Investigating the impacts of urbanization on soil ecology in Berlin, Germany

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Selbstständigkeitserklärung

I hereby declare that this dissertation was written and prepared by me independently. Furthermore, no sources and aids other than those indicated have been used. Intellectual property of other authors has been marked accordingly. I also declare that I have not applied for an examination procedure at any other institution and that I have not submitted the dissertation in this or any other form to any other faculty as a dissertation.

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Thesis Summary

Urban landscapes, whilst only accounting for a small proportion of total land surface, are now home to the majority of humanity. The functioning of soils within these systems is therefore highly influential for human wellbeing; these soils mediate flood risk, cycle nutrients, and determine whether healthy plant life can be supported. This plant life in turn brings not only aesthetic benefits to cities, but can also yield food and boost the mental wellbeing of inhabitants. These soils are also the basis of urban agriculture and provide aesthetically pleasing spaces for cultural events.

This thesis examines three different aspects of soil ecology across a series of urban and peri-urban grasslands, scattered across Berlin and its neighbouring federal state, Brandenburg. Due to their varied surroundings, these sites represent a gradient of urbanization. Throughout this thesis Principal Component Analysis is used to extract axes of variation from a substantial dataset of environmental parameters, in order to make these grasslands practically comparable. The most significant axis of variation within this environmental data is consistently shown to be a selection of urban-related parameters. Examples being the degree to which soil is sealed, the proximity of roads, and the local population density. We term the axis “urbanity” and use it as a metric to score how urban a grassland is.

The first aspect of these soils that we explore is their physico-chemical properties. This investigation, presented in Chapter One of this thesis, consisted of a selection of field and laboratory tests. In-situ, we used an infiltrometer to measure the infiltration rate of the soil, and ex-situ we used wet-sieving to establish the percentage of water stable aggregates within samples, and dry sieving to establish the particle sizes present. Finally, we used the molarity-of-ethanol-drop method to investigate levels of hydrophobicity present in soil samples. By using the aforementioned urbanity metric, we were able to understand how these properties were impacted by urbanization. We found that the most urban soils tended to have lower percentages of stable aggregates, larger particle sizes, and more rapid infiltration rates.

In Chapter Two of this thesis, we explore the fungal colonisation levels of plant roots, again using samples taken from our study sites. By staining short sections of root, and then examining them under a microscope, it was possible to establish colonisation rates of both arbuscular mycorrhizal fungi, and non-arbuscular filamentous fungi. We also examined the degree of root-hair presence on roots. Again, we compared these parameters to the urbanity score for each site to explore how urbanization impacted root colonisation and morphology. We found that roots in the most urban soils had increased levels of colonisation by non-arbuscular filamentous fungi and higher numbers of root hairs.

Finally, in Chapter Three, we use molecular techniques to explore how microbial communities changed across our study sites. We used high-throughput illumina sequencing to record the bacterial, fungal, and cercozoan communities, and then used advanced statistical techniques to investigate how the community composition and species richness of these groups shifted across our urban gradient. We also considered the distribution of some highly abundant species and identified potential urban-indicator species. We found that fungal richness was increased at the most urban sites, and that a variety of environmental variables, including urbanity, drove microbial community composition.

Zusammenfassung

Städtische Landschaften machen zwar nur einen kleinen Teil der gesamten Landfläche aus, beherbergen aber die Mehrheit der Menschheit. Das Funktionieren der Böden in diesen Systemen ist daher von großer Bedeutung für das menschliche Wohlergehen. Diese Böden beeinflussen das Hochwasserrisiko und den Nährstoffkreislauf und bestimmen, ob eine gesunde Pflanzenwelt gedeihen kann. Dieses

Pflanzenleben wiederum bringt nicht nur ästhetische Vorteile (für die Städte) mit sich, sondern kann auch Nahrungsmittel liefern und das psychische Wohlbefinden der Bewohner*innen fördern. Diese Böden sind auch die Grundlage der städtischen Landwirtschaft und bieten ästhetisch ansprechende Räume für kulturelle Veranstaltungen.

