2020)*. These insights highlight that increasing soil carbon globally may still be feasible, especially through sustainable local or regional management coordination.

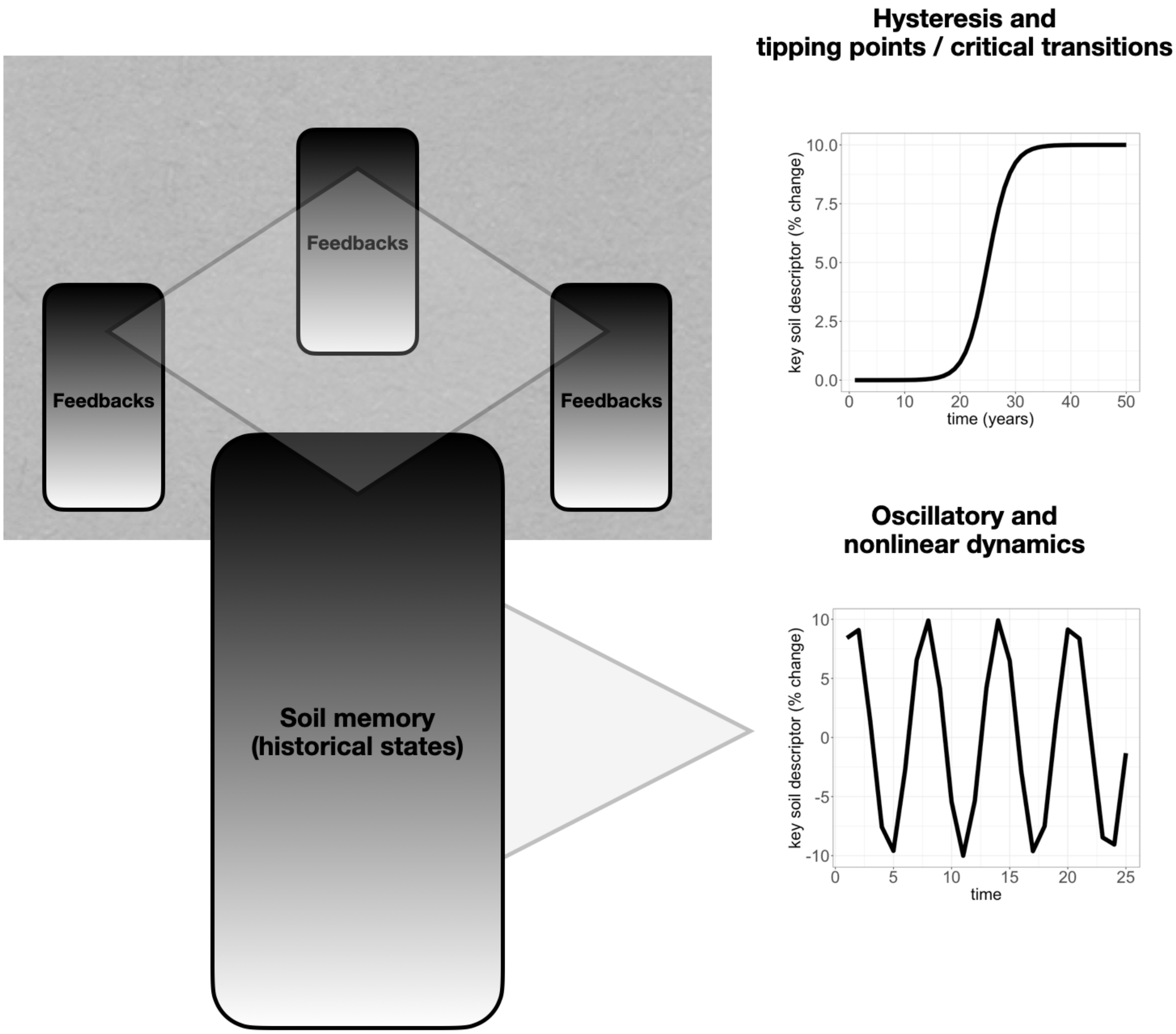

Figure 2.1: Conceptual diagram highlighting (bottom) that past states incorporated down a soil profile, like buried or subsoil horizons that are plowed, primed and nutrient-poor, or instead deeply fertile (black gradient, bottom-left), may induce oscillatory dynamics (bottom-right) in key soil variables, like moisture and microbial biomass, and that (top) similarly continuous feedbacks across many soil profiles and pedons in a landscape (top-left) may produce nonlinear regional patterns including tipping points (top-right) for key soil variables, like soil organic matter, not only during land degradation, but also during coordinated collective management toward regeneration.

Accordingly, even social processes that affect regional land management, such as in agriculture, represent a source of uncertainty affecting soil organic matter dynamics. However, for soil ecology, the understanding offered by systems and complexity theory comes less from understanding farmers' individual decision-making processes, which is still relevant *(Mestre et al., 2020)*, and more from how information spread and collective decision-making processes affect regional agricultural management regimes and related soil processes. Given the modern corporate food regime *(Campbell, 2009; McMichael, 2009)* and reliance on plantation land systems *(Wolford, 2020)*, widespread deficit narratives about the ecology of agroecosystems have been somewhat normalized. However, a liberal interpretation of a systems approach to soil ecology, by virtue of normalizing and aiming to integrate hard-to-anticipate critical transitions *(Scheffer et al., 2012; Scheffer et al., 2015)*, motivates the vision for a counter-narrative – one that focuses on supporting the world's small-scale farmers. This suggests that local and regional cooperative efforts toward ecosystem restoration and regenerative agriculture could also be strong drivers of future global changes to key soil variables, such as soil carbon.

Rather than continuing to degrade, regional soil organic matter levels could potentially increase rapidly, assuming most soils are far from carbon (or other nutrient) saturation, with agroecological soil management practices like cover crops, compost additions and other soil conservation techniques (add references). This could be directly supported by local social movements that have formed to protect small-scale agriculture and land tenure via local neighboring cooperation and collaborative governance. Studies in Brazil in areas where the landless workers movement (MST) is active, show that soil fertility is positively associated with the recent adoption of and transition to sustainable land management practices *(Stratton et al., 2022)* like cover crop diversity, which could also be promoted by stronger social recognition of benefits *(Williams et al., 2021)*. This perspective, together with other studies directly linking soil organic matter to crop yield *(Oldfield et al., 2022)*, ultimately ties soil regeneration to environmental justice issues of land tenure. Integrating socio-ecological processes thus offers an additional means by which key soil variables and anthropedogenesis can show critical non-linear transitions, especially in positive directions, challenging common narratives that depict humans as forces of soil degradation. Some ideas of how social processes affect nutrient cycling have emerged as biogeo-socio-chemistry especially for urban settings *(Pataki et al., 2011; Kaushal et al., 2014)*, and others may help address additional soil ecological dimensions of multi-functionality *(Creamer et al., 2022)* beyond nutrients, like spatial patterns of faunal diversity and soil food web network structures. Recent studies of urban ecology already point to interesting patterns that challenge deficit narratives of societal relationships with local soils and agriculture *(Bonilla-Bedoya et al., 2022; Nugent and Allison, 2022; Pindral et al., 2022; Zhang et al., 2022)*. Novel insights on soil socio-ecological dynamics may help guide how to tailor sustainable development initiatives by individual countries to achieve international soil governance initiatives *(Farnese, 2022; García et al., 2022)* like through the UN FAO Global Soil Partnership, Global Soil Biodiversity Initiative *(Wall et al., 2015)*, and other working groups generally addressing UN sustainable development goals of combating soil and habitat degradation to enhance ecosystem services via dynamic key soil ecological indicators *(Pradhan et al., 2017; Bennich et al., 2020)*.

2.6 Conclusion

This synthesis applies a complex systems framework to analyzing key uncertainties about soil processes and habitats, drawing from various analytical tools used across interdisciplinary fields, and presenting how they have and will better address key research questions in the field of soil ecology and agroecology. Concepts highlighted include soil memory, or legacy effects of management history and past ecological states; oscillations over time, which are observed in many key soil descriptive variables; and, tipping points and hysteresis, when several correlated variables change at different times and/or rates. Together, these three principles should help decrease uncertainty around soil model structures *(Bradford et al., 2016)* by pointing toward how to improve model generality for key soil processes of interest, such as soil respiration and particulate organic matter storage via occlusion by microbial community byproducts produced during soil aggregation. The complexity perspective on soil agroecology also inspires a positive outlook on the potential feasibility of collective societal solutions to the soil degradation crisis *(Montgomery, 2007; Richter, 2021)*, by including and better anticipating drastic unexpected changes that often emerge from the combined effects of many interacting variables and processes. Because farms are social-ecological systems, forward steps include collaborating with social sciences, humanities such as history or literature *(Schloss and Handelsman, 2007)*, or non-equilibrium or statistical physics *(Bak et al., 2002)* including geophysics, to expand and improve the set of potentially useful analytical tools and perspectives needed to inclusively and robustly describe the extreme heterogeneity and complexity of soil habitats.

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2.8 Declaration of interests

No conflicts of interest declared.

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2.10 Data statement

Not applicable; All references to literature data are made in-text.

2.11 References

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Chapter 3 Soil Aggregation Promotes Microbial Biodiversity by Favoring Random Ecological Drift Processes

3.1 Abstract

Soils hold one quarter of Earth's biodiversity, but the relative influences of underlying processes, such as natural selection or, in contrast, random ecological drift, often remain inconsistent. Soil structure, including particle aggregate formation, is increasingly acknowledged as a key factor shaping species interaction networks, and likely also how species consortia assemble. This study analyzes how soil structure affects fungal and bacterial community assembly processes by parsing the relative influences of processes underlying community diversity and compositional patterns across soil aggregate size fractions from a coffee agro-forest. Results show that as soil aggregate size increased, soil fungal biodiversity accumulated, including decomposers and pathogens, and increased faster than bacterial biodiversity; both community types also tended to diverge in taxonomic composition from each other, as well as from communities among smaller aggregates, and also diverged from randomly simulated community compositions. These results suggest that soil aggregation tends to increase the relative influence of random ecological drift on aggregate-associated microbial communities, which overall aligns with the traditional ecological theory of island biogeography, but contrasts microbial diversity patterns observed in other soils. Overall, this study highlights the biodiversity benefits of improving agricultural soil structure – for instance, by using organic amendments instead of synthetic sprays – and points to future studies that might refine understanding of the ecological interactions among species co-occurring together in soils.

3.2 Introduction

Most biodiversity is microbial, and soils contain one-quarter of all biodiversity as Earth's most biodiverse habitat *(Bahram et al. 2018; Averill et al. 2022; Baldrian et al. 2022)*, containing relatively novel taxa *(Talbot et al. 2014; Tedersoo et al. 2020)*, key for important soil functions, including decomposition and biodiversity maintenance *(Glassman et al. 2018)*. Accordingly, the research question of how this vast biodiversity is maintained is fundamental to biodiversity and soil microbial ecology research *(Martiny et al. 2006; Liu, Bearup, and Liao 2022)*, having implications for future genetic variation *(Escalas et al. 2022)* and functions *(Nelson, Martiny, and Martiny 2016; Wilhelm et al. 2023)* such as nutrient cycling *(Crowther et al. 2019; Högberg et al. 2020; Sokol et al. 2022)* and climate change *(Větrovský et al. 2019; Jansson and Hofmockel 2020)*. Despite some environmental filtering *(Glassman et al. 2017)*, microbial biodiversity and likely complexity *(Galiana et al. 2022)* tends to follow predictable increasing patterns with and across larger spatial scales *(Horner-Devine et al. 2004, 2007; Kenneth J. Locey and Lennon 2016; Grilli 2020)*, which agrees with traditional ecological theory explaining species-area curves *(Macarthur and Wilson 1963)*. Locally, however, these patterns may not hold or even be reversed, such as in soils *(Tedersoo et al. 2003; Bach et al. 2018)*. To resolve potential deviations from traditional species-area predictions, island biogeography *(Glassman et al. 2017)*, and broader effects of spatial scale on microbial communities *(Bahram, Peay, and Tedersoo 2015; V. Guerra et al. 2020)*, it is useful to parse out the contributions of various underlying processes shaping soil microbial community structures.

Previous conceptual frameworks identify basic eco-evolutionary microbial community assembly processes as including drift, selection, dispersal, and diversification *(Vellend 2010; Hanson et al. 2012; Nemergut et al. 2013; Vila et al. 2019)*, with previous study *(Evans,* *Martiny, and Allison 2017; Mougi 2019)*. Modern frameworks of coexistence *(Chesson 2000; Barabás, D'Andrea, and Stump 2018; Levine 2000)* originally draw from the ability of an introduced species to pack into an existing community, implying limited potential niche overlap among co-existing species, but also implies that coexistence depends on the potentially stochastic persistence of new immigrants *(Kinnunen et al. 2018)*. The eco-evolutionary inclusion of selection as a community assembly process, in practice, can be seen as akin to testing for environmental filtering, analogous to statistical definitions of fitness *(Orr 2009)*. However, recent perspectives *(Hawlena 2022)* add more complex processes to close gaps in fuller explanations of biodiversity maintenance *(HilleRisLambers et al. 2012)*, which include the effects of habitat refugia on small populations *(Hopf and Hopf 1985; Vandermeer 2021)*, intransitive (i.e. rock-paper-scissors style) loops of conflicting pairwise competition among various species *(Allesina and Levine 2011; Vandermeer 2011; Levine et al. 2017; Gallien et al. 2017; Soliveres et al. 2015)* and higher-order indirect interactions *(Werner 1992; Bairey, Kelsic, and Kishony 2016; Gibbs, Levin, and Levine 2022)*, all of which can also occur within microbial consortia *(DeAngelis, Lindow, and Firestone 2008; Heilmann, Krishna, and Kerr 2015; Swain, Fussell, and Fagan 2022)*. Continuing to parse the relative importance of contributing processes, as well as how they change across environmental gradients, remains an active line of research *(Chase and Myers 2011; Vellend et al. 2014; Dini-Andreote et al. 2015; Blaser et al. 2016)*.

Spatial separation is increasingly reported as a key factor shaping overall community structures *(Levin 1992)* and other interdisciplinary phenomena *(Pachepsky and Hill 2017)*, although it remains unclear how it affects the relative importance of underlying community assembly processes. Motivated by widespread habitat fragmentation *(Haddad et al. 2015)*, early spatial analyses led to ecological theories of island biogeography *(Macarthur and Wilson 1963)*, meta-population *(Levins 1969)*, meta-community *(M. A. Leibold et al. 2004)*, and meta-ecosystems *(Loreau, Mouquet, and Holt 2003)*. In soil habitats specifically, the complex spatial structure of micro-habitats in between air-filled pores and organic particle aggregates is increasingly presented as important for key ecosystem functions, such as nutrient cycling *(Six and Paustian 2014; Kravchenko et al. 2019)*, diversification *(Rillig, Ingraffia, and Machado 2017)*, and trophic interactions in soil food webs *(Erktan et al. 2020)*. Soil components that tend to self-organize into hierarchies, such as aggregates *(Tisdall and Oades 1982)*, likely mediate overall community composition and assembly *(Mathew A. Leibold et al. 2022)* by changing spatial connections between populations, which could be in pairwise competition, mutualistic co-metabolism *(Lopez and Wingreen 2022)*, dispersing, and experiencing stochastic fluctuations in size. Indeed, soil microbial spatial structure is likely in part shaped by a tendency for micro-aggregates under 250 um to hold relatively more stable and processed mineral-associated organic carbon, while macro-aggregates over 250 um can occlude more labile and energy-rich particulate organic carbon *(Totsche et al. 2018)*. However, while chemical variation in reactive functional side groups is theoretically infinite, much evidence shows functional redundancy in most microbial groups *(Allison and Martiny 2008)*, especially for widespread processes such as decomposition. Therefore, it likely remains insufficient to hypothesize that soil microbial community variation can be exaplained primarily by fine-scale chemical variation in abiotic niches across soil particles. Instead, soil microbial community diversity and compositional changes across fine spatial scales traversing soil aggregates likely require invoking more complex and higher-order biological and ecological interactions among the vast array of genetically variable taxa. As a result, soil aggregates likely change species distribution

(Ostling 2000; Harte et al. 2005; Jackson et al. 2014) and similarity of aggregate-associated community compositions, or beta diversity (Anderson, Ellingsen, and McArdle 2006).

This study asked how soil aggregates structure microbial biodiversity and the relative influences of basic underlying processes shaping their communities. Realistically assuming bounded chemical variation among individual soil aggregates within empirical size fractions, and in contrast to traditional island biogeography, which predicts higher biodiversity in larger soil 'islands', i.e. soil aggregates, mainly due to less extinction of immigrating populations, we hypothesized that soil aggregation would increase deterministic processes, such as traditional transitive competition, relative to random stochastic ecological drift, and in turn, would result in predictions of lower alpha (Bach et al. 2018) and beta diversity among larger soil aggregates.

3.3 Methods

3.3.1 Study site selection

This study was conducted using soil collected from Finca Irlanda (15.1732729, -92.3365757), a 45 ha neotropical shaded coffee agroforest in the Soconusco region of Chiapas, Mexico (Philpott 2006) of broad relevance (Toju et al. 2018) and well-studied (Vandermeer et al. 2019). The local ecological network is complex but revolves around interactions with the keystone ant *Azteca sericeasur* (Vandermeer et al. 2021), a widespread species in the neotropics known for ant-plant mutualisms (Janzen and Apr 2017), and has locally dynamic (Vandermeer, Perfecto, and Philpott 2008) yet indiscriminate nesting patterns (K. Li et al. 2016). It nests only in the shade trees of the system and is thus easily located by sampling those trees. Nine sites with ant nest-control tree pairs, whose two trees were up to about 100 m distance from each other, were chosen for sampling (n<=9), and each selected tree had a history of either no nest or an *Azteca* ant nest for the past five years, totaling about 10 trees and 40 soil cores.

3.3.2 Data collection

3.3.2.1 Soil sampling and aggregate isolation

Soils were sampled in quadruplicate per tree, with each core taken one meter in each cardinal direction around the local tree trunk base to 10 cm depth using a 5.5 cm wide slide hammer corer (AMS Inc, American Falls, ID, USA). Core samples were collected in the plastic liners inside the metal core cylinder, sealed with rubber caps, and transported on ice within 6 h to the local field station, where soil was released from plastic liners along existing fault lines by tapping the side with a rubber hammer and composited by tree. Soil cores were oven dried at 60 C for 24-72 h, weighed, and pooled by tree to amass enough hardened soil that can withstand mechanical sieving, and more clearly define subsequent soil aggregate size fractions. Soil aggregate size fractions were distinguished by passing all soil collected through through a stack of meshes that were 2000, 500, 250, 125, and 63 µm wide by shaking across approximately 10 cm at 30 oscillations per min.

3.3.2.2 DNA extraction and sequencing

Approximately 0.15 g soil was sub-sampled from each tree's pooled soil aggregate mass fraction (35 samples total across about seven trees and five soil aggregate size fraction each) and run through the manufacturer protocol for the DNeasy PowerLyzer PowerSoil kit (QIAGEN, Ann Arbor, MI, USA). Protocol modifications to improve yield, due to DNA sorption to fine soil particles, included replacing half (300 µL) of the listed Powerbead solution with 1 M sodium-potassium phosphate buffer (pH 7) before initial shaking for bead disruption, and after this cell lysis step, an additional water bath incubation at 65 C for 10 min (noted in protocols.io). Final extracts were eluted with the standard manufacturer solution C6, stored at -20 C.

Library prep and amplicon next-generation MiSeq sequencing (Illumina Inc, San Diego, CA, USA) was done at the University of Michigan Microbiome Sequencing Core (Ann Arbor, MI, USA) using standard protocols for community profiling. Specifically, protocols amplified bacterial 16S via V4 *(Kozich et al. 2013; Caporaso et al. 2012)* and fungal internal transcribed spacer ITS2 ribosomal regions *(Schoch et al. 2012)* via 5.8S-Fun/ITS4-Fun primers *(D. L. Taylor et al. 2016)* for standard PCR conditions and more sensitive touchdown PCR as needed.

3.3.2.3 Bioinformatics

Sequence data was resolved to exact or amplicon sequence variants (ASVs) *(Benjamin J. Callahan, Mcmurdie, and Holmes 2017)* over other methods *(Schloss et al. 2009; J. Chen et al. 2013; Dufresne et al. 2019; Schloss 2020)* to help analyze fine scale genetic diversity *(Van Rossum et al. 2020)* with potentially low impacts on results *(Nguyen et al. 2015; Glassman et al. 2017)*, though resolving microbial diversity remains a challenge for bigger data sets *(Y. P. Wang et al. 2016; Lücking et al. 2020; Phukhamsakda et al. 2022)*. Sequences were processed with 'DADA2' 1.28.0 *(Benjamin J. Callahan et al. 2016)* (https://benjjneb.github.io/dada2/ITS_workflow.html), including suggested changes for ITS, mainly using higher quality forward reads *(Argiroff et al. 2022)*, variable-length unwanted sequence trimming with 'cutadapt' in python 3.10 *(M. Martin 2011)*, and fine tuning parameters minimum read quality score 'truncQ' and maximum expected base errors 'maxEE' both increased from 2 to 8 *(Rolling et al. 2022)*. Taxonomy was assigned with DADA2's naive Bayesian classifier method *(Q. Wang et al. 2007)* and the bacterial SILVA *(Quast et al. 2012; Glöckner et al. 2017) v132 (B. Callahan 2018)* and fungal UNITE *v9.0 (Abarenkov et al. 2010;*

Nilsson et al. 2019) databases, namely *v16.10.2022* with singleton fungal references *(Abarenkov et al. 2022)*.

3.3.3 Statistics

Analyses were done in R version 4.3.1 (2023-06-16) *(R Core Team 2023)*. A total of 6215 unique bacterial and 3948 unique fungal ASVs were observed across all soil samples, and each sample was rarefied to the minimum number of unique sequences inferred per sample, near 500 for bacteria and 400 for fungi. Alpha diversity was measured as (unique) ASV richness, per-aggregate values calculated by dividing richness per million soil particles assuming cubic shape and average density of aggregates per mass fraction *(Perfect, Rasiah, and Kay 1992)*, and soil aggregate size effects were assessed with Kruskal-Wallis tests on cumulative richness (and physical soil mass) distribution parameters across soil aggregate fraction sizes.

Beta diversity was measured as centered pairwise Bray-Curtis distances between samples within the same soil aggregate size fraction (*n*=15-50). To estimate significant influences of deterministic versus stochastic (i.e. drift) processes, observed beta diversity values were also compared to the beta diversity values among 99 random sub-samples of the rarefied community per soil sample, thereby also maintaining sample meta-data such as aggregate size fraction, but within each sample instead giving random yet likely presence (i.e. 0) and abundance values *(Anderson, Ellingsen, and McArdle 2006)*, as a useful null model for beta diversity *(Chase and Myers 2011; Vass et al. 2020)* to combat sample heterogeneity effects *(Armitage and Jones 2019)*. Significant deviations from random stochastic (drift) processes were inferred in soil aggregate fractions where simulated and observed error bars for beta diversity did not overlap *(Stegen et al. 2013)*, with magnitudes estimated as one minus the absolute value of observed minus simulated distance. Additionally, increases in beta diversity with aggregate size were considered indicative of habitat heterogeneity effects *(Chase et al. 2019)*.

Individual taxon responses to soil aggregate size were practically measured with Pearson correlations among taxon abundance data pooled across tree site soil samples, despite some inference limits *(Carr et al. 2019)*. Broadly, other useful R packages assisting reproducibility *(Nilsson et al. 2019)* included 'here' *(Müller 2020)*, 'dplyr' *(Wickham et al. 2023)* and 'tidyr' *(Wickham, Vaughan, and Girlich 2023)*, 'rstatix' and 'vegan' *(Dixon 2003)*, 'ggpmisc' *(Aphalo 2022)*, 'ggpubr' *(Kassambara 2023)*, and 'bookdown' *(Xie 2023)*.

3.4 Results

3.4.1 Alpha diversity

Overall, richness increased with soil aggregate size *(Fig 3.1)*. The rates of increase were explained by scaling (power) law functions, as well as similar long-tailed distribution functions, namely log-normal, exponential, and poisson. Total and per-aggregate rates of increase in richness varied significantly across soil physical mass and microbial richness variables, under scaling law models by 0.56 parameter units (df=2, n=25, p=0.0008), under exponential models by 0.71 parameter units (df=2, n=25, p=0.00015), with fungi showing faster rates of increase by about double under these two distribution models, and physical soil aggregate sizes showing faster rates of accumulation by about at least double under poisson and log-normal models.

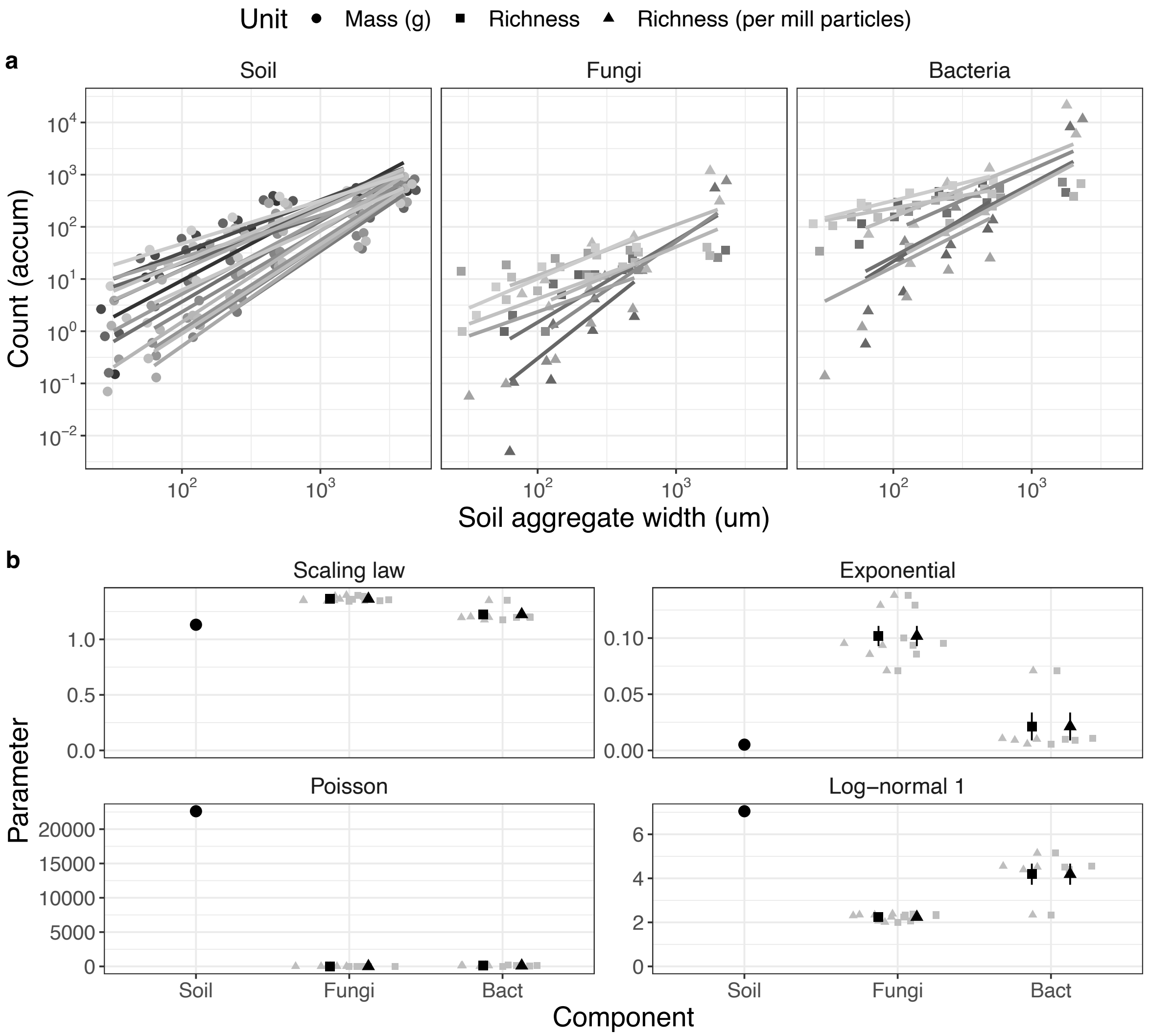

Figure 3.1: Plot of (a) cumulative soil mass (circles), total ASV richness (squares), and richness per million soil aggregates (triangles), showing tree or sampling area replicates (greyscale); and (b) distribution family parameters by domain or component of soil, including scaling (or power) law, exponential, poisson, and lognormal functions, with sample data (grey) and means ± 1 standard error (black).

3.4.2 Beta diversity

Overall, community composition varied by soil aggregate size, marginally significantly for observed fungi (df=5, p=0.1) and significantly for both simulated bacteria (df=5, p=<0.0001) and fungal communities (df=5, p=0.012) *(Fig 3.2)*. Observed bacterial communities were also significantly different from simulated ones (df=1, p=0.00034), with largest difference appearing at 250 um. Beta diversity community distances differed significantly by soil aggregate size, in bacteria by 0.017 (df=5, p=0.0032) and fungi by 0.48 distance units overall (df=5, p=<0.0001) .

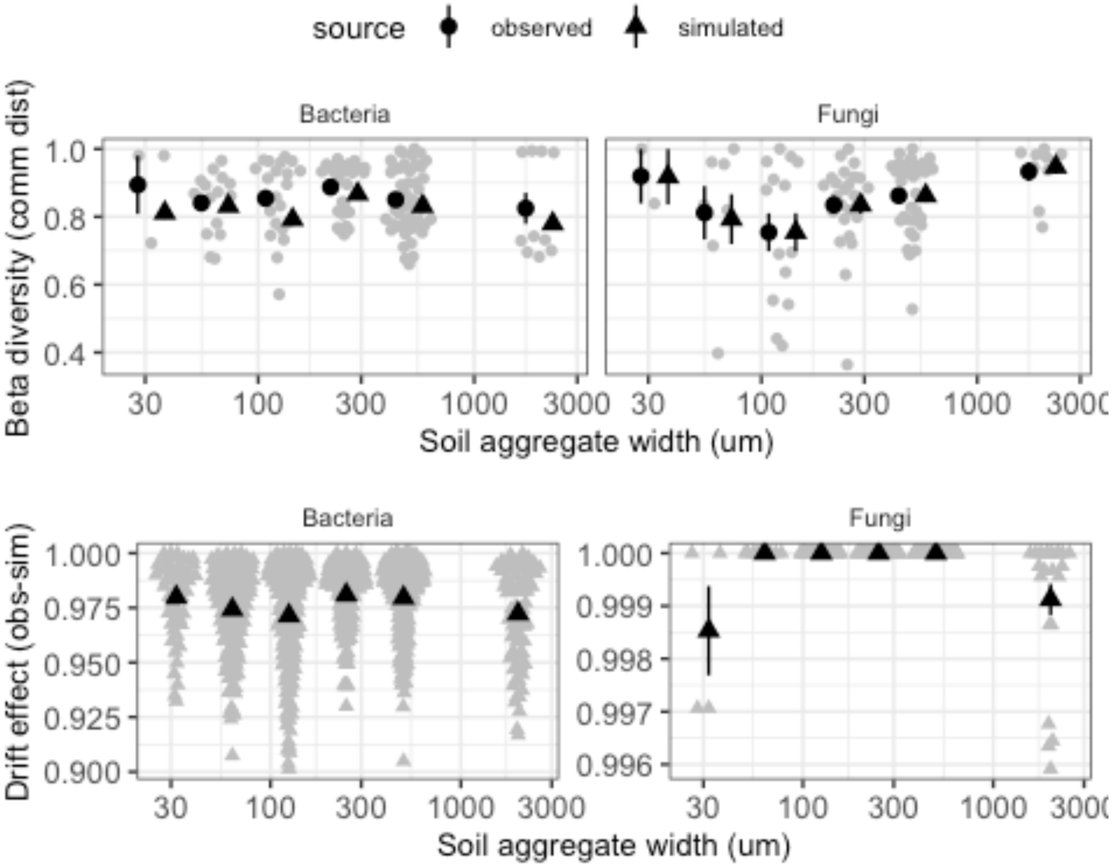

Figure 3.2: Beta diversity calculated as pairwise bray-curtis distances between simulated (triangles, 99 iterates) and observed (circles) rarefied bacterial (left) and fungal (right) communities across sampling sites and soil aggregate sizes (top), as well as differences between observed and simulated community distances (bottom), all showing underlying data (grey) and bin size center point means ± 1 standard error (underlying simulated hidden to clarify observed data in top panel).

3.4.3 Taxonomic composition

Of observed genera in both fungi and bacteria showing significant correlations with soil aggregate size, most correlated positively *(Fig 3.3)*. Specifically, nine bacterial genera increased in abundance (descending correlation estimate): *Acidothermus* (p=0.0111, r=1), *Gemmata* (p=0.0766, r=0.99), *Candidatus Udaeobacter* (p=0.102, r=0.99), *Nocardioides* (p=0.013, r=0.95), *Gaiella* (p=0.0757, r=0.84, p=0.0703, r=0.93), *Polycladomyces* (p=0.0665, r=0.85), *Luedemannella* (p=0.0614, r=0.73), *Bryobacter* (p=0.0995, r=0.62), and *Pedomicrobium* (p=0.021, r=0.43) ; as well as 15 fungal genera: *Xylodon* (p=0.00022, r=0.41), *Tremella* (p=0.00022, r=0.41), *Paraconiothyrium* (p=0.00022, r=0.41), *Neopyrenochaeta* (p=0.00022, r=0.41), *Microdiplodia* (p=0.00022, r=0.41), *Ceriporia* (p=0.00022, r=0.41), *Castanediella* (p=0.00022, r=0.41), *Aquidictyomyces* (p=0.00022, r=0.41), *Absidia* (p=0.00022, r=0.41), *Phanerochaete* (p=0.0125, r=0.28, p=0.00022, r=0.41), *Calvatia* (p=0.0135, r=0.28, p=0.00022, r=0.41), *Saitozyma* (p=0.00603, r=0.31), and *Ganoderma* (p=0.104, r=0.19) .

Significant negative correlations with soil aggregate size included five bacterial genera – *Chthoniobacter* (p=0.12, r=-0.7), *Bacillus* (p=0.0503, r=-0.71), *Romboutsia* (p=0.0748, r=-0.93), *Streptomyces* (p=0.0134, r=-0.95, p=0.0082, r=-0.99), and *Conexibacter* (p=0.0235, r=-1, p=0.0516, r=-1) – as well as two fungal genera – *Rhizomucor* (p=0.0906, r=-0.19) and *Lamprospora* (p=0.089, r=-0.19) . *Thermoactinomyces* was the only bacteria that responded significantly and neutrally on average to soil aggregate size (p=0.0588, r=0.44, p=0.117, r=-0.56) .

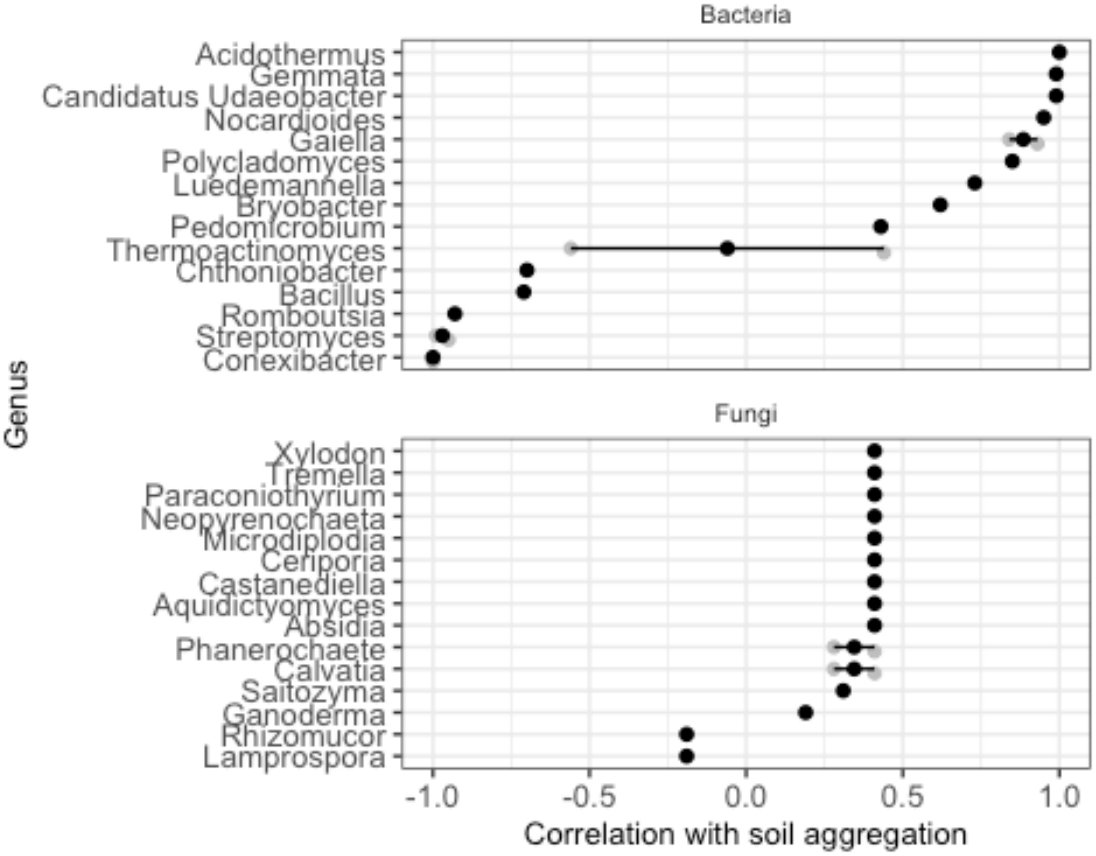

Figure 3.3: Significant pearson correlation estimates for bacterial (top) and fungal (bottom) ASV abundances among all soil samples across soil aggregate sizes, showing presence in either single or multiple tree site soil samples (grey) and center means ± 1 standard error (black).

3.5 Discussion

Overall, this study shows that soil aggregation promotes both microbial biodiversity and stochastic community assembly processes, such as ecological drift and competitive intransitivity. This was shown by higher alpha diversity (i.e., cumulative richness) as soil aggregate size increased, and a detectable divergence in beta diversity (i.e., community dissimilarity) between the empirical and simulated communities, which represented a shift away from randomly assembly community compositions. These results were generally true for both bacteria and fungi, most of whose genera responded positively to soil aggregate size, but fungi also accumulated species notably faster with increasing soil aggregate size (as physically fractionated) compared to bacteria, with relatively more genera increasing abundance with soil aggregate size. Together, this study supports those biodiversity patterns predicted by traditional island biogeography and observed in other microbial studies *(Grilli 2020)*, although underlying biodiversity-maintaining processes likely include more than those originally hypothesized *(Macarthur and Wilson 1963)* and even later developed *(Vellend 2010)*, especially in speciose microbial communities.

There are several ways to explain how soil aggregation accumulates microbial biodiversity, and these relevant mechanisms may vary in some ways between bacterial and fungal communities. First, even with neutral biological interactions, the physical aggregation of soil particles can accumulate microbial biodiversity because microbial populations live on soil aggregate surfaces *(Wilpiszeski et al. 2019)*. However, if this were true, alpha diversity would accumulate at nearly the same rate as soil mass, which does not align with the data presented here. Instead, biological richness accumulated at notably different rates than the physical soil environment, thereby invoking additional, and likely ecological, mechanisms of biodiversity maintenance. Originally, island biogeography invoked relatively faster immigration compared to extinction rates to explain species-area accumulation curves. This may also be true in soils, assuming a revised model tailored to the physically aggregated soil habitat, where larger soil islands are also physically closer together, under the soil aggregate hierarchy model, thereby allowing for faster immigration relative to extinction. Future studies making use of newer fine-scale technologies *(Tan, Saleski, and Lin 2022; Clark et al. 2023)* should help more explicitly test variable immigration rates, and potentially extinction rates using multi-gene sequencing *(Badia-i-Mompel et al. 2023)*.