In dieser Arbeit werden drei verschiedene Aspekte der Bodenökologie auf einer Reihe von städtischen und stadtnahen Grünlandflächen untersucht, die über Berlin und das benachbarte Bundesland Brandenburg verstreut sind. Aufgrund ihrer unterschiedlichen Umgebung stellen diese Standorte einen Gradienten der Urbanisierung dar. In dieser Arbeit wird die Hauptkomponentenanalyse verwendet, um Variationsachsen aus einem umfangreichen Datensatz von Umweltparametern zu extrahieren, was den praktischen Vergleich dieser Grünlandflächen ermöglicht. Die signifikanteste Variationsachse innerhalb dieser Umweltdaten ist nachweislich eine Auswahl stadtbezogener Parameter. Beispiele hierfür sind der Grad der Bodenversiegelung, die Nähe von Straßen und die lokale Bevölkerungsdichte. Wir bezeichnen diese Achse als "Urbanität" und verwenden sie als Maßstab, um zu bewerten, wie urban ein Grünland ist.

Der erste Aspekt der Böden, die wir untersuchen, sind ihre physikalisch-chemischen Eigenschaften. Diese Untersuchung, die im ersten Kapitel der Arbeit vorgestellt wird, besteht aus einer Reihe von Feld- und Labortests. In-situ haben wir mit einem Infiltrometer die Infiltrationsrate des Bodens gemessen, ex-situ haben wir durch Nasssiebung den Prozentsatz der wasserstabilen Aggregate in den Proben ermittelt und durch Trockensiebung die vorhandenen Partikelgrößen bestimmt. Schließlich untersuchten wir mit der Molaritäts-Ethanol-Tropfen-Methode den Grad der Hydrophobie in den Bodenproben. Durch die Verwendung der oben erwähnten Urbanitätsmetrik konnten wir nachvollziehen, wie sich die Urbanisierung auf diese Eigenschaften auswirkt. Wir stellten fest, dass die meisten städtischen Böden tendenziell einen geringeren Anteil an stabilen Aggregaten, größere Partikelgrößen und schnellere Infiltrationsraten aufweisen.

Im zweiten Kapitel dieser Arbeit untersuchen wir anhand von Proben aus unseren Untersuchungsgebieten den Grad der Pilzbesiedlung von Pflanzenwurzeln. Durch das Färben kurzer Wurzelabschnitte und die anschließende Untersuchung unter dem Mikroskop konnten wir die Besiedlungsraten sowohl von arbuskulären Mykorrhizapilzen als auch von nicht-arbuskulären Pilzen ermitteln. Des Weiteren untersuchten wir Wurzelhaare. Auch hier verglichen wir diese Parameter mit dem Urbanitätswert für jeden Standort, um zu untersuchen, wie sich die Urbanisierung auf die Wurzelbesiedlung und die Morphologie auswirkt. Wir stellten fest, dass die Wurzeln in den am stärksten urbanen Böden stärker von nicht-arbuskulären Fadenpilzen besiedelt waren und eine höhere Anzahl von Wurzelhaaren aufwiesen.

In Kapitel drei untersuchten wir schließlich mithilfe molekularer Methoden, wie sich die mikrobiellen Lebensgemeinschaften an unseren Untersuchungsstandorten veränderten. Wir verwendeten Hochdurchsatz-Illumina-Sequenzierung, um die Bakterien-, Pilz- und Cercozoa-Gemeinschaften zu erfassen und untersuchten dann mit geeigneten statistischen Verfahren, wie sich die Zusammensetzung der Gemeinschaft und der Artenreichtum dieser Gruppen über unseren urbanen Gradienten hinweg veränderten. Wir untersuchten auch die Verteilung einiger sehr häufiger Arten und identifizierten potenzielle Stadtindikatorarten. Wir fanden heraus, dass der Pilzreichtum an den am stärksten urbanen Standorten erhöht war und dass eine Vielzahl von Umweltvariablen, einschließlich der Urbanität, die Zusammensetzung der mikrobiellen Gemeinschaft beeinflusste.