Comparing bacterial and fungal diversity accumulation rates, some models detected fungi accumulating richness faster than bacteria and the mass of the soil environment, while other models detected bacteria accumulating richness slower than fungi and the mass of the soil environment. Bacterial richness accumulation could be explained in part physically by a dense spatial footprint of growing populations, since bacteria tend to form nucleated colonies more often than long thin linear chains, which in contrast is more often the structure of fungal cells as hyphae, useful for spatial adaptation *(Orive, Barfield, and Holt 2023)*. Another potential explanation is that microbes can persist under metabolic dormancy for long periods of time *(Lennon and Jones 2011; Rebelo et al. 2021)*, such as when water or nutrient availability is low, which can affect microbial dispersal *(Wisnoski, Leibold, and Lennon 2019; K. J. Locey et al. 2020)*, microbial resilience *(Sorensen and Shade 2020)* and net biodiversity *(Jones and Lennon 2010)* and stability *(Qian and Akçay 2020)*, and soil functions *(Joergensen and Wichern 2018; Salazar, Sulman, and Dukes 2018)*. In cases when populations do not survive to respond to the next favorable environment and die out, remaining relic DNA can persist separate from any cell activity and accumulate noticeably in soils *(Carini et al. 2016)*. Furthermore, metabolic activity and community assembly can be affected by soil viruses *(Braga et al. 2020)*, such as bacteriophages *(Marsh and Wellington 1994; Ashelford, Day, and Fry 2003)* and mycoviruses *(Talbot et al. 2014; Myers et al. 2020)*, which are notably under-studied *(Trubl et al. 2018)*. Adding assumptions of non-neutral interactions among taxa, despite likely mutualisms *(Cai et al. 2020)* and variable genetic *(Nair et al. 2019; Stewart et al. 2022; Hogle et al. 2023)* and demographic dynamics *(Abreu et al. 2019; De La Cruz Barron et al. 2023)*, slower bacterial richness accumulation could also be explained by antagonisms among various taxa, such as competing metabolisms and cross-feeding inhibition, where neighboring taxa are limited in absorbing and gaining energy from the biochemical byproducts of a different taxon's metabolism, such as antibiotics *(Seth and Taga 2014; Liao et al. 2020; Lopez and Wingreen 2022)*, which can emerge spontaneously *(X.-W. Wang and Liu 2021)* and yield bi-stability *(Vet, Gelens, and Gonze 2020)* or coexistence *(Niehaus et al. 2019)* also depending on commensalisms *(Pacheco, Moel, and Segrè 2019)*. Furthermore, at longer time scales, soil aggregates may serve as evolutionary incubators, facilitating biological diversification *(Vellend et al. 2014)* and *de novo* speciation *(Nemergut et al. 2013)*. Currently, the bacterial domain of life is considered to have accumulated more genetic diversity than fungal kingdom *(Hug et al. 2016; James et al. 2020)*, in part due to evolutionary origins and genetic complexities *(Berbee and Taylor 2010; Grossart et al. 2016)* , which can occasionally result in limited adaptation *(Scheuerl et al. 2020)* such as by negative epistatic interactions among genes or their products *(Khan et al. 2011; Porse et al. 2020; A. J. Taylor et al. 2021)*. In fungi, while richness still accumulated, it did so at a faster rate compared to bacteria and physical soil mass, under scaling law and exponential distribution models. This result suggests that commensal or perhaps mutualistic interactions, potentially leading to diversification such as by functional plasticity, may outweigh negative interactions occurring among soil fungal taxa *(Smith et al. 2018)*. Also of note is that bacteria and fungi are known to interact with each other, potentially mutualistically *(Frey-Klett, Garbaye, and Tarkka 2007; Nasslahsen et al. 2022)*. While the results presented support predictions made by island biogeography theory *(Macarthur and Wilson 1963)* and other microbial syntheses *(Kenneth J. Locey and Lennon 2016)*, the hyper-diverse nature of microbial communities in soils may extend beyond the implied equilibrium balances between immigration and extinction, and more toward increasing contributions of more complex mechanisms, such as conflicting *(Soliveres et al. 2015)* yet stable *(Serván et al. 2018)* intransitive loops and indirect higher-order interactions *(Morin et al. 2022)*, to help explain biodiversity maintenance *(Bairey, Kelsic, and Kishony 2016)* and relevant functions *(Albrecht et al. 2021)*.

The result for beta diversity aligns with scale dependence of beta diversity *(Jennifer B. H. Martiny et al. 2011)*, related functions *(Waring et al. 2016)*, and assembly processes *(Bier et al. 2022)*, but results in part ran counter to our original hypothesis, and may be explained by similar processes underlying alpha diversity results, but with some differences. The original hypothesis presented here was that soil microbial communities would be more similar to each other among the largest soil aggregates, and most different from each other among the smallest soil aggregates, in part because of alleviation from strongly competitive individual species when physical soil particles were more separate from each other, one outcome of spatial competition *(Gude et al. 2020)*. Among larger aggregates, soil communities were predicted to show lower turnover *(Rivett et al. 2021)* and converge to the same few dominant taxa, given there is potentially more spatial proximity and connections among populations when soil particles , or at least cells themselves, are physically closer together in clusters, therefore making it easier for competition hierarchies to play out. These processes may still occur, but inferences about community assembly processes *(Stegen et al. 2013)* imply that any competitive interactions becoming stronger as soil aggregates increase in size may end up as unstable, such as among three or more taxa. In other words, there may still be competition increasing in larger aggregates, but the dominant species may vary due to instability *(Tabi et al. 2020)*, leading to greater distance or dissimilarity among samples of soil aggregates. This could occur if ecological dominance was not a fundamental (i.e., genetic) characteristic of a species, but instead dependent on which other available species competitors there were in the surrounding environment. In contrast, among smaller aggregates, species populations may be more dormant, such as from smaller nutrient pools representing lower carrying capacity in smaller patch habitats, or more potential spatial configurations *(Borer, Tecon, and Or 2018)*, and so neutral ecological interactions to be more prevalent, which overall may not favor any particular species, given variable priority effects *(Kennedy and Bruns 2005; Neumann, Echeverria, and Hasenauer 2023; Debray et al. 2022; Dolinšek, Ramoneda, and Johnson 2022)* from abiotic mineral or niche heterogeneity *(F. Li et al. 2018; Muscarella et al. 2019; Finley et al. 2022)* with notable tradeoffs *(Manhart and Shakhnovich 2018; Yonatan et al. 2022; Ontiveros et al. 2023)*, implying that such indeterminate competition could thereby explain why beta diversity (i.e. community dissimilarity values) may be less distinguishable from the random simulations. Furthermore, in the potential case of smaller dormant population sizes, extinction would accordingly be more likely to have a significant effect on local species diversity per small soil aggregate particle, especially since there is less physical space, and therefore also likely fewer nutrients available, on the smaller particles themselves. The role of extinction has also been a key underlying hypothesis from traditional island biogeography theory *(Macarthur and Wilson 1963)*, but with extinction being a fundamentally extreme process to measure (i.e. one must be certain that there is not a single individual remaining from any population of a species), the role of extinction is community assembly and longer-term evolution has been difficult to analyze *(Newman 1996)*. As context, it is also worth considering soil type effects on soil aggregation and microbial diversity patterns, where coarse-textured soils may potentially show weaker self-similarity than finer-textured soils or soils with higher organic matter, which might have more micro-aggregates within macro-aggregates, and thus could potentially predict weaker or stronger drift effects, depending on some fine-scale factors such as group selection *(Jansen 2011)*.

Individual taxonomy findings here agree with the current known ecology of specific genera, as well as generally similar studies, which have also found *Nocardioides* and *Pedomicrobium* in larger soil aggregates *(Z. Chen et al. 2022)*. Bacteria that increased with soil aggregation included common dominant soil genera – *Gemmata (Singh and Bhagwat 2022)*, *Candidatus Udaeobacter (Willms et al. 2021)*, and *Bryobacter (Deng et al. 2018)* – as well as key functional genera, including *Nocardioides* and *Luedemannella* for key enzymes and nutrients *(Zhang et al. 2021)*, *Gaiella* for disease-resistant crop cultivars *(Lazcano et al. 2021; Wongkiew et al. 2022)*, *Bryobacter* for whole microbial communities *(Wu et al. 2021; X.-W. Wang and Liu 2021; Hu and Shen 2022)*, and *Pedomicrobium* for root rot disease aversion *(Jiang et al. 2019)*. Bacteria that decreased with soil aggregation included common soil bacteria, namely *Chthanobacter*, *Bacillus*, *Romboutsia*, and *Streptomyces*. Many observed genera were also typical for weathered acidic tropical agricultural soils, namely *Candidatus Udaeobacter (Willms et al. 2021)*, *Gaiella (Wongkiew et al. 2022)*, *Bryobacter (X.-W. Wang and Liu 2021)*, *Pedomicrobium (Braun, Richert, and Szewzyk 2009)*; *Chthoniobacter*, *Bacillus (Van Dijl and Hecker 2013; Radhakrishnan, Hashem, and Abd_Allah 2017; Saxena et al. 2020; Yahya et al. 2021)*, *Romboutsia (Brown, Appiah, and Berninger 2022; Shang et al. 2022)*, and *Streptomyces (Tarkka et al. 2008; Seipke, Kaltenpoth, and Hutchings 2012; Olanrewaju and Babalola 2019; Cordero, De Freitas, and Germida 2020; Hamid et al. 2020)*. Fungi promoted by soil aggregation included various saprotroph decomposer genera: *Xylodon*; *Neopyrenochaeta (Jayasiri 2019)*; *Ceriporia* which mobilizes phosphorus, iron *(Sui, Huang, and Yuan 2022; Sui et al. 2022; Jun Yin and Porporato 2022)*, and nitrogen *(Jie Yin, Yuan, and Huang 2021)*; *Absidia* with only recently known habitat preferences *(Zhao et al. 2022)*; *Phanerochaete (Davis et al. 1993)*; *Calvatia (Hou et al. 2012; Golubkina et al. 2023)*; and *Ganoderma (Anothai and Chairin*

2020); while *Rhizomucor* responded negatively to soil aggregation. Simultaneously, opportunistic plant and human pathogen genera also increased with soil aggregation: *Paraconiothyrium* (*Verkley 2004; Damm et al. 2008; Gordon et al. 2012; Guarnaccia et al. 2022; Tennakoon et al. 2022*); *Microdiplodia* (*Dietzel et al. 2019*); *Absidia* (*Moon and Jithendran 2018*); but *Rhizomucor* (*Kimura et al. 2009; Ziaee et al. 2016; Ibrahim and Voelz 2017; Cornely et al. 2019; Menzinger, Sid'Amar, and Kaya 2020)* with potential anti-fungal resistance *(Dannaoui 2017)* decreased with soil aggregation. In turn, medicinal genera also increased with soil aggregation, namely *Tremella (Xu, Jiang, and Wang 2021)* and *Ganoderma (Ren et al. 2020)*. The increase in both pathogens and medicinal fungi, along with important saprotroph decomposers *(Vlk et al. 2020; Yang et al. 2021)* and key bacterial groups, aligns with higher richness with soil aggregation, as well as more speciose communities also being more complex and/or stable *(Manhart and Shakhnovich 2018; Averill et al. 2022; Yonatan et al. 2022)*, although this is uncertain with respect to traits *(Chaudhary et al. 2022)*.

In conclusion, this study shows how community assembly processes, most likely more ecological drift shown by diverging observed community compositions from randomly simulated community composition, affect various soil microbial groups differently, thereby helping elaborate on the application of traditional island biogeography theory to soil micro-habitats. In applied contexts, these fundamental ecological insights might also point to some extended implications for practitioners, such as the more improvement in soil structure and aggregation, rather than simply chemical fertility, then the more microbial diversity that may accumulate in soil aggregates, potentially avoiding more local soil erosion and general degradation in soil quality. For future studies, we recommend more direct manipulations that test specific examples of competitive hierarchies or intransitive loops among common soil microbial taxa *(Lozano et al.*

2019) and/or soil invertebrates *(Sprunger 2015; T. Martin and Sprunger 2021)*, as well as important symbiotic mutualisms, which are understudied in ecology compared to competition. Ultimately, this study promotes the further study of key effects of soil structure on hotspots of soil biodiversity and functioning.

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3.7 Data availability

Code stored at github.com/nmedina17/microbes.

3.8 Interests statement

No conflicts of interest to declare.

3.9 References

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Chapter 4 Above- and Below-ground Links Mediated by Arboreal Ants and Host Tree Modifying Soil Aggregation Scaling, Infiltration, and Chemistry

4.1 Abstract

Soils are increasingly recognized as complex systems, emphasizing a need to study unique properties such as long-tailed scaling laws and the role of indirect interactions among arboreal above- and below-ground soil invertebrates. However, few studies consider the above-below-ground connections mediated by invertebrates' activity and behavior compared to tree physiology. Given previous work showing that arboreal ants can compete and affect ground foragers as well as altered foraging behavior on different host trees, it is plausible that persistent above-ground ant nesting could extend to affect soil properties including structure and chemistry, mediated by ground ant exclusion. This study analyzes soil aggregation, water infiltration, and macro-chemical data associated with longer-term (5+ year) ant nesting in a rustic tropical agroforest. Results show that, 1) ant nesting maintained scaling law exponents or fractal dimensions of soil aggregate size distributions, and significantly associated with relatively larger micro-aggregate diameters and log-normal variance in macro-aggregate size distributions, suggesting more consistent (less variable) underlying aggregation processes similar to host tree species effects; 2) areas in the vicinity of trees with no dominant ant nests had three-times faster water infiltration than in the vicinity of trees with dominant ant nests; and 3) a tendency toward changes in soil carbon and nitrogen stocks by one-quarter depending on host tree. These patterns are consistent with the hypothesis linking above-belowground processes via ground ant suppression by an aggressive keystone arboreal ant, and are supported by previous studies

reporting positive ground ant nest effects on soil chemistry and documenting ground ant foraging as a source of soil aggregate fragmentation. This study presents new ecological processes affecting ecosystem-scale functions, and suggests that future research on indirect interaction cascades would be beneficial to advance fundamental understanding of drivers of whole-ecosystem processes.

4.2 Introduction

Soils are a basis for both ecosystem functioning *(V. C. Yang, Papachristos, and Abrams 2019)* and human civilizations *(Montgomery 2007; Richter 2021)*. Accordingly, they are increasingly acknowledged as complex systems *(Young and Crawford 2004)* with many key interacting descriptive natural variables *(Kuzyakov and Zamanian 2019)*, as well as less-studied social ones *(Duarte-Guardia et al. 2020)*. However, both soil fauna and agroecological management (i.e. agroforestry polycultures vs. industrial monocropping) dimensions are continually under-studied *(Bottinelli et al. 2015; Grandy et al. 2016)* alongside soil microbial diversity and activity, prompting recent related syntheses *(Lavelle et al. 2016; Barreto and Lindo 2022; Potapov 2022; Medina and Vandermeer 2023)* and empirical efforts *(Maaß, Caruso, and Rillig 2015)*. Specifically, ants are key soil engineers in the tropics *(Griffiths et al. 2018)*, as earthworms are in temperate zones *(Jouquet et al. 2006; Sánchez-de León et al. 2018; Angst et al. 2019)*, and in addition, themselves behave as a complex adaptive organismal system *(Gordon 1996)* whose collective soil engineering behavior is an extended phenotype *(Jouquet et al. 2006)* varying by taxon. Studying ant effects on soils also represents a unique coupling of naturally complex systems, especially in agroecologically managed agroecosystems where ant activity can be favored unintentionally *(Vandermeer and Lin 2008)*. Furthermore, cascading effects to soils from aboveground ants in highly connected ecological networks (*Vandermeer et al. 2019*) can also represent a type of link between above and belowground processes, which remains a fundamental area of study in ecology (*Bardgett and van der Putten 2014*; *Tao, Hunter, and de Roode 2017*; *Meier and Hunter 2018*).

Analyzing soils using complex systems theory (*Newman 2011*) offers both perspective and interdisciplinary statistical tools including mathematical formulations with network structures (*Chen et al. 2022*), spatial explicitness (*Vandermeer and Yitbarek 2012*) for studying soil structure and heterogeneity (*Rabot et al. 2018*; *Vogel et al. 2022*), agent-based modeling (*Waring et al. 2020*), and novel parameters of non-normal skewed distributions, like scaling or power laws (*Clauset, Shalizi, and Newman 2009*), interpreted in part using fractal geometry (*Boddy et al. 1999*; *P. Baveye, Parlange, and Stewart 2000*; *Menéndez et al. 2005*). Previous reviews of how various metrics have been used in soils (*Rieu and Sposito 1991*; *P. Baveye, Parlange, and Stewart 2000*; *Pachepsky and Hill 2017*) explicitly point toward the need for further explanations of how relevant properties like scaling or power laws arise, as well as how they both reflect and are shaped by ongoing biological processes. The need for interdisciplinary interpretation may explain the ubiquity of more intuitive measures of soil aggregation like mean weighted diameter and water-stable aggregate proportion over others like fractal dimension (*Caruso et al. 2011*). However, fractal dimensions can offer useful, complementary, and more holistic representation of soil structured environments (*Vogel et al. 2022*), that are similar across soil phases and diverse components (*Peng, Horn, and Hallett 2015*), and also integrate combined effects of aggregate or pore size, density, and stability (*Caruso et al. 2011*) or related functions (*Tisdall and Oades 1982*), including implications for fine-scale biodiversity patterns (*Alexandra N. Kravchenko et al. 2014*; *Rillig, Ingraffia, and Machado 2017*; *Bach et al. 2018*), particulate and aggregate-associated organic matter storage *(O'Brien and Jastrow 2013; Ananyeva et al. 2013; Alexandra N. Kravchenko et al. 2015; Alexandra N. Kravchenko and Guber 2017; Quigley and Kravchenko 2022)*, and local greenhouse gas emissions *(Y. Li et al. 2019; J. Wang et al. 2019)* and/or microbial co-metabolism *(A. N. Kravchenko et al. 2019; Franklin et al. 2021)* across hotspots of activity *(Kuzyakov and Blagodatskaya 2015)*. Indeed, changes in scaling law exponents are generally considered indicators of more fundamental changes in underlying rules (i.e., 'regime' shifts) of a system, which for soils, can be shifts in relative aggregate formation versus fragmentation, such as those caused by more fungal agglomeration versus invertebrate burrowing, but also represents a generalized mechanism that can apply to cluster-forming systems more broadly such as networks. Beyond the physical limits of natural patterns *(Halley et al. 2004)*, shared scaling patterns across biology and ecology have been generally interpreted as indicators of habitat complexity *(Loke and Chisholm 2022)*, biodiversity maintenance *(Ostling et al. 2004)*, entropy *(Harte, Umemura, and Brush 2021; Klöffel et al. 2022)*, and comparable solutions to optimizing information flow or conserving energy during system changes or adaptation *(West, Brown, and Enquist 1997)*. As a result, analyses of soils that draw from complex systems theory, such as fractal dimensions of soil aggregate size scaling or power law distributions, or similar long-tailed families including log-normal and exponential arising from partially hierarchical soil aggregation *(Tisdall and Oades 1982; Melo, Figueiredo, and Filho 2021)*, continue to offer integrated information about how soils function and respond to key soil biota, like aggregate fragmentation by invertebrates during niche or nest construction *(Maaß, Hückelheim, and Rillig 2019)* and formation by associated microbial activity *(Maaß, Caruso, and Rillig 2015)*.

Invertebrate soil ecosystem engineers are widely recognized for their fundamental roles in shaping ecosystems *(Perfecto, Vandermeer, and Philpott 2014)*, soil habitats *(Jouquet et al. 2006; Lavelle et al. 2016),* biodiversity *(Thakur et al. 2020; Lavelle et al. 2022)*, and related processes *(McGlynn and Poirson 2012; Filser et al. 2016)*, although they are less studied compared to soil microbes *(van der Heijden, Bardgett, and van Straalen 2008; Banerjee and van der Heijden 2022; Charlotte et al. 2022)*. Extended phenotype engineers *(Jouquet et al. 2006)*, namely ants, have been shown to affect soil geomorphology *(Whitford and Eldridge 2013)* including: changes in texture and lower bulk density *(Dostál et al. 2005; Cammeraat and Risch 2008)*; an increase in general microporosity *(Tschinkel 2005),* water infiltration *(Cerdà, Jurgensen, and Bodi 2009)* and water availability *(X. R. Li et al. 2014)*; increased soil nutrient availability *(Wagner, Brown, and Gordon 1997; Wagner, Jones, and Gordon 2004; Shukla et al. 2013; Kotova, Umarov, and Zakalyukina 2015; Sankovitz and Purcell 2022)* but lower availability of metals *(Gramigni et al. 2013)*; increased plant root and shoot growth *(Farji-Brener and Werenkraut 2017)*; and recently, also increase microbial diversity *(Delgado-baquerizo et al. 2019; Baker et al. 2020)* and changed community structure, including even by non-fungal specialist ants *(Lindström et al. 2019)*, in addition to fungal specialist (i.e. Attine) ants *(Meyer 2013; Reis et al. 2015)*. Although, some studies present soils (compaction, granulometric composition, and pH) as being what affects ant biodiversity and community structure *(Rocha-Ortega and García-Martínez 2018; Costa-Milanez et al. 2017)*, most other available studies support the reverse, ant modifications of soil properties *(Shukla et al. 2013; Kotova, Umarov, and Zakalyukina 2015; Sankovitz and Purcell 2022)*, ultimately aligning with the context behind this study here. More specifically, clearer evidence of ant effects on finer-scale soil structural properties, such as soil aggregate or pore size distributions, remains rare, but may be useful for interdisciplinary ecological models (*Bennett et al. 2019*). Ant effects on soil aggregate size distributions, which tend to have long tails and are thus tied to fractal geometry and self-organization (*Young and Crawford 1991*, *2004*; *Perfect, Rasiah, and Kay 1992*), could be considered as resulting from skewed balances between positive formation and negative fragmentation processes, analogous to some early explanations of scaling laws in ecology (*Macarthur and Wilson 1963*; *Triantis, Guilhaumon, and Whittaker 2012*; *Chase et al. 2019*). This research gap presents an opportunity to assess how ants, and soil invertebrates more generally, affect soil system processes as a whole (*Levin 1992*).