0.1 General introduction

The interaction between humankind and the rest of the natural world, whilst always present, can often feel attenuated, or even absent. Indeed, throughout much of recent history the capacity for humans to shape the natural world has been poorly understood. This has historically been particularly true in some western cultures, which have viewed the world as an inexhaustible Eden, provided for the sole benefit of humanity by a loving god, and therefore impossible to over-exploit (White, 1967). However, ever since the works of Huxley and Darwin amended the position of humankind from being dominant over nature, to being intrinsically linked to our animal relatives, there has been a gradual acceptance of humanity's role within the natural world. In recent decades, climate change has caused a further shift in perspective; to humans as ecosystem engineers with the capacity to destroy the Eden in which we find ourselves. Recent research suggests that for at least 12,000 years human activities have impacted most of the terrestrial biome (Ellis *et al.*, 2021); meaning that the Anthropocene was significantly underway by the time anyone noticed its existence. Alongside recent shifts in our understanding of the relationship between humans and the natural world, the field of ecology has also transformed; in a world such as this, any boundary between the human world and a natural world becomes blurred, if not entirely irrelevant. Of all the ecosystems shaped by humans, perhaps those which most define us, and are in turn most defined by us, are urban landscapes. These pinnacles of human endeavour have spawned the field of urban ecology; the study of how organisms interact in ecosystems often built for the sole benefit of their human inhabitants.

In this thesis we will examine one element of the urban landscape: the soil. This complex matrix of organic and inorganic matter provides a home for countless organisms and microorganisms, which cycle nutrients, and in doing so support the functioning of all terrestrial ecosystems. The microorganisms that live within urban soils face not only the stresses faced by all soil microorganisms (eg. predation, competition), but also a host of environmental stresses inflicted due to the activities of their above-ground human neighbours.

This thesis explores the impacts of urban stresses upon three different aspects of urban soils. First, we explore how the physico-chemical properties of soils change with increasing levels of urbanisation. Second, we explore how the colonisation of roots by symbiotic fungi is affected by these same urban stresses. Finally, we use molecular techniques to explore the ways in which fungal, bacterial and cercozoan communities change in richness and composition in response to urban stresses.

Before going into these subjects in detail, we shall first provide some context and background for the subject of urban soil ecology.

0.1.1 Urban ecosystems

In order to study urban ecosystems, it is first important to define what one is. This is difficult, as there is no single parameter that can be said to define a location as being urban. Additionally, many variables which could be said to be intrinsic elements of urban landscapes (eg. travel infrastructure, high population densities, and sealed soils) are unlikely to have, themselves, defined the survival strategy of organisms due to their relatively recent appearance in evolutionary history. Even for organisms with an exceptionally fast generational time and rate of evolution (eg. bacteria), linking individual and community traits to urban variables can be challenging due to a lack of clear ecological connection and high degrees of collinearity between environmental variables. For example; areas with high population densities are likely to also have large amounts of travel infrastructure. Generating testable hypotheses which are backed up by ecological understanding can therefore be challenging in urban systems.

Due to the unique selection of environmental pressures within urban ecosystems, and the relatively recent occurrence of these systems in evolutionary history, urban communities can contain Frankenstein-esque mixes of species which have not co-evolved (Andrade *et al.*, 2021). One key

question that urban ecology aims to answer is whether these new communities are globally similar. In other words; are the communities that can be described as urban-specific?

Urban ecosystems are hotspots for both environmental heterogeneity and collinearity. Whilst this statement may at first appear to be a contradiction, it is not. Urban systems are a heterogeneous patchwork of biotopes; grasslands, forest patches, ponds, expanses of tarmac, and buildings. However, concealed within this heterogeneity is collinearity; areas with more buildings tend to have higher population densities, resulting in larger densities of roads and more thoroughly managed parklands.

When studying urban systems, these elements of their character make them hard to statistically describe. One common approach to countering this problem is to circumvent finer-scale variation by using a paired-system approach. This technique entails selecting geographically close sites, of which one is urban and the other is not. Through replication, this technique has been used to provide evidence for shifts in communities between urban and rural sites at a global level (Delgado-Baquerizo *et al.* 2021). One benefit of this technique is that it reduces the likelihood of pseud-replication. In other words, by making large-scale studies, encompassing multiple urban landscapes from separate continents and latitudes, practically feasible, findings are more likely to be truly indicative of a purely urban-related phenomenon. Alas, a profound problem with this technique is a lack of precision; when sites within cities are directly compared with rural counterparts, it is highly likely that alongside the impacts relating directly to urban systems, a wealth of other variables will come into play. Unfortunately, poor site selection can occasionally result in fundamentally different biotopes being compared (eg. urban parkland vs. agricultural fields). When done well, these studies can answer fundamental questions about urban systems, however, due to their categorical approach the causation of observed patterns is hard to elucidate from their results.

Some urban studies also provide globally relevant data by focussing on biotopes that are thought to be globally comparable. A good example of this is graveyards, which have been the source of much urban ecological research in recent years (Loki *et al.*, 2019). This, naturally, comes with the limitation of only reflecting one, very specific, biotope, and is consequently more useful for conservation studies, than studies exploring ecological drivers.