Indirect or higher-order interactions are also increasingly recognized as important for ecosystem network dynamics (*Werner and Peacor 2003*; *Perfecto, Vandermeer, and Philpott 2014*) and biodiversity maintenance (*Bairey, Kelsic, and Kishony 2016*), and those tied specifically to keystone ant taxa have accordingly been shown to affect ecosystem stability (*Vandermeer et al. 2021*). Direct and indirect competition among ant taxa, occurring across above- and belowground plant, litter, and soil nesting sites, can generate persistent landscape-level spatial patterns in species occupancy (*Vandermeer and Yitbarek 2012*; *Perfecto and Vandermeer 2013*; *Perfecto, Vandermeer, and Philpott 2014*; *Vandermeer and Perfecto 2020*; *Vandermeer et al. 2022*). In some cases, this can lead to a halo effect, where some local competitive suppression and/or exclusion occurs within a limited range near a focal nest site, although coexistence may be maintained at a larger spatial scale (*Ennis 2010*; *Ennis and Philpott 2017*; *Ennis, Perfecto, and Vandermeer 2023*; *Salinas, Vandermeer, and Perfecto 2019*). Furthermore, similar to effects of leaf-cutting ants (*Hudson et al. 2009*; *Sousa-souto et al. 2012*), when ant-plant mutualisms are involved (*Vandermeer and Perfecto 2019*; *Vandermeer et al. 2019*), individual ant taxon foraging behaviors can be altered by the presence of extra-floral nectaries in tree canopies *(Godschalx et al. 2015; Passos and Leal 2019; Nogueira et al. 2020)*, which can benefit the tree *(Wagner and Nicklen 2010)*, but may replace other existing phloem-feeding insect-tending relationships nearby *(Perfecto and Vandermeer 2006; Styrsky and Eubanks 2007)*. In cases where ants have very noticeable effects on their surrounding habitat *(Morris, Vandermeer, and Perfecto 2015; Vannette, Bichier, and Philpott 2017; MacDougal 2019)*, even arboreal nesting activity has been shown to be associated with litter communities *(Donoso et al. 2013)* and decomposition *(Schmitt and Perfecto 2020)*, soil nutrients *(Clay et al. 2013; Godschalx et al. 2015; J. M. Lucas, Clay, and Kaspari 2018)*, soil microbiomes *(J. Lucas et al. 2017)*, and host trees *(Livingston, White, and Kratz 2008)*. Surprisingly, These effects show ecological variation by individual colony or diet, similar to co-evolutionary specialization like in Attine ants *(Schultz and Brady 2008)* and termites *(Nobre et al. 2011)*. Ultimately, nesting and activity patterns of even aboveground keystone ants have the potential to extend belowground to affect soil properties.

Given separate but related evidence of invertebrate effects on soils and their limited study, this study analyzes potential above-belowground throughline linkages between a tropical keystone arboreal-nesting ant *Azteca sericeasur* and adjacent agroforest soil properties. The motivating hypothesis is that persistent *A. sericeasur* nests affect soil properties by out-competing other nearby ground-dwelling ants *(Ennis 2010; Salinas, Vandermeer, and Perfecto 2019; Vandermeer et al. 2019)*, that would otherwise have positive effects on nearby soils, such as those previously mentioned including increased burrowing and thus opening soil macropore space. Specifically, it was predicted that soils under persistent *Azteca* ant nests would show evidence of less beneficial ground ant activity, namely: smaller parameters of long-tailed soil aggregate size distributions including scaling laws, reflecting weaker self-similarity in the soil aggregation process and potentially less efficient soil structural dynamics; slower soil water infiltration, due to lower porosity; and fewer available nutrients, due to limited water infiltration; when compared to soils not under ant nests. Furthermore, these *Azteca* ant nest effects would be mediated by host tree species, and be stronger near host trees without extra-floral nectaries, where competitive suppression of ground ant activity would be stronger.

4.3 Methods

4.3.1 Study site and system

This study took place at Finca Irlanda *(15.1732729, -92.3365757)*, a 300 ha tropical, organic, biodynamic, shaded coffee agroforest farm in the highland Soconusco region of Chiapas, Mexico. The agroforest is 950-1150 m.a.s.l. and gets ~4500 mm MAP, with one six-month rainy season. The region is known for high coffee production relative to nearby areas. The dominant soil types are Ultisols, with topsoil showing subangular blocky aggregates and sub-soil showing columnar structure *(Fig. 4.5a)* . Fine yellow-red mottling can also be observed at times, likely reflecting seasonally variable redox conditions.

A permanent 45-ha long-term (~20 year) census plot (see *Philpott et al. (2008)*) has over 90 shade tree species, mostly uniformly distributed, except along roads *(Vandermeer and Lin 2008)*. Most canopy trees are *Inga*, notably *I. micheliana*, which are maintained by periodic pruning *(Philpott and Bichier 2012)* as a Mayan cultural legacy *(Valencia et al. 2015)* and also later promoted by local extension agencies *(Peeters et al. 2003)* in part for nitrogen (N) benefits *(Romero-Alvarado et al. 2002)* from N-fixing bacterial root symbionts *(Grossman et al. 2005)*. *I. micheliana* is notable for hosting both extra-floral nectaries and helmet scale (*Octolecanium*), which can also affect symbiotic ant foraging patterns *(Livingston, White, and Kratz 2008)*. In contrast, *Alchornea latifolia*, the second-most abundant tree *(unpublished data)*, is not a legume and does not have extra-floral nectaries.

Azteca sericeasur is a local keystone arboreal ant *(Vandermeer et al. 2010; Vandermeer et al. 2019; Vandermeer 2021)* that nests relatively indiscriminately in shade trees in this census plot *(Kevin Li et al. 2016)*, mostly in trunks or occasionally constructed carton nests *(Clay et al. (2013); pers. obs.)*. *A. sericeasur* foraging can extend to nearby coffee bushes within 10 m *(Schmitt and Perfecto 2020)*, having cascading effects on coffee plant-associated arthropods, via predation, competitive exclusion, and aggression *(Vandermeer, Perfecto, and Philpott 2010; Perfecto, Vandermeer, and Philpott 2014; Vannette, Bichier, and Philpott 2017)*; via mutualisms with extra-floral nectaries and Hemiptera, like *Coccus viridis (Hsieh et al. 2012; Hsieh 2015)* and *Octolecanium (Salinas, Vandermeer, and Perfecto 2019; Livingston, White, and Kratz 2008)*; and even have cascading effects on litter-dwelling ant communities *(Ennis 2010; Salinas, Vandermeer, and Perfecto 2019; Schmitt and Perfecto 2020)*.

4.3.2 Data collection

4.3.2.1 Nest tree selection and variables recorded

Sampling sites were chosen based on census records of trees with resident active *A. sericeasur* nests for at least seven years. Paired control sites were trees less than or equal to 50 m from a chosen nest without a recorded *Azteca* nest for at least five years *(Fig. 4.5b)* . Five nest-control pair sites were *I. micheliana* trees, and another five were *A. latifolia*, totaling 20 trees.

Ground slope, leaf litter depth, tree diameter at breast height (DBH, ~*2.7* m), number of coffee bushes closer than three meters from the target tree, distance to nearest trail, and *A.*

sericeasur activity, as the number of ants passing a fixed point on the trunk in one minute, were recorded for each tree.

4.3.2.2 Soil sampling

Four soil samples were taken around each tree one meter from the trunk base along the cardinal directions. Each sample was taken down to 10 cm depth using a 5.5 cm diameter bulb transplanter and collected in small plastic bags in 2018, and in 2019 with a 5.5 cm diameter slide-hammer corer *(404.05, AMS Inc., American Falls, ID)* lined with inner plastic sleeves, and each tool sampled similar volumes. Soils were oven dried at ~50 C (over three to four days) and final weights was used to estimate bulk density.

4.3.2.3 Aggregate isolation

Each dried sample was sieved through a six mm wire mesh, and individual aggregates were carefully arranged equidistantly on a light-colored flat surface, similar to other approaches *(Oades and Waters 1991)*, and the arrangement was digitally photographed. Photos were visually color-corrected to detect separate soil aggregates and analyzed for pixel cluster area measurement using the color-threshold settings *(Fig. 4.5c)*, followed by the *'particle size analysis'* function in the public Java-based software *ImageJ v1.52a* (NIH, imagej.nih.gov), with the resulting tables exported and composited by tree identity (i.e., individual).

Sites were re-sampled in 2019 to amass ~500 g soil, which was sieved to 6 mm, and cores were composited by tree site and further sieved through a stack of metal wire meshes 4000, 2000, 500, 250, 125, and 63 µm wide, shaken for two minutes at ~100 oscillations per minute over five cm radius by hand to standardize across samples and resemble other studies, and remaining size fractions weighed.

4.3.2.4 Infiltration and chemistry

To assess infiltration potential, subsamples of unsieved soil was placed into infiltrometers made from 2-inch-wide PVC pipes, wire mesh, and plastic collection cups *(Fig. 4.5d)*. One-quarter liter (250 mL) water was added to each column, and two measures, namely the time for 100 mL to pass through the column, as well as how much total water collected after five minutes, were both recorded.

Soil aggregations greater than six mm (collected on the largest sieve) from a subset of sites was composited by tree ID, and to align with previous chemical analyses at soil aggregate-level (not shown), the 10 largest aggregates, plus 10 smaller aggregates weighing ~20 g, were homogenized by mortar and pestle and sent for Olsen available phosphorus (P) analysis *(Olsen et al. 1954; Horta and Torrent 2007)* to the local university plant and soil laboratory *(ECOSUR, San Cristóbal, Mexico)* in 2018, plus organic carbon (C) and total nitrogen (N) analysis to the base university lab using a LECO Trumac CN combustion analyzer *(LECO Corp, St Joseph, MI)* in 2019.

4.3.3 Statistical analysis

Analyses were primarily done in R version 4.2.3 (2023-03-15) *(R Core Team 2023)* after ImageJ and described below *(Nieminen 2020; Davis and Kay 2023)*. All dependent variable measurements were tested for both separate and interactive associations with ant nests and host shade tree species using a linear mixed effects model, to improve effect detection power given a paired-site design *(X. Yang et al. 2014)*. Two fixed effects were ant nest presence and host shade tree species, and random effects were site pair number along with co-variates ground slope angle magnitude, leaf litter depth, host tree diameter, number of nearby coffee bushes, and distance to nearest used walking path. Significance was set at *alpha*=0.05 and marginal significance at 0.05

< *alpha* < 0.1, and only models that met residual normality and equal variance assumptions using Shapiro-Wilk normality and Levene homoscedasticity tests were used, which was sufficient for this analysis (compared to generalized models with different distributions).

Soil aggregate size distributions were analyzed for scaling law fits based on goodness-of-fit tests and bootstrapping methods *(Clauset, Shalizi, and Newman 2009)*. Briefly, size frequencies were accumulated, scaling function x-min and exponent parameters were estimated for the long-tailed region, and bootstrapping was done to assess how likely the slope parameter was, given the data. The same was done assuming other similar long-tail distributions, namely exponential, log-normal, and poisson families, and these were followed by pairwise comparisons testing if fitted scaling laws with given exponent and x-min parameter values described the data either better or equally well compared to similar alternative distribution families. Pre-analysis, large macro-aggregation (>6 mm) size values generated from image analyses were filtered for viable sizes based on the empirical mesh size used, and converted from area to diameter by calibrating the smallest viable aggregate detected with the sieve mesh size. Micro-aggregate data was initially converted from mass to frequency assuming spherical aggregate shapes, and that the mass proportion of each sieve fraction to about half the collected soil mass was similar to the mass fraction's proportion of soil by volume, which generally aligns with other methods assuming constant aggregate density *(Perfect, Rasiah, and Kay 1992)*. However, to minimize any potential compounding calculation effects on resulting distribution parameters, original soil aggregate mass fraction data were ultimately used for distribution parameter estimations to minimize data transformations *(O'Hara and Kotze 2010)*.

Nutrient values were converted to stocks by multiplying raw percents by bulk density as typically done, and given a significant correlation between carbon and nitrogen values, these data were bound together into a MANOVA and tested for fixed effects of ant nest presence, host tree species, and paired site. Phosphorus and carbon-nitrogen ratios were tested separately with a similar linear mixed-effects model with paired site as a random effect. A general ordination was done using all dependent variables above as well as environmental co-variates except fitted poisson parameters and measured ant activity using a PERMANOVA with ant nest presence, host tree species, and paired site as fixed effects. Analysis code was produced with the help of R packages *here (Müller 2020)*, *poweRlaw 0.70.6 (Gillespie 2015)*, *rstatix* from *tidyverse (Wickham et al. 2019)*, *lme4 0.70.6 (Bates et al. 2015)* and *lmerTest 3.1.3 (Kuznetsova, Brockhoff, and Christensen 2017)*, *vegan (Dixon 2003)*, *bookdown (Xie 2023)*, *grateful (Rodriguez-Sánchez and Jackson 2023)*, and stored at github.com/nmedina17/azteca.

4.4 Results

4.4.1 Aggregation

All soil aggregate size distributions followed a scaling law among both micro- and macro-aggregates, with exponents mainly ranging between 1.5 and 2 *(Fig. 4.1)* . Indeed, nearly all sample soil aggregate size distributions were better described by the scaling law function family than by the poisson function family. While scaling laws did accurately describe the data, nearly all samples could also be described by log-normal and exponential functions, which share long-tail qualities with power functions but need more parameters defined.

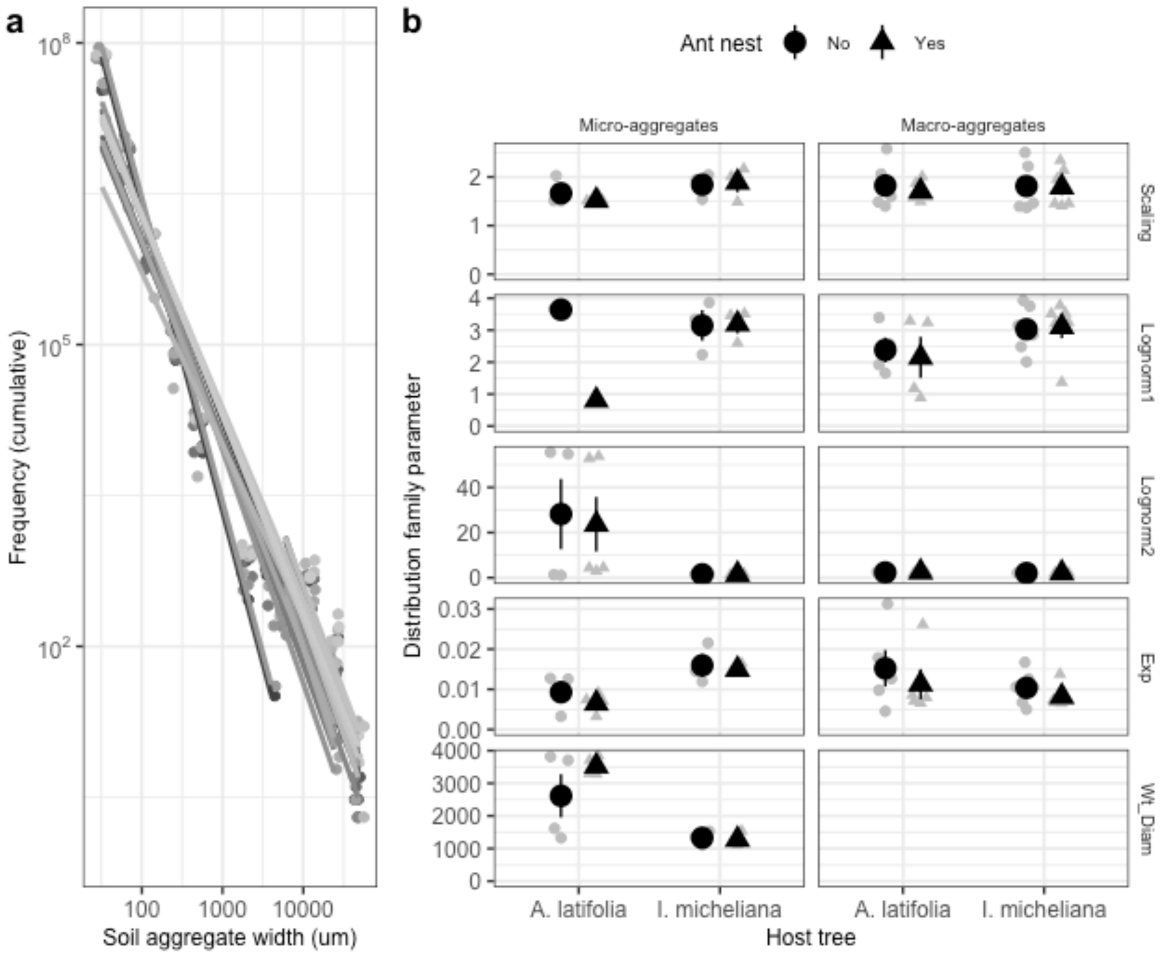

Figure 4.1: Soil aggregate size distribution (a) cumulative frequencies (log-transformed to show long tails) colored by local tree IDs where soils were sampled, and (b) long-tailed function family raw values (gray) and center means ±1 standard error (black) grouped by ant nest presence, categorized by host tree species, and facetted by both aggregate size class and distribution family.

Ant nests were not significantly associated with scaling law exponents, but ant nests were significantly associated with 0.35 ± 0.13 units lower variance in macro-aggregate size, under a log-normal distribution model *(df=11.797, t=2.7, p=0.019*). Ant nests also marginally significantly increased mean weighted diameter by 903.82 ± 455.38 um *(df=6.2, t=<0.0001, p=0.092)*.

Host shade tree species was significantly associated with micro-aggregate size distribution parameters under all relevant models tested – scaling law, log-normal, and exponential function families. Specifically, micro-aggregate scaling law exponents were marginally significantly 0.33 ± 0.15 units lower under *Alchornea* than under *Inga (df=9.6, t=2.1, p=0.061)*, and associated exponential function base value parameters were 0.01 ± 0.0017 lower under *Alchornea* than under *Inga (df=5.9, t=5.2, p=0.0021)*. Both micro-and macro-aggregate central tendency parameters (but not variance parameters) under log-normal model representations were also lower under *Alchornea* than under *Inga*, by 1795.46 ± 107.74 for micro-aggregates *(df=0.91, t=17, p=0.049)* and 1.17 ± 0.48 for macro-aggregates *(df=6.8, t=2.4, p=0.046)*. Finally, mean weighted diameter of soil aggregates were 2248.97 ± 526.84 g larger under *Alchornea* than under *Inga (df=11, t=<0.0001, p=0.0014)* .

4.4.2 Infiltration

Overall, soil water infiltration rates were significantly explained by both ant nesting and host shade tree species, both separately and interactively *(Fig. 4.2)*. Ant nesting alone significantly increased soil water infiltration by 5.54 ± 0.84 mL per sec initially soon after water entry and by ~ 0.05 ± 0.01 mL per sec at near-complete infiltration (i.e., after most water had passed through the soil column).

Host shade tree species were significantly associated with water infiltration rates only at near-complete drainage, with *Alchornea* increasing rates by 0.11 ± 0.04 mL per sec *(df=13, t=<0.0001, p=0.028)*. Nest-tree interactions marginally significantly explained 4.74 ± 2.35 mL per sec of initial soil water infiltration rates *(df=12, t=2, p=0.066)*, which appeared as clear increase under ant nests on *Alchornea* but not under *Inga (Fig. 4.2)*.

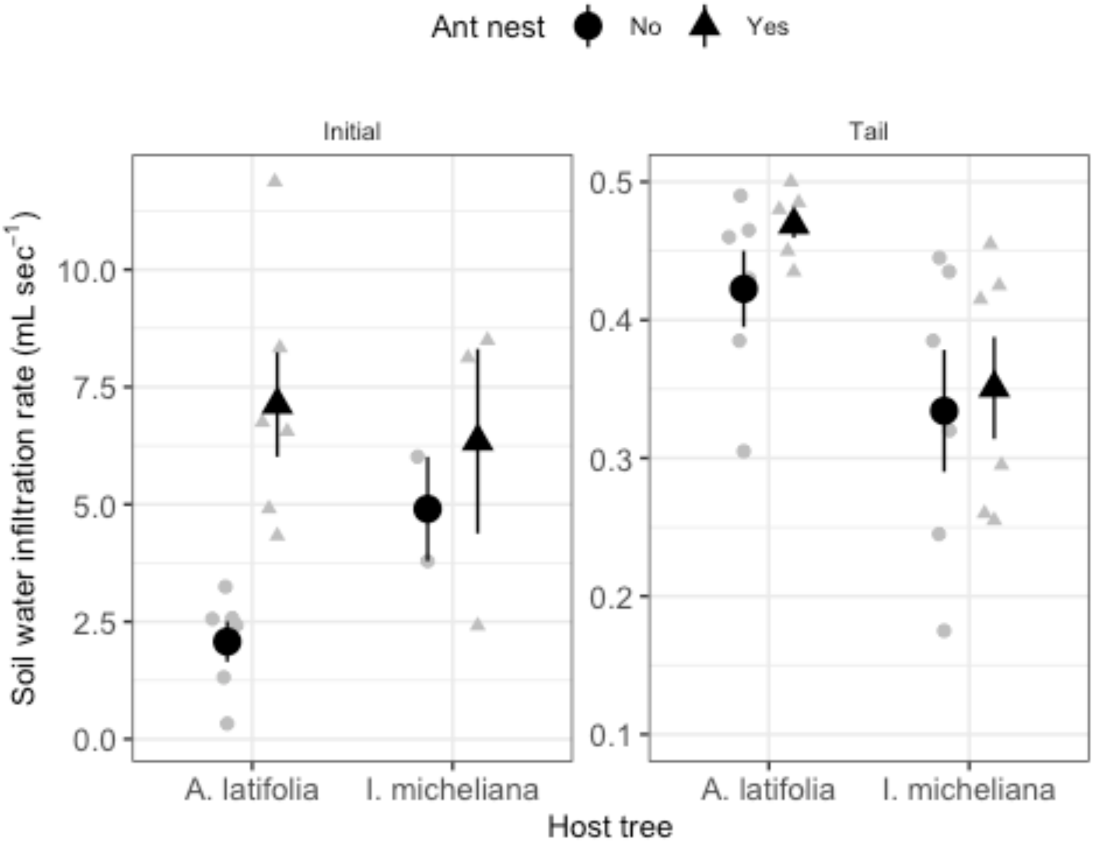

Figure 4.2: Soil water infiltration under *Alchornea* and *Inga* trees with (triangles) and without (circles) persistent (5+ year) arboreal *Azteca* ant nests, showing raw (grey) and center means (black) ± 1 standard error, during initial stages when water entered the soil column (left) and final stages of water percolation after most water exited the soil column (right).