One way of obtaining continuous data to explore a complex system is to use a proxy. Such a technique can commonly be seen in studies where no causal link between the predictor and response is required. An example of this in urban systems is the use of street-light density as a proxy for the urbanisation of a system (Hu *et al.*, 2020). As with all proxies, there will be cases where this is not an accurate measure of how urban a location is, which may result in biases (eg. highly urban, but poor, communities may be much less well-lit than less urban, but richer, communities). Additionally, in an ecological context, unless the proxy in question is likely to have a link with the research question, an understanding of causation is again lacking. For example; streetlight density could have a causal link to communities of urban glow-worms, but no such link can be easily made to urban plant seed dispersal.

In order to create an accurate depiction of the myriad variables in urban systems, the problem of collinearity once more appears. When multiple variables are measured, such as the aforementioned population density and road density, if one of these variables is a causal factor for a pattern (eg. road density results in heightened seed dispersal), it may be impossible to statistically prove this due to the other (i.e population density) also correlating with the parameter measured. Unfortunately, in field studies, due to the wide range of statistical noise within data, disentangling collinear variables is often impossible.

Whilst it may not be possible to discover the individual drivers responsible for ecological patterns using highly collinear datasets, it is possible to group variables together into ‘syndromes’. Using these syndromes of collinear variables alongside ecological knowledge, it is possible to understand, at least in broad terms, how aspects of the environment drive ecological patterns. One method for discovering such syndromes is Principal Component Analysis (PCA). This statistical technique, pioneered for use

in urban ecology by du Toit and Cilliers (2011), extracts the main axes of variation from within a dataset, providing scores for sites according to their position on each axis. In doing so, it creates a series of orthogonal data series which each explain a separate syndrome of environmental parameters. It is then a simple matter to discover which of these syndromes is a significant driver of the ecological pattern in question. It should be noted, however, that care should still be taken in selecting variables to include in PCA. For example; if a parameter is likely to be temporally variable, it should not be included in the PCA dataset for studies where data was collected at other time points.

0.1.2 Berlin as a case study for urban ecology

Berlin is a location of particular note within the history of urban ecology. One reason for this is that it was the location of the notable naturalist Herbert Sukopp, and his brainchild, the Berlin School of Urban Ecology (Kowarik, 2020). In recent years, Berlin has been the location of the CityScapeLabs research platform (von der Lippe *et al.*, 2020). This platform consists of fifty-six grassland sites spread across Berlin and its neighbouring federal state, Brandenburg. By limiting sites to representing only one biotope, the aforementioned issues relating to urban heterogeneity are mostly avoided. A huge array of data is available for these sites, including parameters reflecting their urban setting (eg. population density, soil sealing, road density), and others which are more universally relevant (eg. soil pH, organic carbon and nitrogen levels). Throughout the chapters in this thesis, we use PCA to extract usable environmental syndromes from this data, including one syndrome representing the "urbanity" of locations.

History and geopolitics have shaped Berlin over centuries, and have consequently impacted its ecology. Berlin can trace its history back to the 12th century when the land between the Elbe and Oder rivers was first inhabited by a cluster of interconnected villages. The city formed in 1709 through the merging of the settlements of Cölln and Berlin. As the capital of Prussia under Frederick the Great (1712-1786), Berlin transformed into a metropolis, seeing a rapid increase in its population. Berlin was selected as the capital of the newly formed nation of Germany in 1871, a wise choice, as it was by then Germany's most heavily industrialised city, and one of Europe's largest (Arandjelovic and Bogunovich, 2014). At the turn of the 20th century Berlin was one of the most technologically advanced cities in the world, however, the following century was to see Berlin face destruction and division on an unprecedented scale.

Berlin's role as the epicentre of two world wars shaped the city profoundly. During the first world war, food shortages caused by the blockade of Germany resulted in vast swathes of land, previously owned by the Catholic church, to be gifted to the state. These sites can still be seen scattered across Berlin in the form of large "garden colonies". Strict rules are still in place requiring certain proportions of these sites to be used for food production. Whilst the construction of small sheds and summer-houses is permitted, long-term residence is not. As such, these sites provide valuable access to green space for Berlin's human and non-human residents.