4.4.3 Chemistry

Overall, soil nutrients did not differ based on presence of ant nests but varied significantly among tree species and site *(Fig. 4.3)*. Soils showed lower nutrient stocks under *Alchornea* compared to *Inga* trees, specifically showing lower carbon significantly by nearly half *(df=1, F=28, p=0.0062)*, lower nitrogen significantly by also nearly half *(df=1, F=34, p=0.0043)*, and lower phosphorus marginally significantly by 9 ± 4.2 *(df=8, t=2.2, p=0.062)* , but not carbon-nitrogen ratio. There were also significant differences between host tree species by overall site pair (irrespective of ant nesting) in soil carbon *(df=4, F=9.1, p=0.028)* and nitrogen *(df=4, F=8.6, p=0.031)*.

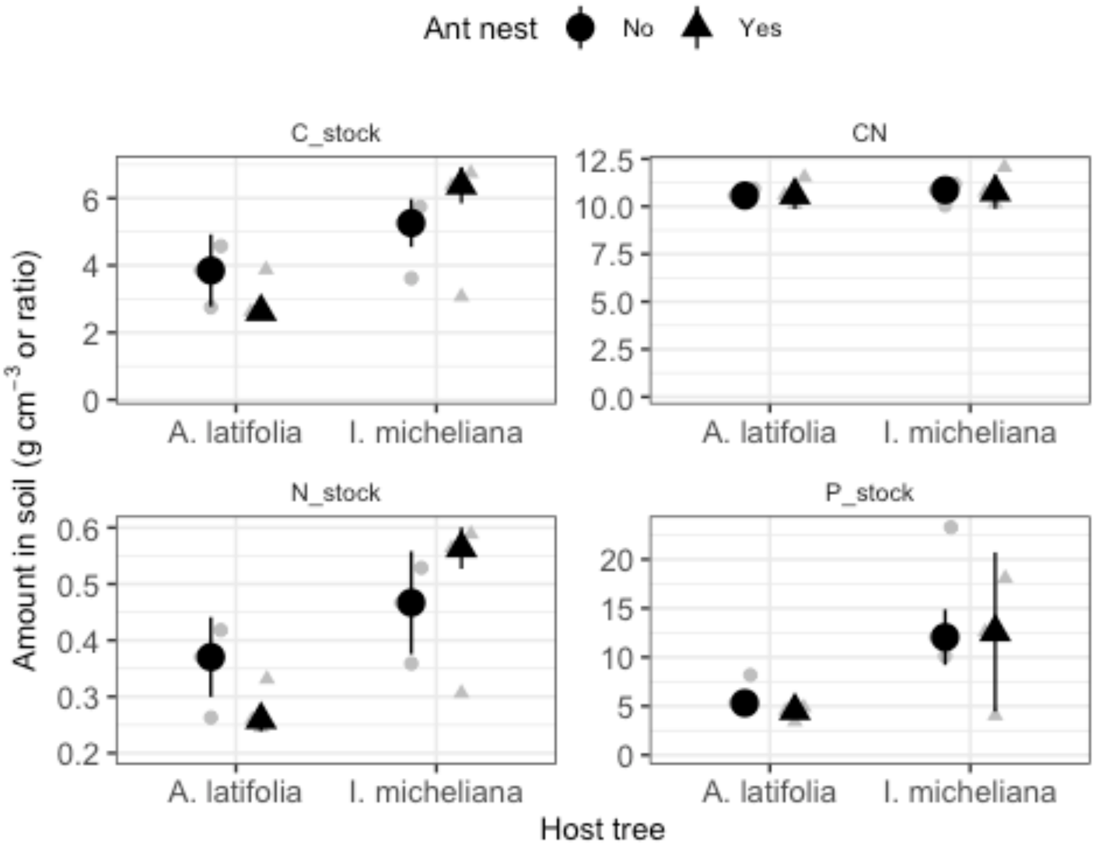

Figure 4.3: Soil carbon (top-left), nitrogen (bottom-left), phosphorus (bottom-right), and carbon-to-nitrogen ratio (top-right) in soils under *Alchornea* and *Inga* trees with (triangles) and without (circles) persistent (5+ year) arboreal *Azteca* ant nests, showing raw (grey) and center means (black) ± 1 standard error.

4.4.4 Co-variates

Including environmental co-variates, host tree species was significantly associated with ranked sample dissimilarities, explaining 13.8 % of variation in sample distances *(stress=0.089, df=1, F=3.5, p=0.044, R2=0.14) (Fig. 4.4)*.

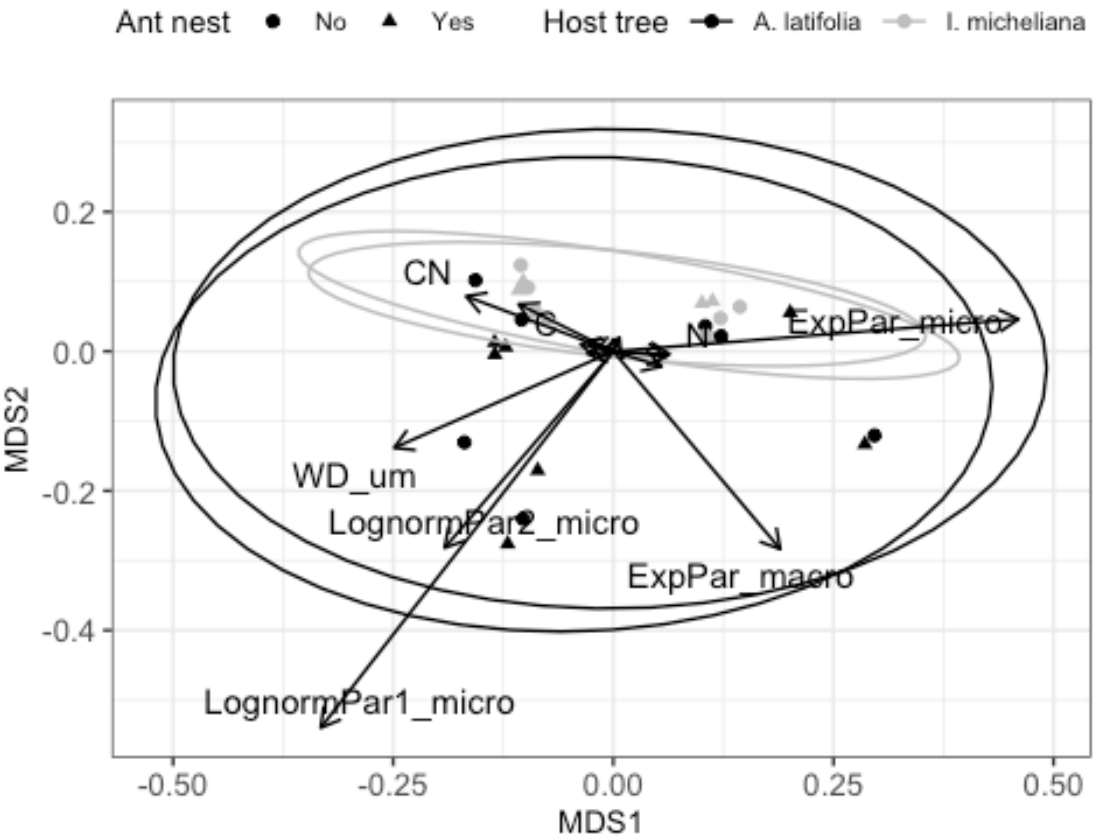

Figure 4.4: Plot of principle component analysis using all relevant numerical data grouped by points with (triangle) and without (circle) *Azteca* ant nests on non-leguminous *A. latifolia* (dark) and leguminous *I. micheliana* (light) shade trees in a coffee agro-forest.

4.5 Discussion

Taken together, these results show that behavior-associated arboreal ant nesting patterns are associated with significant differences in soil structure, water infiltration, macro-chemistry, and environmental co-variates. Specifically, soil aggregate size distributions were described by distribution families with long tails, and their descriptive parameters were in various cases different across ant nest and host shade tree sites. *Azteca* ant nests alone marginally significantly increased mean weighted diameter and log-normal variance of soil aggregate sizes significantly. Alternatively, host shade tree *Alchornea* marginally significantly lowered micro-aggregate scaling law exponents, significantly lowered exponential function bases and log-normal mean soil aggregate diameters, while increasing log-normal variance and mean weighted diameter – all of which suggests consistent soil aggregation producing relatively larger aggregates, generally improving soil structure. Soil water infiltration rates were also significantly higher under ant nests, marginally significantly interacting with host shade tree species, and, in line with faster infiltration, total soil nitrogen concentrations were also lower under ant nests, which may have been released in soil water. Overall, host tree species was a significant predictor of sampling sites across the focal tropical agro-forest. Collectively, results were somewhat inconsistent with initial hypotheses: *Azteca* ant nests were indeed partially associated with smaller soil aggregate distribution parameters, indicating relatively larger soil aggregates, which aligned with exclusion of ground ant burrowing and soil aggregate fragmenting activity; however, rather than infiltration being slower due to excluded ground ant burrowing activity, the opposing result of faster infiltration and lower soil nitrogen, potentially from leaching, aligned with the larger macro-aggregate results, which also suggested larger pores likely occurring between them. This suggests that arboreal ant nest effects on soils can appear direct, amid both potentially direct *(Clay et al. 2013)* and emerging indirect *(Ennis 2010; Salinas, Vandermeer, and Perfecto 2019; Schmitt and Perfecto 2020)* underlying processes.

Soil aggregation results here differed by response distribution parameter, suggesting that both arboreal ant nests and leguminous host shade trees with extra-floral nectaries can promote soil aggregation, but in different ways. Fundamentally, each metric of aggregate size distributions presented here reflects slightly different underlying processes. Scaling or power law distribution families by definition represent compounding multiplicative iterations among components governed by just one exponent parameter, also referred to as self-organization

(Brown et al. 2002; Young and Crawford 2004), and can be generated by a preferential attachment process where 'rich get richer' *(Newman 2001)*. In soils, this process could explain observed scaling laws if, as soil aggregates get larger, they also increase their chance of assimilating nearby smaller aggregates. Results here showed equivalent scaling exponents among macro-aggregates across both ant nest sites and host tree species, but not for micro-aggregates across host tree species, suggesting an interpretation that micro-aggregates were about one-third more likely to continue enlarging smaller, previously aggregated soil clusters into larger soil aggregates, thereby increasing self-similarity of all fine-scale soil aggregates, under leguminous nitrogen-fixing *Inga* trees, through two mm but before six mm. This faster scaling rate under a legume tree species could be explained by more fungal growth given higher nitrogen availability *(Gou et al. 2023)*, since bacteria *(Rashid et al. 2016)* and fungi are well-studied soil aggregators *(Ritz and Young 2004; Siddiky et al. 2012; Leifheit et al. 2014; Baumert et al. 2018; Lehmann and Rillig 2015; Lehmann et al. 2020)*, until soil aggregates get massive enough to be influenced more by plant roots than by thinner fungal hyphae *(Rillig et al. 2015)*. Additionally, it is increasingly recognized that scaling laws can also be generated by other processes *(Simkin and Roychowdhury 2011)*, such as exponential functions composed together, which has been shown to also potentially represent exponentially distributed sampling times of components that themselves grow exponentially, as another related example mechanism underlying these long-tailed distributions *(Reed and Hughes 2002)*. Exponential soil aggregation would reflect a relatively consistent soil particle accretion process, which is independent of aggregate size, as well as in this study ant nesting, but vary for micro-aggregates by host tree species. In line with a faster scaling law rate, there may also be some variation in size of smaller micro-aggregates used in the aggregation process, thereby equivalently explaining more

fractional mass undergoing accretion under an exponential soil aggregation model. Similarly, other long-tailed distributions such as log-normal distributions can also be generated by multiplicative processes, such as if the rates of multiplication or interaction strengths between components are themselves random and independently normally distributed *(Mitzenmacher 2004)*. In practice, the log-normal peak or centrality parameter could also be thought of as translated into the start or minimum cutoff for scaling law shapes in empirical data *(Clauset, Shalizi, and Newman 2009)*. Such an idea could help explain the significant host tree species effects on soil micro-aggregate log-normal centrality observed here, as could a possible underlying interaction with ant nest presence, but host tree effects on both log-normal centrality and variance in macro-aggregates suggests more change in process. Beneficial effects of nitrogen-fixing bacteria on fungal growth and soil binding may also be more variable in macro-aggregates, due to general increases in intra-aggregate heterogeneity, such as in hyper-local microbial community taxa and activity *(Alexandra N. Kravchenko et al. 2014; P. C. Baveye et al. 2018; A. Kravchenko et al. 2019)*. In soils, the aggregate hierarchy concept provides some theoretical support for such multiplicative exponential processes leading to long-tailed distribution families, given that different categories of processes from bacteria to plants roots are responsible for aggregating soil at different micro-spatial scales *(Tisdall and Oades 1982; Melo, Figueiredo, and Filho 2021)*. In this case, each underlying processes such as clay flocculation, bacterial polysaccharides, and fungal enmeshment, would occur at a different rate. This is plausible, given that as soil aggregation proceeds hierarchically, the component sub-aggregates increase in mass and decrease in number, potentially slowing down the aggregation process, and is consistent with corresponding decreases in density and increases in porosity *(A. N. Kravchenko et al. 2011; W. Wang et al. 2012)* and heterogeneity *(Barbosa and Gerke 2022)* among larger macro-aggregates. Mean weighted diameter has also been discussed as an intuitive measure of soil aggregation *(Caruso et al. 2011)* and likely mathematically biased toward larger values *(Pachepsky and Hill 2017)*. In cases where larger aggregates are more strongly affected, such as in this study for *Azteca* ant nests and shade tree species, this can be more likely to reflect significant group differences. In the field, relatively larger aggregates are consistent with the hypothesis of competitive suppression by aggressive ants such as *Azteca (Ennis and Philpott 2017)* of ground ant worker activity, whose mandibles fragment soil aggregates during tunneling and excavation for nest construction *(Mikheyev and Tschinkel 2004; Tschinkel 2005; Cerquera and Tschinkel 2010)*. Overall, considering scaling laws as the most parsimonious model with fewest parameters, in this study, soil aggregate size distribution exponents were around two, which agrees with image-based values of structural heterogeneity *(Armstrong 1986; Perfect, Rasiah, and Kay 1992; Peyton et al. 1994; Kun Li et al. 2018)*, but less so soil water-based exponents reported from other studies *(Tyler and Wheatcraft 1990, 1992; Kozak, Sokotowski, and Sokotowska 1996; Perfect et al. 1996; Perrier et al. 1996)* and approaches *(Young and Crawford 1991; Menéndez et al. 2005)*, barring methodological exponent calculation differences *(Newman 2005)*. These exponents around two can be interpreted as when soil aggregates double in diameter, the chance of subsequently doubling again cuts nearly in half, and consistently across several orders of magnitude in soil aggregate size, as well as across varying ecological conditions. This process could either be extended to consider the whole soil mass and the pool of aggregates smaller or larger than a certain diameter, and/or zoomed in to consider that a single soil aggregate will typically be comprised of two slightly smaller aggregates, regardless of binding mechanism. Such a process in soils could be explained in a variety of ways, and warrants future research to improve fundamental understanding of universal ways that soils behave.

Faster soil water infiltration is also consistent with overall soil aggregation results of relatively larger soil aggregates, despite this study's original hypotheses. Geometrically, larger soil aggregates make larger pores between them, allowing faster water flow and drainage *(Franklin et al. 2021)*. Faster water infiltration could also occur due to optimal arrangement of soil aggregates, which also creates ideal mixes of macro- and micro-pores, and which optimally would increase connectivity and shorten longest path length among soil pore networks *(Gastner and Newman 2006)*. Significantly faster soil water infiltration under *Alchornea* host trees, which do not have extra-floral nectary structures in the canopy, is consistent with suppression of ground ant activity. Similarly, unchanged water infiltration rates under *Inga* trees, which have extra-floral nectaries in the specific species used here, is consistent with un-altered ground ant activity, primarily as a source of soil aggregate fragmentation over formation. Taken together, this hypothesis was supported with a significant interaction between ant nest presence and host tree species, in the directions predicted by the original hypothesis based on previous related studies. Slower infiltration under *Inga* could be explained by a more self-similar soil aggregation process, where more smaller aggregates are combined to produce aggregates of the same size, possible due to increased fungal enmeshment *(Ritz and Young 2004)*, potentially increasing soil compaction.

Generally, soil nutrients, namely carbon and nitrogen, results tended to show some support for the hypothesis, and were consistent with results of other measured variables. Soils had less total carbon and nitrogen, and plant-available phosphorus under non-leguminous *Alchornea* host trees, where aggregates were observed to be simultaneously relatively larger and water infiltration was also faster. This suggests that available elements may be more easily lost than stored in soil aggregates, despite the suggested importance of aggregates for organic matter stability *(Johan Six et al. 2002; J. Six et al. 2002, 2004; Alexandra N. Kravchenko et al. 2015)* and microbial diversity *(Alexandra N. Kravchenko et al. 2014; Rillig, Muller, and Lehmann 2017)*. Alternatively for phosphorus, fungal scavenging and phosphorus provision may help stimulate nitrogen-fixation under *Inga* legumes *(Püschel et al. 2017)*. While showing no detectable difference, ant nests appeared to exacerbate these effects, nearly showing even lower element concentrations under *Alchornea* where these *Azteca* ants forage more on the ground impacting the ground ant community, and slightly higher concentrations under *Inga* where they forage some more in the canopy. These effects would agree with previous studies showing altered soil chemistry *(Sankovitz and Purcell 2022)* and heterogeneity *(Wagner, Brown, and Gordon 1997)* near ground ants, which *Azteca* excludes near non-leguminous host trees *(Ennis 2010; Ennis and Philpott 2017; Salinas, Vandermeer, and Perfecto 2019)*, showing specifically higher leachable dissolved carbon and nitrogen and available base cations alongside lower organic carbon levels *(Bierbaß, Gutknecht, and Michalzik 2015)*, possibly mediated by local soil microbiome changes *(Dauber and Wolters 2000; Lindström et al. 2019)*. This evidence may suggest shade tree effects on soil nutrients may be present and possibly context-dependent *(Romero-Alvarado et al. 2002)* related to local invertebrate and/or microbial activity.

Overall, this study shows significant effects reaching from above- to belowground activity, mediated by persistent (5+ years) arboreal nesting and extended community effects of a keystone ant ecosystem engineer on different host tree species. This study highlights the importance of trait-mediated indirect interactions *(Werner and Peacor 2003; Schmitz, Krivan, and Ovadia 2004)* for soil and ecosystem functions, including above-ground linkages *(Bardgett and Wardle 2003)*, and frames these effects using analytical tools from complex systems theory *(Perfecto, Vandermeer, and Philpott 2014; Newman 2011)* applied to soils *(Young and Crawford*

2004; Lavelle et al. 2016; Medina and Vandermeer 2023) affected by invertebrate ant activity *(Hsieh et al. 2012)*. Future research may further incorporate indirect interactions, including functional *(Martin and Isaac 2015)* and behavioral traits, as relatively novel mechanisms unique to including soil fauna in model representations of ecosystem functioning *(Grandy et al. 2016)*, with additional implications for land management, and potentially diversifying agro-pedogenic trajectories if such ecological management was to be applied at larger local or regional scales *(Phillips 2017; Kuzyakov and Zamanian 2019)*.

4.6 Funding

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4.7 Declaration of interests

No conflicts of interest declared.

4.8 Acknowledgements

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4.9 Data statement

Code stored at github.com/nmedina17/azteca .

4.10 Supplementary information

4.10.1 Methods

4.10.1.1 Site

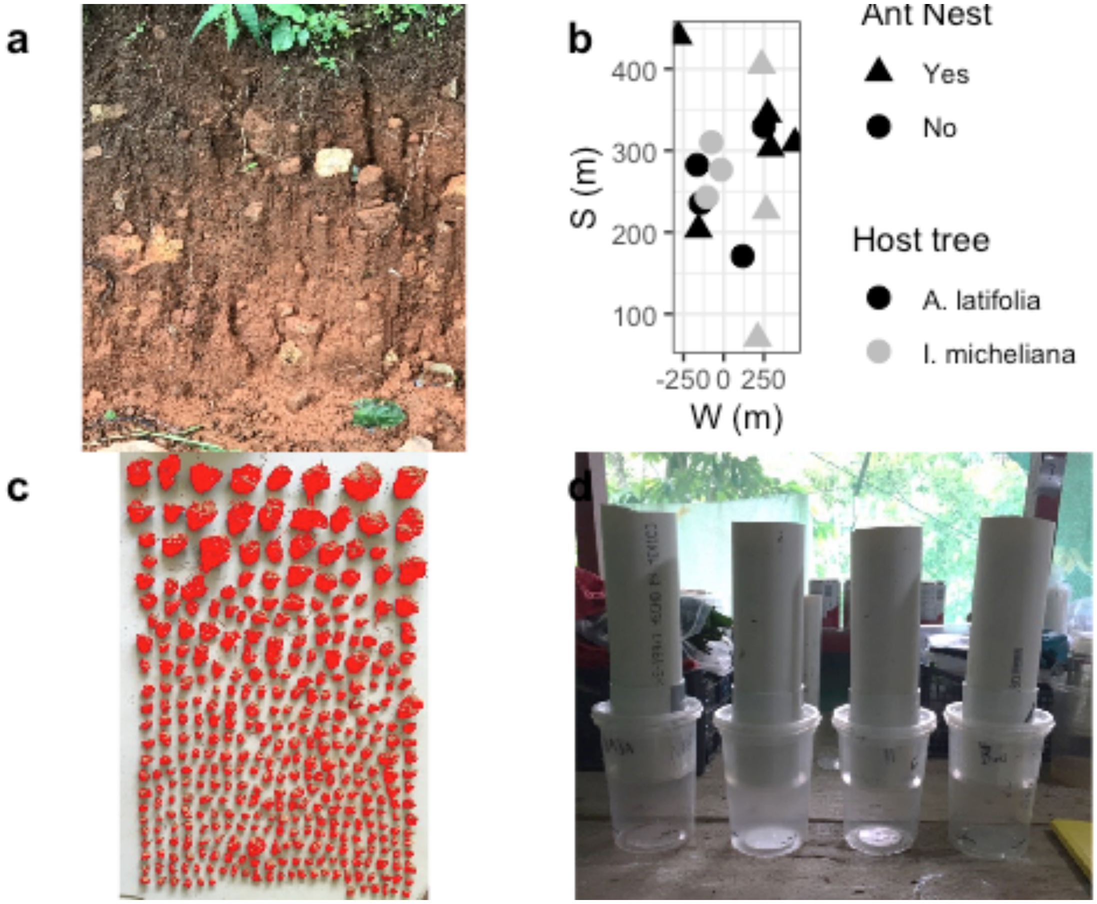

Figure 4.5: Field site and methods images, showing (a) road-side soil profile, (b) map of selected trees near sampling sites, (c) sample large macro-aggregate manual separation and cluster detection by ImageJ software (NIH), (d) constructed lab soil infiltrometers used in a remote diverse neo-tropical wet coffee agro-forest.

4.10.2 Results

4.10.2.1 Aggregate size distributions

Table 4.1: Scaling law fit and comparisons to similar distribution families for soil macro-aggregate samples. Null hypotheses H0 = sampled data follow a scaling law, and the scaling model is equally likely vs. comparison family.

Shade tree	*Ant nest*	*Tree ID*	*Size*	*P(scaling)*	*P(=exp)*	*P(=log-norm)*	*P(=pois)*
A. latifolia	Yes	023	Micro	0.677	0.0641	0.591	<0.0001****
A. latifolia	No	084	Micro	0.636	0.0075**	0.908	<0.0001****
A. latifolia	No	155	Micro	0.394	0.74404	0.788	0.002429**
A. latifolia	No	155	Macro	0.758	0.34562	0.395	0.000596***
A. latifolia	Yes	268	Macro	0.889	0.24623	0.408	<0.0001****
A. latifolia	No	381	Macro	0.879	0.25923	0.613	<0.0001****
A. latifolia	No	474	Macro	0.657	0.33694	0.308	0.00083***
A. latifolia	Yes	574	Micro	0.576	0.12839	0.59	<0.0001****
A. latifolia	No	854	Macro	0.687	0.42519	0.626	0.00649**
A. latifolia	No	900	Micro	1	0.13781	0.424	<0.0001****
A. latifolia	No	900	Macro	0.667	0.39125	0.62	0.003172**
A. latifolia	Yes	975	Micro	0.667	0.00655**	0.875	<0.0001****
A. latifolia	Yes	023	Macro	0.667	0.52072	0.475	0.067437
A. latifolia	No	381	Micro	0.717	0.59573	0.67	0.00638**
A. latifolia	Yes	404	Micro	0.97	0.11859	0.398	<0.0001****
A. latifolia	Yes	574	Macro	0.586	0.39724	0.585	0.00477**
A. latifolia	Yes	917	Micro	0.697	0.15262	0.672	<0.0001****
A. latifolia	Yes	917	Macro	0.475	0.50945	0.645	0.002033**
A. latifolia	Yes	975	Macro	0.586	0.36298	0.553	0.002918**
I. micheliana	Yes	092	Macro	0.717	0.42899	0.353	0.003615**
I. micheliana	No	125	Micro	0.939	0.46964	0.486	0.000536***
I. micheliana	No	125	Macro	0.535	0.5262	0.666	0.00172**
I. micheliana	Yes	135	Micro	0.818	0.52376	0.52	0.003804**
I. micheliana	Yes	151	Micro	0.778	0.5158	0.749	0.000822***
I. micheliana	No	327	Macro	0.515	0.50078	0.657	0.000732***
I. micheliana	Yes	423	Macro	0.677	0.41191	0.629	0.005735**
I. micheliana	No	479	Micro	0.687	0.5709	0.563	0.000167***
I. micheliana	No	479	Macro	0.626	0.535	0.444	0.0718
I. micheliana	No	658	Micro	0.778	0.50905	0.716	0.00148**
I. micheliana	Yes	092	Micro	0.687	0.43536	0.457	0.004197**
I. micheliana	Yes	108	Macro	0.293	0.46553	0.651	<0.0001****
I. micheliana	Yes	135	Macro	0.737	0.50468	0.438	0.074786
I. micheliana	Yes	151	Macro	0.253	0.4952	0.661	0.000241***
I. micheliana	Yes	207	Macro	0.778	0.41452	0.338	0.003432**
I. micheliana	No	537	Macro	0.606	0.46974	0.65	0.007533**
I. micheliana	No	658	Macro	0.697	0.41477	0.36	0.003039**
I. micheliana	No	898	Macro	0.747	0.34082	0.331	0.001017**

4.10.3 Statistical output

4.10.3.1 Aggregation and infiltration

Table 4.2: Statistical results for main soil aggregation and water infiltration data, using fixed nest and tree effects and random site pair and covariate effects.

variable	*term*	*Estimate*	*Std..Error*	*df*	*t.value*	*P*
LognormPar1_macro	TreeSpeciesI. micheliana	1.17	0.48	6.76	2.4	0.05
LognormPar2_macro	AztecaNestNo	0.35	0.13	11.80	2.7	0.02
PlPar_micro	TreeSpeciesI. micheliana	0.33	0.15	9.59	2.1	0.06
ExpPar_micro	TreeSpeciesI. micheliana	0.01	0.00	5.95	5.2	0.00
LognormPar1_micro	TreeSpeciesI. micheliana	1795.46	107.74	0.91	16.7	0.05
Db_gcm3	TreeSpeciesI. micheliana	0.24	0.07	11.97	3.3	0.01
Infl_mLsec	AztecaNestNo	-5.54	0.84	6.54	-6.6	0.00
Infl_mLsec	AztecaNestNo:TreeSpeciesI. micheliana	4.74	2.35	12.43	2.0	0.07
Infl_mL300sec	AztecaNestNo	-0.05	0.02	10.71	-2.5	0.03
Infl_mL300sec	TreeSpeciesI. micheliana	-0.11	0.04	12.58	-2.5	0.03
WD_um	AztecaNestNo	-903.82	455.38	6.25	-2.0	0.09
WD_um	TreeSpeciesI. micheliana	-2248.97	526.84	10.87	-4.3	0.00

4.10.3.2 Chemistry

Below: R code showing statistical model formula and result output for soil nutrient response variables.

```
statTblchem <- aov(cbind(C_stock, N_stock) ~ AztecaNest * TreeSpecies + Par,
                   data=chemStatTbl) %>% summary(); statTblchem #%>% kable()

##  Response C_stock :
##                         Df Sum Sq Mean Sq F value Pr(>F)
## AztecaNest               1   0.02    0.02    0.06 0.8195
## TreeSpecies              1   9.26    9.26   27.82 0.0062 **
## Par                      4  12.09    3.02    9.09 0.0275 *
## AztecaNest:TreeSpecies  1   1.11    1.11    3.34 0.1417
## Residuals                4   1.33    0.33
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##  Response N_stock :
##                         Df Sum Sq Mean Sq F value Pr(>F)
## AztecaNest               1 0.0010  0.0010    0.49 0.5242
## TreeSpecies              1 0.0713  0.0713   33.90 0.0043 **
```

```
## Par                     4 0.0721  0.0180    8.56 0.0305 *
## AztecaNest:TreeSpecies  1 0.0085  0.0085    4.06 0.1143
## Residuals               4 0.0084  0.0021
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
statTblchemP <- lmer(P_stock ~ AztecaNest * TreeSpecies + (1|Par),
                     data=chemStatTbl) %>% summary(); statTblchemP
```

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: P_stock ~ AztecaNest * TreeSpecies + (1 | Par)
##    Data: chemStatTbl
## 
## REML criterion at convergence: 53
## 
## Scaled residuals: 
##    Min     1Q Median     3Q    Max 
## -1.474 -0.334 -0.055  0.250  1.592 
## 
## Random effects:
##  Groups   Name        Variance Std.Dev.
##  Par      (Intercept)  0.0     0.00    
##  Residual             26.1     5.11    
## Number of obs: 12, groups:  Par, 6
## 
## Fixed effects:
##                                         Estimate Std. Error   df t value
## (Intercept)                                 6.12       2.95 8.00    2.08
## AztecaNestYes                              -1.85       4.17 8.00   -0.44
## TreeSpeciesI. micheliana                    9.03       4.17 8.00    2.17
## AztecaNestYes:TreeSpeciesI. micheliana     -1.74       5.90 8.00   -0.30
##                                         Pr(>|t|)  
## (Intercept)                                0.072 .
## AztecaNestYes                              0.669  
## TreeSpeciesI. micheliana                   0.062 .
## AztecaNestYes:TreeSpeciesI. micheliana     0.775  
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## 
## Correlation of Fixed Effects:
##             (Intr) AztcNY TrSI.m
## AztecaNstYs -0.707              
## TrSpcsI.mch -0.707  0.500       
## AztNY:TSI.m  0.500 -0.707 -0.707
## optimizer (nloptwrap) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

```
# statTblchemCN <- lmer(CN ~ AztecaNest * TreeSpecies + (1|Par),
#                        data=chemStatTbl) %>% summary(); statTblchemCN
```