Following the abdication of Kaiser Wilhelm II at the end of the first world war, the establishment of the Weimar Republic resulted in the creation of the Berlin we see today; in 1920 the current boundaries for Berlin were established. The century since has seen little urban growth into Berlin's surrounding federal state, Brandenburg, and as such there is a steep gradient in urbanisation levels at the border of the city.

Germany witnessed extreme political turmoil during the economic crisis of the 1920s, resulting in the rise of fascism. Fortunately, the grandiose redesign project for Berlin to become the Third Reich's capital, "Germania", was never realised. In an interesting case of Berlin's soil shaping its architecture, one of the few traces of Germania that can still be seen in Berlin is the "heavy load-bearing object", a massive concrete cylinder constructed to test if Berlin's sandy soil would be capable of supporting the immense dome conceived as the centre point for the National-Socialist capital.

Germany's defeat in the second world war resulted in Berlin suffering the destruction of almost one third of its housing. The debris resulting from aerial bombing and exceptionally destructive fighting in the last days of the war created enough raw material to construct several hills, significantly increasing the levels of topographic variation that can be seen within the city.

Following the second world war Germany was divided between four of the victorious nations, resulting in American, British, French, and Soviet administered sectors. A split rapidly arose between the western-administered regions and that of the Soviets, resulting in the creation of two nations in 1949. Berlin, itself divided into sectors, was rendered in two. Only the Eastern half of Berlin maintained its status as a capital city, the western half losing this privilege to Bonn. Initially only administrative, this split became physical in 1961 with the construction of the Berlin wall. The creation of the Berlin wall cut a swathe through the city, which can still be seen in many places today. Following the fall of the Berlin wall in 1989, and the subsequent reunification of Germany, many areas formerly used as the death strip were redeveloped into parks and nature reserves. Reunification, and the collapse of the Soviet Union resulted in the abandonment of many buildings, for which it was often hard to determine ownership. Whilst some of these have since been demolished, others have been left derelict. This period of division has resulted in a range of habitats spread throughout Berlin, providing niches for a variety of organisms and contributing heavily to the 44% of Berlin surface area which is classified as green space (Kalandides and Grésillon, 2021). The "Berlin Urban Landscape Strategy" (Senatsverwaltung für Stadtentwicklung und Umwelt, 2016) aims to preserve this urban green space in the face of Berlin's current rapid growth in population.

Certain sites within Berlin can be used as examples of how the city's history has shaped its green spaces. One of these, represented within the CityScapeLab platform, is Tempelhofer Feld. Dating back as an open space until at least 1350, the militaristic Frederick the Great initiated its long-term use as a parade ground. In the 20th Century, Tempelhofer Feld became a centre for aviation; initially home to the balloon detachment of the Prussian army in 1884, the site saw a flight by Orville Wright in 1909 and was established as an airport in 1923. Even after this, the site was still used for rallies during the Nazi period, and subsequently passed through Soviet and American hands before being handed back to German administration in 1993. The airport closed in 2008 and reopened as a park in 2010 (Tempelhof Project GmbH, 2022). Even since then, the park has performed different roles, including hosting 2500 migrants during the migrant crisis of 2015-16. These changing functions of a site, with resultant alterations in site-management and consequent impacts on its ecology, demonstrate the difficulty entailed when attempting to categorise urban study-sites, especially in a city with a history as tumultuous and varied as that of Berlin.

0.1.3 Urban soils and their use

When describing soils, the term "Urban" is loosely defined. It is something of an umbrella term referring to soils which human activity has impacted. Interestingly, whilst this could also be said of agricultural soils, the human impacts in urban soils have more of a tendency to be accidental, and non-food production related. However, exceptions to both of these conditions occur: a huge amount of food is produced from urban farms and gardens each year, particularly in the far-east, where urban farming is common (Li *et al.*, 2018).

Urban soils can take many forms, and several definitions have appeared over the past few decades. The term "anthroposol" (a.k.a "anthrosol") has been used interchangeably with "urban soil", with more specific soil types falling within this umbrella. Meanwhile, the word "technosol" has also been used to refer to soils defined by human usage, including those sealed by human activity, such as soils existing under roads and buildings (Dutta *et al.*, 2022). Additionally, the term "paleotechnosol" has been used to refer to their ancient counterparts (Markiewicz *et al.*, 2013). Also discussed in the literature are soils which have been transported by humans, these tend to be lumped together with other anthropogenically-influenced soils to make "human altered and transported", or HAHT, soils (Glabraith *et al*., 2018). In a more specific category, "necrosols" are those soils found in graveyards (Sobocká, 2004).