4.10.3.3 Co-variates

Below: R code showing statistical model formula and result output matrix ordination of environmental co-variates.

```
## ordRespTbl ~ AztecaNest * TreeSpecies * Par_num

##  [1] "LitterDepth_cm"    "Slope_deg"         "TrailDist_m"
##  [4] "DBH_cm"            "CoffeeBushes"      "Par_num"
##  [7] "PlPar_macro"       "ExpPar_macro"      "LognormPar1_macro"
## [10] "LognormPar2_macro" "PlPar_micro"       "ExpPar_micro"
## [13] "LognormPar1_micro" "LognormPar2_micro" "PlPar"
## [16] "ExpPar"            "LognormPar1"       "LognormPar2"
## [19] "Db_gcm3"           "Infl_mLsec"        "Infl_mL300sec"
## [22] "C"                 "N"                 "CN"
## [25] "WD_um"

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## vegan::adonis2(formula = ordModel, data = ordVarTbl)
##                               Df SumOfSqs    R2    F Pr(>F)
## AztecaNest                     1     0.11 0.018 0.45  0.575
## TreeSpecies                    1     0.83 0.138 3.52  0.047 *
## Par_num                        1     0.49 0.081 2.07  0.148
## AztecaNest:TreeSpecies         1     0.11 0.018 0.46  0.544
## AztecaNest:Par_num             1     0.01 0.002 0.05  0.992
## TreeSpecies:Par_num            1     0.20 0.033 0.85  0.406
## AztecaNest:TreeSpecies:Par_num  1     0.02 0.003 0.08  0.971
## Residual                      18     4.28 0.707
## Total                         25     6.05 1.000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

4.11 References

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Chapter 5 Biochar and Compost Improve Urban Soil Compaction and Organic Matter While Tending to Lower Microbial and Invertebrate Diversity

5.1 Abstract

Urban soils are often degraded by industrial activities, but are increasingly re-purposed for small-scale agriculture. Applying biochar, or pyrolized biomass, can improve various soil physical and chemical properties, although underlying mechanisms remain less clear, and net effects on soil biodiversity remain understudied. This study tested how biochar affects physical, chemical, and biological properties of an urban Technosol soil that was recently transitioned for small-scale cultivation over two growing seasons in Detroit, MI, USA. Specific hypotheses about biochar particle size, application rate, and comparison to compost were tested by sieving manufactured biochar at one mm and co-applying with compost across various treatment plots. Biochar-compost co-additions significantly lowered soil bulk density, increased soil organic matter by 5% , generally increased bacterial richness and variability while lowering fungal phyla abundances, and tended to lower invertebrate richness and similarity, with broadly consistent interactions with soil depth. These results suggest that biochar may improve some key soil conditions to promote plant growth, but longer term effects on soil biological activity may be somewhat detrimental. Overall, this study presents valuable practical data and trends to inform urban growers about the benefits and tradeoffs of adopting biochar amendments as an effective short-term growing strategy.

5.2 Introduction

Soils are being degraded and eroded ten times faster than they form *(Richter 2021)* due to industrial agriculture and intensive urbanization *(Beniston, Lal, and Mercer 2016)*, thereby posing risks to regional food systems . Yet, degraded urban soils that are re-purposed for cultivation after being engineered for construction can also potentially regenerate fertility relatively quickly *(Mukherjee, Lal, and Zimmerman 2014)*. By definition, degraded soils are far from soil carbon saturation *(Six et al. 2002; Stewart et al. 2007, 2009)*, meaning that increases in soil organic matter occur faster than those occuring while soil carbon is already high. Furthermore, urban soils being re-claimed for small-scale agriculture, such as in vacant lots, are subject to a wide range of individual management strategies, as well as broader coordinated efforts toward ecosystem multi-functionality similar to rural settings *(Stratton et al. 2022)*. As a result, urban soils are likely to show quick responses to positive interventions, but their magnitudes, underlying mechanisms, and potential trade-offs, especially among biological variables, remain under-studied, including in applied contexts.

Biochar, or pyrolized biomass, has spread widely in modern use since the archaeological documentation of *terra preta* ("dark earth") anthropogenic Anthrosol soils *(Woods et al. 2009)* primarily formed by indigenous settlement activity *(Silva et al. 2021; Lombardo et al. 2022; Silva et al. 2022)* in the Amazon and globally, which show some relatively distinct properties from surrounding soils *(Glaser 2007; Joseph et al. 2021)*. Biochar can affect soil carbon content, soil structure and aggregate size distributions, as well as aggregate stability and carbon concentrations inside them *(D. Wang et al. 2017)*. Biochar-amended soils can also show high phosphorus *(P)* and calcium, which has served as evidence of co-amendments, such as fish bones on original sites *(Lombardo et al. 2022)*, and compost in modern applications. Higher chemical fertility is also likely to affect soil microbial communities *(Anderson et al. 2014; Lévesque et al. 2020; Han et al. 2022; F. Zhang et al. 2022)*, such as arbuscular mycorrhizal fungi who heavily scavenge for soil P *(Warnock et al. 2007)*, and accordingly also their invertebrate grazers. However, despite these potential propagated effects through the soil food web, few studies report how biochar affects soil microbial biodiversity *(O'Neill et al. 2009; Lucheta et al. 2017)*, including as mediated by biochar particle size which have differing effects on soil moisture *(Edeh et al 2022)* and could have effects on biodiversity akin to soil aggregates sizes *(Chapter 2)*, and furthermore very few studies report biochar effects on invertebrates *(Domene 2016)*.

This study investigates how biochar amendments affect soils in the context of urban agriculture *(Nugent and Allison 2022)*, namely soil physical aggregate structure, nutrient concentrations, and microbial and invertebrate biodiversity. We hypothesized that biochar would improve overall soil fertility by alleviating physical compaction and increasing nutrient availability. We expected these physical and chemical properties to be associated with differences in soil microbial and invertebrate community structure. More specifically, we expected that the biochar would mediate these effects based on mean particle size of the biochar, and would have effects that were different compared to controls or compost-only additions. Accordingly, we predicted that soils with added biochar would show higher organic matter, nutrient concentrations and more neutral pH, and microbial and invertebrate alpha and beta diversity, compared to control (unamended) soils.

5.3 Methods

5.3.1 Study site

This study was done on a 50 x 50 ft area of the Michigan State University Extension Detroit Partnership for Food Learning and Innovation in the Brightmoor neighborhood of Detroit, MI, USA. The site lies approximately 2 mi from a river and the neighborhood contains a greenway collection of other small scale sustainable urban farms. This growing area had been minimally amended since the demolition of the former school on the property in 2017, keeping the soils in need of optimization for plant growth by amendment, rather than soil optimized for structural stability for construction. Baseline soil tests for the site show low levels of heavy metals (though nearby areas may have higher levels) and 2-3% organic matter, but high pH and low aggregate stability (Edwards, Medina, and Asker 2023).

5.3.2 Study design

Approximately one cubic yard (about 100 gal) of pine wood biochar *(Wakefield Biochar, Columbia, MO, USA)* was sieved manually at one mm (1000 um) into two distinct particle size classes, small (<1 mm) and large (>1 mm), for two weeks. Sieved biochar was then mixed 1:1 by volume with compost *(Tuthill Farms & Composting Inc, South Lyon, MI, USA)* and let sit outside for two days to allow for some initial activating and inoculating processes. The study area tarp was removed and it was roto-tilled to 10 cm (4") depth using a shared Brightmoor community roto-tiller *(BCS 749)*. The following season no-till management was used and weeds were controlled using a shared community weed whacker. The study area was divided into a grid of 64 adjacent 1.5 x 1.5 m (5 x 5 ft) plots using one foot wood chip and/or paper mulch pathways, representing at least four replicates per treatment across an equivalent number of adjacent blocks. Blocks primarily accounted for any coarse spatial effects within the study area, so plot locations sufficiently varied across the blocks in study area but plots were not fundamentally random. The biochar-compost mix was applied at 5% by volume of topsoil in the plot (150 x 150 cm x 5 cm depth) two days before seeding. The following season one-quarter of the plots were maintained with repeat particle size treatments, and the other plots used for additional treatments. One-quarter was used for unmanipulated, biochar-only, compost-only, and (mixed-size) biochar-compost treatments, which could discern between direct biochar effects and those from co-application with compost. The remaining half was used to apply biochar at zero, five, ten, and 20% by volume, to test maximal potential soil responses.

Cowpea was chosen as a legume with bacterial-root symbioses that provide biological nitrogen fixation, adding soil nitrogen that biochar lacks, being a decay-resistant form of carbon, making a combination that studies show improves the effectiveness of biochar (urea is also an alternative nitrogen source). Cowpea also has African origins, making it of cultural importance to local Detroit urban farmers. Buckwheat was chosen as a companion crop for its ability to scavenge soil phosphorus, grow quickly, and attract pollinators via high floral density (Clark 2007). The following season an early cover crop mix of red clover and hairy vetch was planted to continue supplying nitrogen and root carbon to soils. Seeds *(Keep Growing Detroit, MI, USA; Johnny's Selected Seeds, Albion, ME, USA)* were planted by hand and planted into the soil using a hand hoe, arranged into two alternating rows of each species per plot at recommended distances apart (Clark 2007) totaling eight buckwheat seeds and five cowpea seeds per row per plot (approximately 16 buckwheat and 10 cowpea seeds per plot). The following season plots were seeded with beets and field peas in four alternating rows of eight to ten seeds each ~2.5 cm (1") deep.

5.3.3 Data collection

Soil samples were collected in triplicate per plot using a 5.5 cm-wide metal corer and sand-filled dead-blow hammer for minimal disturbance (AMS Inc, American Falls, ID). Given early natural pedogenic profile development of this Technosol, each core sample down to 10 cm included both A horizon and some of that below it. To better describe treatment effects appearing in the field by soil depth beyond the one inch of estimated topsoil of treatment application, each core sample was split along a natural horizontal fault line midway down, and treated separately throughout downstream analyses. A total of 192 soil core samples from 32 plots (*n* = 8) were collected in the Fall of the first year. In the subsequent season, soils were collected in duplicate from 48 plots, in the same manner as before, after mowing the following Spring. All soil samples were stored at 4 C within 24-72 h of collection.

To facilitate the total soil mass requirements for multiple lab analyses per plot and treatment, triplicate plot sub-samples were not pooled together but instead each core was allocated to a separate lab analysis. Specifically, after collection of all soil samples from the study area, both depths of the first sub-sample from each plot, in the first season specifically selected from every other row (*n* = 4) and in the next season soil cores were used from every plot sampled (124 samples total), were sent to the Michigan State University Soil and Plant Nutrient Laboratory (East Lansing, MI, USA) for a standard suite of garden soil analyses including 1:1 soil-water pH, extractable phosphorus (Olsen et al. 1954), and ammonium-acetate extracted potassium (K), magnesium (Mg), and calcium (Ca). The second sub-sample from these same plots in every other row (*n* = 4, 32 samples total) were sent for only wet aggregate stability analysis to Cornell University Soil Health Lab *(Ithaca, NY, USA)*. Lab chemical analyses were done for bulk soils due to soil mass requirements for lab protocols.

Approximately 0.15 g soil was sub-sampled from each depth of the second soil core (of triplicate soil cores per plot in the first season and of duplicate soil cores per plot in the second season). These lab sub-samples were then run through the manufacturer protocol for PowerLyzer

PowerSoil kit *(QIAGEN)*, with some modifications including replacing half (300 µL) of recommended Powerbead solution with 1 M sodium-potassium phosphate buffer (pH 7), an additional water bath incubation at 65 C for 10 min, followed by a rapid freeze at -80 C for 5 min, to improve nucleic acid de-sorption from fine soil particles (noted in protocols.io). In total 160 DNA extractions were stored at -20 C.

Sample libraries were generated using primers for bacterial ribosomal v4 regions *(Caporaso et al. 2011)* and fungal ITS regions *(Taylor et al. 2016)* in standard PCR and troubleshooted with touchdown PCR, with final sequencing done on the MiSeq platform at the University of Michigan Microbiome Sequencing core (Ann Arbor, MI, USA). DNA sequences were cleaned following the standard operating procedures for using DADA2 *(B. J. Callahan et al. 2016)* on bacteria and fungi (https://benjjneb.github.io/dada2/ITS_workflow.html), which clusters sequence information with 99% similarity into exact amplicon sequence variants (ASVs). Suggested modifications for the fungal ITS pipeline included excluding reverse reads and reads with quality scores below eight, trimming unwanted sequences (i.e. primers, repeats) of variable lengths with ‘cutadapt’ for python 3.10 (Martin 2011), and allowing eight expected sequence errors (Rolling et al. 2022). Taxonomy was assigned overnight with the naive Bayesian classifier method implemented in DADA2 (Q. Wang et al. 2007) matching to the bacterial SILVA (Quast et al. 2012; Glöckner et al. 2017) *v132* (B. Callahan 2018) and fungal UNITE v9.0 (Abarenkov et al. 2010; Nilsson et al. 2019) *v16.10.2022 (29.11.2022)* (Abarenkov et al. 2022).

Arthropods (i.e., insects and springtails and mites) were surveyed using one pitfall trap placed in the center of each plot, which is informative for smaller body sizes and includes potentially preferential movement activity for larger sizes (see Lee 2021 for exploration). Traps were made of wide plastic cups with soapy water at the bottom, covered with propped paper plates to create cool shade while allowing crawlers to fall in by foraging. Traps were left outside for one week and then collected, sealed, and all trapped invertebrates transferred to smaller vials filled with 70% alcohol for preservation. Specimens were identified to Order and morpho-species under a microscope and abundance tallied by plot.

5.3.4 Statistics

Field and lab data was recorded in Excel and analyzed in R programming language for statistical computing *(R Core Team 2023)*. Data were centered at the plot level using means and treatment effects decomposed using linear mixed effects models with treatment and depth as fixed effects and spatial block as a random effect. Key open-source packages included 'here' (Müller 2020), 'dplyr' and 'tidyr' and 'rstatix' (Wickham et al. 2019) for data cleaning, 'microeco' (Liu et al. 2021) and 'tblhelpr' for bioinformatics and cleaning, 'vegan' (Dixon 2003) and lmerTest (Kuznetsova, Brockhoff, and Christensen 2017) for statistical testing, 'ggplot2' and 'ggpubr' (Kassambara 2023) for plotting, and 'rmarkdown' (Allaire et al. 2023), 'knitr' (Xie 2023b), 'bookdown' (Xie 2023a), and 'grateful' (Rodriguez-Sánchez and Jackson 2023) for text and code integration.

5.4 Results

5.4.1 Physical

Overall, soil bulk density differed significantly by biochar treatments *(Fig 5.1)* . Soil bulk density was marginally significantly lower with 10% biochar by -0.15 ± 0.09 (df=56, t=-1.69, p=0.1) and significantly lower with 20% biochar by -0.2 ± 0.09 (df=56, t=-2.36, p=0.02) . Soil bulk density was also marginally significantly higher at depth by 0.01 ± 0.01 under biochar particle size treatments (df=241, t=1.86, p=0.06) . Furthermore, soil bulk density showed both significant direct interactive responses to biochar-compost treatments compared to control soils: significantly lower with compost by -0.19 ± 0.09 (df=56, t=-2.26, p=0.03) and interacting with depth by 0.02 ± 0.01 (df=56, t=1.64, p=0.1) , lower with biochar by -0.31, 0.03 ± 0.09, 0.01 (df=56, t=-3.64, p=<0.0001, df=56, t=2.97, p=<0.0001) and interacting with depth by 0.03 ± 0.01 (df=56, t=2.97, p=<0.0001), and lower with both compost and biochar by -0.31, 0.03 ± 0.09, 0.01 (df=56, t=-3.6, p=<0.0001, df=56, t=3.18, p=<0.0001) interacting with depth by 0.03 ± 0.01 (df=56, t=3.18, p=<0.0001) .

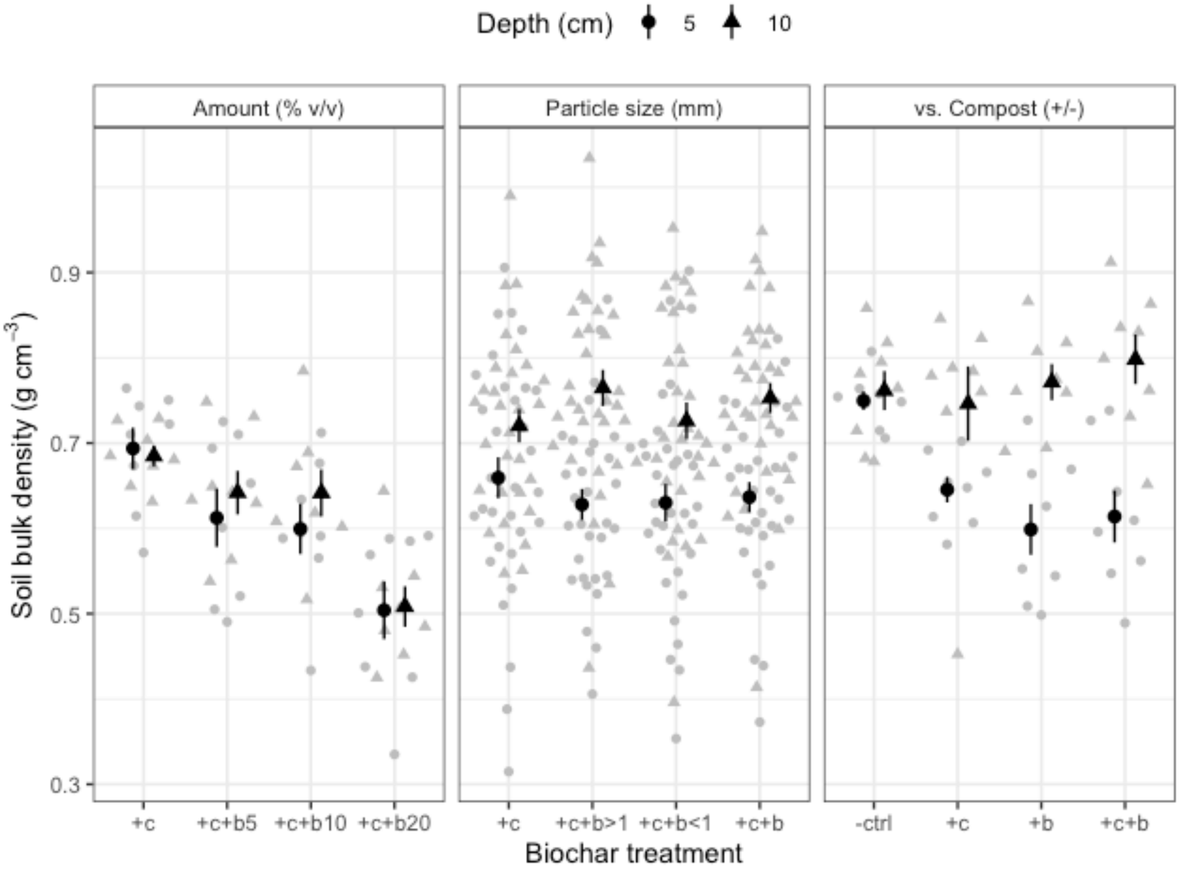

Figure 5.1: Urban soil bulk density by depth and type of field biochar application treatment (+c = compost, +b = biochar at stated % v/v or 5% otherwise, >1 = >1 mm biochar, <1 = <1 mm biochar, and -ctrl = unmanipulated), showing means ± 1 standard error (black) and underlying data (grey) (n = 8).

5.4.2 Chemical

Soil organic carbon responded significantly to biochar application treatments, including where compost additions were equal *(Fig 5.2)* . Soil organic carbon was significantly lower at depth by -0.59 ± 0.19 (in plots focusing on particle size treatments, where compost was added at equal rates across plots) (df=53, t=-3.08, p=<0.0001) . Soil organic carbon also significantly responded to biochar-compost additions at 20% by 28.05 ± 11.34 % (df=21, t=2.47, p=0.02) interacting with depth by -2.74 ± 1.43 (df=21, t=-1.91, p=0.07) . Similarly, soil organic carbon responded significantly to biochar-compost co-application at 5% v/v by 10.71 ± 4.4 (df=23, t=2.43, p=0.02) interacting with depth by -1.05 ± 0.54 (df=23, t=-1.93, p=0.07).

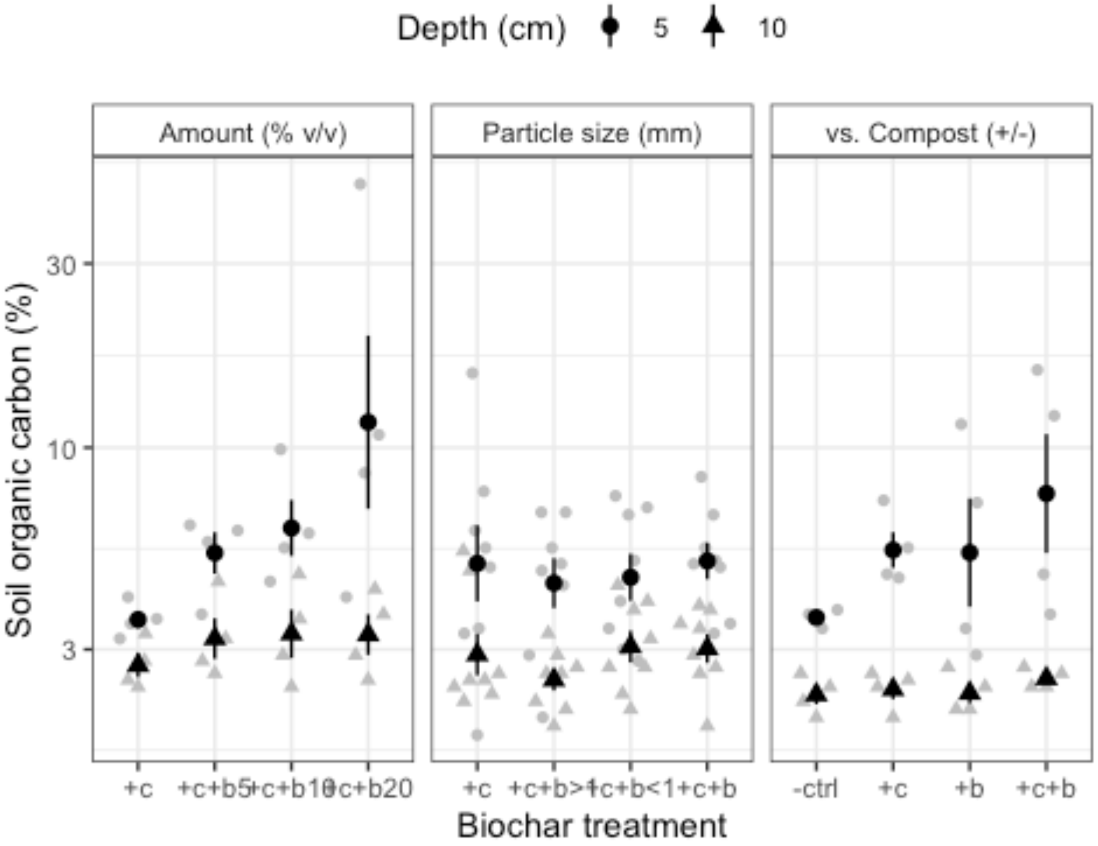

Figure 5.2: Urban soil organic matter concentrations by depth and type of field biochar application treatment, showing means ± 1 standard error (black) and underlying data (grey) (n = 4).

Biochar treatments significantly affected soil chemical fertility *(Table 5.1)* , specifically available phosphorus increased with 5% and 10% biochar co-application with compost along with potassium increasing at 10% biochar co-application. However, somewhat surprisingly, cation exchange capacity decreased with all biochar doses and smaller biochar particle sizes but in contrast increased interactively with soil depth, and magnesium also decreased with sieved biochar co-applications but increased interactively with soil depth .

Table 5.1: Urban soil chemistry linear mixed effects statistical model results, showing elemental response estimates, error, and other parameters across various field biochar compost co-addition treatments (+c = compost, +b = biochar at stated % v/v or 5% otherwise, >1 = >1 mm biochar, <1 = <1 mm biochar, and -ctrl = unmanipulated).

ELEMENT	*STUDY*	*FACTOR*	*Estimate*	*se*	*df*	*t*	*p*
Ca_ppm	Amount (% v/v)	TREAT+c+b20	-1698.75	678.01	24	-2.51	0.02
Cax_pct	Amount (% v/v)	TREAT+c+b20:DEPTH_CM	0.67	0.23	21	2.90	0.01
	Amount (% v/v)	TREAT+c+b20	-7.15	1.83	21	-3.91	<0.0001
Kx_pct	Amount (% v/v)	TREAT+c+b20	2.90	1.10	21	2.64	0.02
Mg_ppm	Particle size (mm)	TREAT+c+b<1	-70.25	33.83	53	-2.08	0.04
P_ppm	Amount (% v/v)	TREAT+c+b20	65.50	23.47	21	2.79	0.01
	vs. Compost (+/-)	TREAT+c+b	35.08	16.43	23	2.13	0.04

5.4.3 Biological

5.4.3.1 Microbial

Community

Soil bacterial and fungal communities responded marginally significantly to some biochar treatments *(Fig 5.3)* . Bacterial richness marginally significantly increased by 102.1 ± 49.9 ASVs (df=17.7, t=2, p=0.06) with only compost additions and interacted with depth marginally significantly by -11.5 ± 6.2 (df=17.5, t=-1.9, p=0.08) , in addition to a marginally significant direct effect of depth by 4.4 ± 2.6 (df=43.3, t=1.7, p=0.1) . Fungal richness also marginally significantly decreased by -28.3 ± 16.6 (df=16, t=-1.7, p=0.1) with 5% biochar co-additions , while decreasing marginally significantly by -2.8 ± 1.5 (df=16, t=-1.8, p=0.09) directly with soil depth . Furthermore, bacterial community compositional distances responded marginally significantly to biochar dose response treatments, namely at 5% by 0.46 ± 0.09 (t=5.33, p=<0.0001) interactively with soil depth by -0.05 ± 0.01 (t=-4.9, p=<0.0001) , and also 20% biochar, by 0.17 ± 0.09 (t=2.02, p=0.05) . Bacterial community distances also marginally significantly responded to depth by -0.02 ± 0 (t=-5.01, p=<0.0001) .

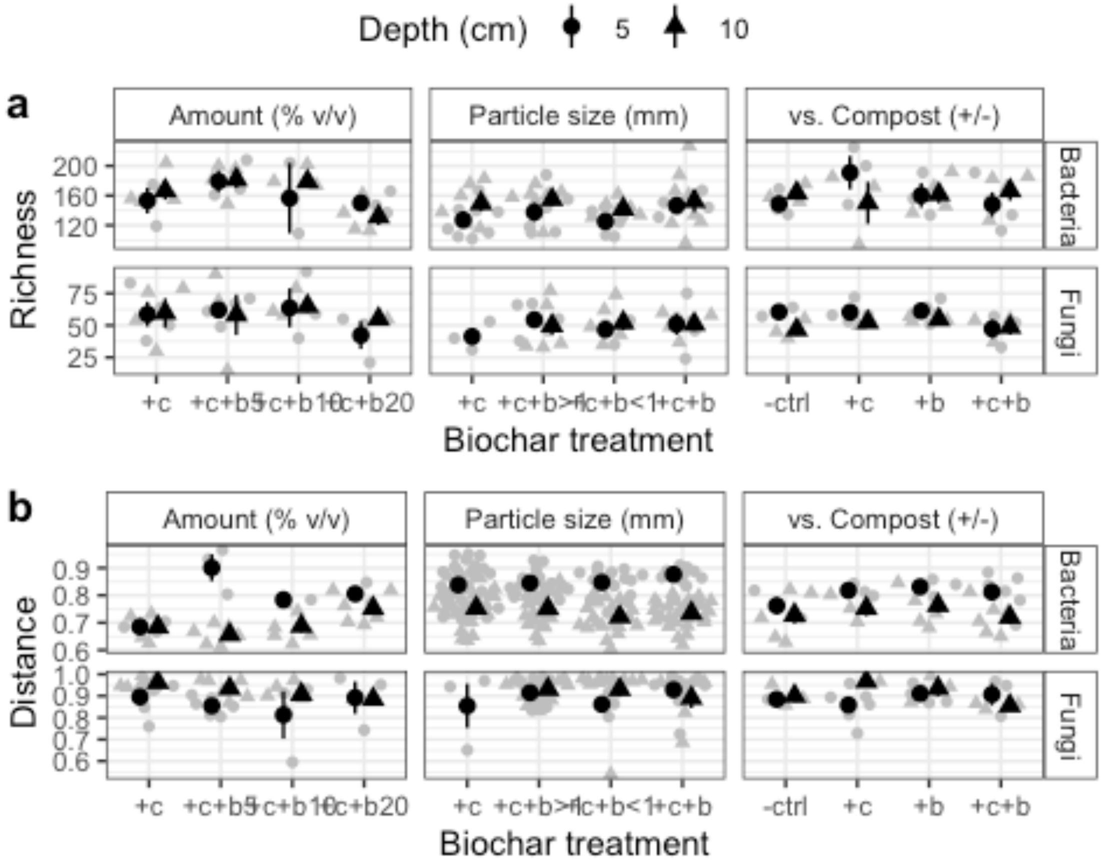

Figure 5.3: Urban soil bacterial and fungal community (a) richness and (b) distance (dissimilarity) across field biochar and compost treatments (+c = compost, +b = biochar at stated % v/v or 5% otherwise, >1 = >1 mm biochar, <1 = <1 mm biochar, and -ctrl = unmanipulated), showing means ± 1 standard error (black) and underlying data (grey) (rarefied to 1000 reads, n = 0-4).

Composition

Community composition of phyla also changed significantly with field biochar treatments, specifically six in Bacteria and five in Fungi *(Fig 5.4)* . Acidobacteriota responded marginally significantly to 10 and 20% biochar co-applications by -19.28 ± 10.73 (df=19, t=-1.8, p=0.09) and -35.84 ± 9.76 (df=19, t=-3.67, p=<0.0001) respectively, alongside depth by 1.05 ± 0.62 (df=43.28, t=1.68, p=0.1) under particle size treatments, and interactions at 10% by 2.33 ± 1.27 (df=19, t=1.84, p=0.08) and 20% co-application by 3.94 ± 1.18 (df=19, t=3.33, p=<0.0001) .

Bacteroidota responded significantly to 5 and 20% biochar co-applications by 25.48 ± 11.33 (df=19, t=2.25, p=0.04) and 38.7 ± 11.33 (df=19, t=3.42, p=<0.0001) respectively, with interactions at 5% by -3.04 ± 1.37 (df=19, t=-2.21, p=0.04) and 20% co-application by -4.26 ± 1.37 (df=19, t=-3.1, p=0.01) . Chloroflexi responded to 10% biochar co-application by -7.35 ± 4.4 (df=16.91, t=-1.67, p=0.1) along with compost only additions by -9.32 ± 4.66 (df=17.55, t=-2, p=0.06) bearing an interaction with depth by 1.01 ± 0.58 (df=17.12, t=1.76, p=0.1) . Planctomycetota responded to 20% biochar by -9.52 ± 3.02 (df=16.34, t=-3.15, p=0.01) interactively with depth by 0.9 ± 0.37 (df=16.19, t=2.46, p=0.03) . Proteobacteria responded most broadly, doing so at 5% by 14.84 ± 8.51 (df=19, t=1.74, p=0.1) , 10% by 31.13 ± 9.36 (df=19, t=3.33, p=<0.0001) , and 20% by 30.64 ± 8.51 (df=19, t=3.6, p=<0.0001) biochar co-application, with corresponding interactions with depth by -1.86 ± 1.03 (df=19, t=-1.8, p=0.09) , -3.31 ± 1.1 (df=19, t=-3, p=0.01) , and -3.38 ± 1.03 (df=19, t=-3.27, p=<0.0001) . Proteobacteria also responded to compost only additions by 11.36 ± 5.91 (df=17.16, t=1.92, p=0.07) as well as depth by -0.86 ± 0.5 (df=17.13, t=-1.72, p=0.1) with an interaction by -1.33 ± 0.73 (df=17.06, t=-1.83, p=0.09) . Finally, Verrucomicrobiota responded to smaller biochar particle size co-application <1 mm by -9.1 ± 4.51 (df=46, t=-2.02, p=0.05) , as well as 5% by -12.93 ± 4.93 (df=19, t=-2.62, p=0.02) and 20% biochar co-application by -10.41 ± 4.93 (df=19, t=-2.11, p=0.05) and depth by -0.82 ± 0.42 (df=19, t=-1.93, p=0.07) interactively by 2.03 ± 0.6 (df=19, t=3.4, p=<0.0001) and 1.35 ± 0.6 (df=19, t=2.25, p=0.04) .

Ascomycota responded to compost-biochar mixtures by -46.57 ± 28.43 (df=13.37, t=-1.64, p=0.1) interactively with depth by 5.98 ± 3.47 (df=13.28 t=1.72 p=0.1) . Basidiomycota responded to 20% biochar co-application by 60.65 ± 31.31 (df=17.12, t=1.94, p=0.07) with no depth interaction. Chytridiomycota responded most widely to 5% biochar co-application by

14.91 ± 6.66 (df=18, t=2.24, p=0.04) with a depth interaction by -1.71 ± 0.86 (df=18, t=-1.99, p=0.06) , as well as compost only additions by -8.98 ± 5.32 (df=15, t=-1.69, p=0.1) also interacting with depth by 1.21 ± 0.68 (df=15, t=1.76, p=0.1) . Glomeromycota responded to unsieved biochar co-application by 21.65 ± 13.58 (df=23, t=1.59, p=0.1) ; and finally Mortierellomycota responded to biochar-compost mixtures interactively with depth by -9.1 ± 5.24 (df=16, t=-1.74, p=0.1) .

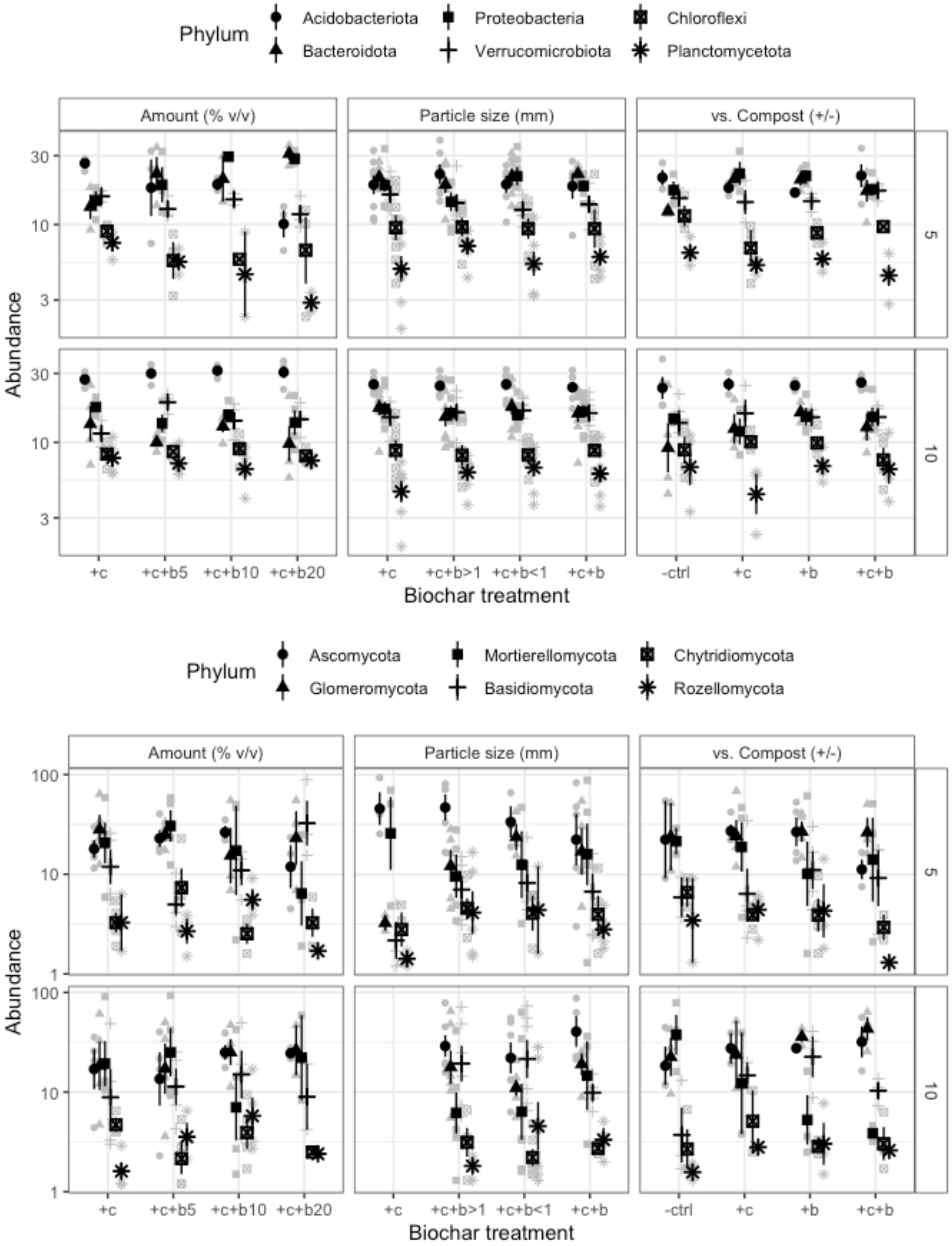

Figure 5.4: Urban soil bacterial (top) and fungal (bottom) phylum abundances across various field biochar treatments (+c = compost, +b = biochar at stated % v/v or 5% otherwise, >1 = >1 mm biochar, <1 = <1 mm biochar, and -ctrl = unmanipulated), showing means ± 1 standard error (black) and underlying data (grey) (rarefied to 1000 reads, n = 0-4).

5.4.3.2 Macrobial

Community

Soil invertebrate communities responded significantly to biochar treatments *(Fig 5.5)* . Soil invertebrate richness was marginally significantly lower with smaller sized biochar particle co-additions by -1 ± 1 (df=748, t=-2, p=0.0984) and with typical unsieved biochar co-additions by -1 ± 1 (df=748, t=-2, p=0.05) . Soil invertebrate richness was somewhat significantly higher with larger sized biochar particle co-additions with compost by 2 ± 1 (df=748, t=3, p=0.0032) . In contrast to richness, soil invertebrate community distances significantly increased with various biochar treatments, namely regular unsieved biochar co-additions 0.043 ± 0.005 (df=5452, t=8.121, p=<0.0001) as well as larger sized 0.025 ± 0.005 (df=5452, t=5.029, p=<0.0001) and smaller sized biochar particle co-additions 0.046 ± 0.005 (df=5452, t=9.411, p=<0.0001) . Despite these differences in community diversity, there more no detectable differences in taxonomic composition *(data not shown)* .

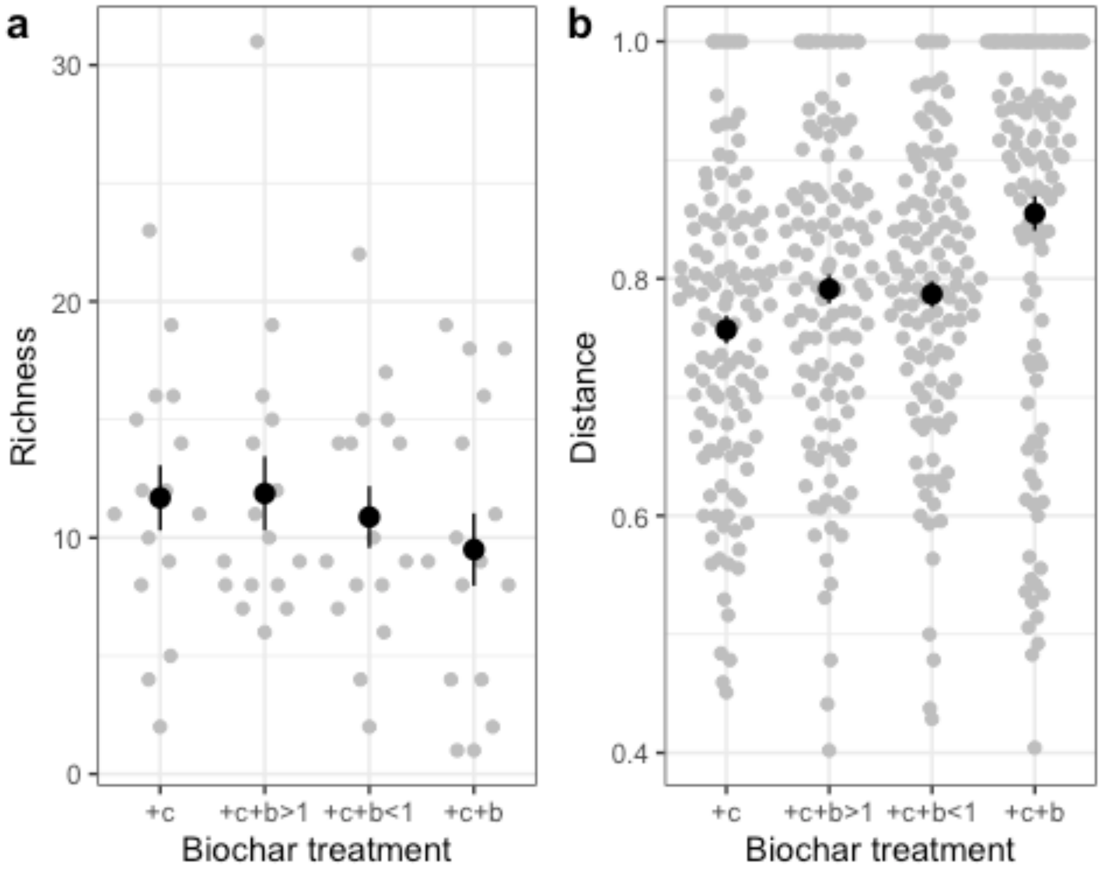

Figure 5.5: Urban soil invertebrate community (a) richness and (b) pairwise distances after biochar particle size field applications with compost (+c = compost, +b = biochar at stated % v/v or 5% otherwise, >1 = >1 mm biochar, <1 = <1 mm biochar, and -ctrl = unmanipulated), showing means ± 1 standard error (black) and underlying data (grey) (n = 16, 120).

5.5 Discussion

Overall, this study finds that short-term biochar amendments are effective strategies under low-till management systems for improving post-construction urban soils for sustainable agriculture. Specifically, results here from various biochar treatments showed that biochar significantly lowered bulk density, increased organic matter and phosphorus and potassium but generally lowered calcium and magnesium concentrations, increased bacterial but lowered fungal richness, and also lowered invertebrate richness while increasing beta diversity or community dissimilarity. Taken together, these results tend to agree with original predictions, supporting the hypothesis that biochar improves urban soils, partially due to physical effects on structure. Biochar co-applications performed here were notably beneficial for lowering physical soil bulk density and alleviating soil compaction issues. This benefit increased with biochar application, similar to other studies (Blanco-Canqui 2017; Wong, Levy-Sakin, and Kwok 2018), although there also appeared some tradeoffs with heavy application on more direct biological properties (Joseph et al. 2021). Soil bulk density was not affected by biochar particle size, but its effect tended to be more than that of compost alone, suggesting it may be more efficient or faster compared to compost only for alleviating soil compaction, though practitioners must also consider slightly higher local cost of ~0.25 USD more per sq ft for biochar versus compost depending on acreage and total quantity needed (*pers. comms.*). Significantly lower soil bulk density can be explained in part by the low density of biochar itself (Werdin et al. 2020), in part due to irregular geometric arrangements of the char particles (Brewer et al. 2014), which can extend to geometric arrangements with native soil aggregate particles. These effects on soil bulk density compared variably to our hypotheses. Increasing application amount lowering bulk density agreed with the original hypothesis present here, while the effects of particle size were surprising. For this fine-textured soil, it was hypothesized that large particles help counteract the consolidating effects of many fine soil particles. However, it may be that 1 mm biochar particles were still relatively too large to help work at the micrometer scales of sand and silt (i.e. <0.02 mm), and that instead the small particles used here <1 mm are actually the more appropriate size to counteract collective soil particle behavior at these small scales, which agrees with other reported effects on soil moisture (Liao and Thomas 2019) and thus this result here might not change with a longer study period. Accordingly, there may still be real differences in mechanisms occurring between 20 and 2 micrometers, for example, but this stretches beyond the limit of detectability in more empirical studies (Brewer et al. 2014). Overall, these bulk density results suggest that even small volume applications of biochar can help transition post-construction urban soils for cultivation, in low-till systems.

Biochar was also effective for improving soil organic matter content and some nutrient levels beyond compost, in line with some studies (Saarnio et al. 2018; Siedt et al. 2021). While more biochar also adds more compost to maintain a 1:1 mixture, the presence of a difference in organic carbon at 5% biochar co-application compared to compost only controls suggests that the presence of biochar itself increased organic carbon concentrations. Lower bulk density may have occured by increasing soil moisture, packing compost particles more tightly together, and/or also adding relatively more pore space, all of which would lower overall soil bulk density. Furthermore, compost is known as a key source of phosphorus in urban soils (Small et al. 2019), and results here suggest that while phosphorus increased more with more biochar co-application at 20%, phosphorus also increased in biochar-compost mixtures compared to biochar and compost-only controls, suggesting that biochar accounted for about half of the increase in phosphorus during co-applications, likely indirectly, as biochar is not a source of nutrients itself (Q. Zhang et al. 2015), which is consistent with the lack of significant effect in biochar-only treatments. Together with the slight increase in potassium levels, but despite lower magnesium, biochar co-amendments may be able to replace synthetic NPK fertilizer usage in small-scale agricultural systems as a more locally sourced alternative input.

Microbial communities responded differently based on phyla to biochar-compost co-additions. Bacterial richness increased most notably with compost only additions, implying that biochar is not a main source of bacterial diversity at the phylum level. However, biochar did consistently correspond with slightly higher bacterial community dissimilarities, suggesting that biochar may re-shape the community compositions that are already there in the soil and/or compost. Specifically, biochar favored bacteria in the phylum Proteobacteria and Bacteroidota, while lowering the abundance of bacteria in others, including Acidobacteriota, Chloroflexi, Planctomycetota, and Verrumicrobiota. Consistent responses of Proteobacteria align with it being the most diverse phylum (Spain, Krumholz, and Elshahed 2009), and lower responses of Acidobacteria align with expected higher pH from biochar use (Sait, Davis, and Janssen 2006; Ward et al. 2009; Greening et al. 2015; Ivanova et al. 2020; Kalam et al. 2020; Sikorski et al. 2022). In Fungi, three of five phyla responded positively to soil amendments, while the others responded negatively. Ascomycota and Mortierellomycota responded negatively, while Basidiomycota, Chytridiomycota, and Glomeromycota responded positively. Of interest for agriculture are Glomeromycota, containing arbuscular mycorrhizal fungi that associate obligately with most plants and also help scavenge phosphorus including with biochar (Mowrer et al. 2022), and Basidiomycota, which contain the mushroom-forming macro-fungi as well as various saprotrophic decomposer groups (Tanesaka, Masuda, and Kinugawa 1993; Floudas et al. 2020), as does Ascomycota (Ma et al. 2013). Interestingly, Chytridiomycota, like Proteobacteria, also responded consistently to treatments, which may similarly be influenced by relative genetic and evolutionary diversity of the group (James et al. 2006), and suggests that biochar co-additions can support soil as a reservoir for fungi other than just decomposers.

Results here also add to sparse studies on soil invertebrate responses to biochar (Singh et al. 2022; Zhu et al. 2023). In contrast to the original expectation, ground soil invertebrates responded negatively to biochar application, and also tend to diverge in composition, or become more dissimilar. This result may help explain top-down effects for microbial predators, and/or cascades for predators of smaller invertebrates. While loss here was small (about one taxon), this was statistically detectable and may be worth considering in newer vacant lots where tilling is useful and invertebrate communities have not fully established themselves yet, potentially representing local extinction debt where compost need to be continuously added to maintain early diversity until other biodiversity maintenance forces can become more self-reinforcing *(Hylander and Ehrlén 2013)*. Separate but related literature in aquatic ecotoxicology implies negative effects of dissolved biochar derivatives in soil water (Gomez-Eyles et al. 2011; Godlewska, Ok, and Oleszczuk 2021). Multiple of these processes may be occurring, but these results imply that negative chemical effects may outweigh positive food web effects on soil invertebrates, and that microbial communities appear negatively correlated with invertebrate community shifts in biochar-amended urban soils. Here, biochar co-applications had a slightly negative effect on invertebrate diversity, but this should still be weighed against other likely benefits of biochar.

In summary, this study provides multiple dimensions of how soil components respond to biochar application with and compared to compost strategies in temperate urban agricultural contexts. Tradeoffs between physical and biological benefits are highlighted, allowing biochar use and discussion to be better informed and grounded in scientific analysis of collected data. More broadly this study also points to potentially more generalized tradeoffs among soil physical and ecological components, raising more nuanced discussion about traditional physical niche dimensions versus more dynamic constructed niche dimensions affected by internal feedbacks among co-existing taxa (Vandermeer 2020). Further studies testing ecological hypotheses of soil dynamics in urban agricultural settings would uniquely contribute to understanding of fundamental and ongoing changes to soils as a part of complex socio-ecological systems.

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5.8 Interests statement

No conflicts of interest declared.

5.9 Data statement

Code and data available at Zenodo via github.com/nmedina17/biochar.

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Chapter 6 Conclusion

Altogether, this dissertation offers a scaling framework to study soil ecology based in complex systems theory, helping to advance fundamental physical, microbial, and applied agricultural purposes. More specifically, it revives the soil aggregate hierarchy model and highlights novel hypotheses to more fundamentally understand soils as dynamical systems rather than static ones, as they are continuously responding to human activities over relatively observable timescales. Specifically, the first study highlights soil memory and time lags, oscillatory dynamics, and tipping points and hysteresis as key but hard to detect phenomena that have important implications for actively responding to soil degradation, but are typically only detectable when analyzing model systems under complexity theory. The second study presents scaling patterns for microbial biodiversity across soil aggregate units of soil structure, showing that soil aggregate particle clustering not only promotes microbial biodiversity, but does so by increasing the relative likely influence of random ecological drift on separate microbial taxonomic group and underlying populations. The third study draws links between above- and below-ground processes, showing how consistent nesting by a keystone tropical ant can have cascading effects on soils, which depend on tree species that shape its local foraging behavior. Finally, the fourth implicitly ties in social and political aspects to consider when studying soils from a complex systems perspective; namely, how multiple land amendment options can work cooperatively to help re-purpose and regenerate post-construction urban soils.

Adjacent questions to this research show promise for future research projects. Future projects include validating new formulations for models of soil aggregate structural dynamics, such as low-dimensional systems of ordinary differential equations with multiplicative terms, or network formulations that dynamically dis- and re-assemble. This also involves key decisions: including about which soil concepts/variables will occur relatively slowly, i.e., formulated as fixed parameter values defined by empirical literature, and deciding which variables will be modeled over shorter time scales, drawing the focal attention of model analyses and study results – both questions involve a priori decisions affecting results framings and related implications. Related to how soil microbial communities assemble, lab studies of pairwise competition trials among culturable taxa would help test theoretical predictions about community matrix determinants or co-variances with microbial players dominant in soils. There are also emerging technologies for single-cell and small population sequencing that allow testing how spatial separation at the finest empirically tractable scales affect not just microbial community diversity or composition, but also within-taxon genetic population diversity. These could offer additional ways to test for selection effects and would require more targeted sequencing techniques. Future studies in invertebrate-soil interactions may involve more direct sampling and sequencing of ant exoskeletons and neighboring soil regions, as has been done some with fungus-farming ants. Finally, introducing social and/or political variables directly into studies of soils in small-scale agriculture could offer ‘long-range’ tests of how open, socio-ecologically active soil systems respond to global and local perturbations together, for example, making use of layered multi-level network structures. Overall, this dissertation motivates the application of phenomena observable in complex ecological systems to other fields of study, and ideally daily life across working sectors.