Urban soils tend to share certain characteristics; they almost universally contain detritus such as waste from construction and everyday life, somewhat euphemistically referred to in the literature as "artefacts" (Dutta *et al.* 2022. They are also generally relatively alkaline, at least when compared to less anthropogenically-impacted soils of the same type, due to the deposition of alkaline building dust (O'Riordan *et al.*, 2021). Beyond this though, they often express highly variable characteristics, which we discuss in more detail in Chapter One.

Urban soils provide the same ecosystem services as non-urban soils, plus some extra. The services provided by soils have previously been categorised as: provisioning services, regulating services, and cultural services (Dominati *et al.*, 2010; O'Riordan *et al.*, 2021). Provisioning services not only relate to the products harvested from soils, such as food, wood and fibres, but in an urban context also relate to the physical use of soils for supporting buildings, and as a raw material for the construction of earthworks and canals. The regulating services of soils, in terms of flood mitigation and filtering nutrients, pollutants and mitigating the impacts of pests and diseases are also heavily impacted in an urban context. Soil sealing in urban landscapes has major implications for both local and down-stream flooding, although we present in Chapter One evidence that urban soils in close proximity to sealed areas may potentially buffer this. Interestingly, many of these regulating processes are linked; for example, the presence of "black carbon" (which is a common by-product of incomplete combustion) in urban soils can act as a filter, absorbing other chemical pollutants (O'Riordan *et al.*, 2021). As a result, filtered through urban soils can sometimes contain fewer impurities than water in less-urban soils. Additionally, the aforementioned alkalinity of urban soils can majorly reduce the availability of heavy metal contaminants; meaning that whilst there may be a higher total quantity of these in urban soils due to pollution, their availability can be lower than in non-urban soils. However, past a critical threshold of pollution urban soils can lose this ability to buffer, and can simply become a reservoir for toxic compounds and polluted run-off. This can have major ramifications for local residents who may directly consume these contaminants (e.g whilst playing in playgrounds) or be exposed to them via locally-grown food (Li *et al.*, 2018). The cultural services provided by urban soils are perhaps the visible way in which they differ from non-urban counterparts. These cultural services relate to the provision of space for sports, cultural events, and burials. They can also be less tangible services, such as providing a spiritual link between people and their home, and providing beautiful aesthetics, such as in verdant gardens and parks. These services are linked to human wellbeing, with access to urban greenspaces having been shown to be important for mental health (Chen *et al.*, 2019).

0.1.4 Microbial biogeography of urban soils

Many of the services provided by urban soils are determined by their biological inhabitants. In this thesis we use different techniques to explore the microbial communities within Berlin's soil, and consider what implications these have for soil functioning.

We will dedicate Chapter Two to the investigation to a group of organisms which have a particularly high degree of ecological impact; root endophytes. Arguably the most iconic and well researched fungal root endophytes, arbuscular mycorrhizal (AM) fungi, are found within >80% of all plant species (Wang and Qiu, 2006), and are credited with aiding their conquest of the land (Redecker *et al*., 2000; Corradi *et al.*, 2012). Whilst some evidence has already shown that AM plants can control levels of AM colonisation in response to environmental conditions (Grünfeld *et al.*, 2021), this is controversial and as-yet no evidence of this occurring in response to urban-related variables exists.

Whilst the vast majority of AM fungi are thought to be mutualists, other filamentous fungi also colonise plant roots. For these symbioses, the nature of their relationship with their host is substantially more opaque (Berthelot *et al.*, 2019). Indeed, the position of these relationships on the mutualism-to-parasitism spectrum depends on the species' involved, and can even change depending on the life stage of the plant (Schulz and Boyle, 2005). Whilst it is difficult to prove the relationship between a non-AM endophyte and a plant, a simple investigation of root colonisation rate can still be illuminating, as we demonstrate in Chapter Two. Previous research has demonstrated a greater tendency for non-AM fungal

root endophytes to respond to environmental parameters (Rillig *et al.*, 1998), although due to their complex relationships with plants, the exact reasons for this can be hard to ascertain. For example; challenging environmental conditions may result in increased colonisation rates due to the plant up-regulating symbioses, but could also be due to a reduced ability to suppress endophytic parasites.

Observations of physical structures is, however, only of limited use when exploring the wider microbial ecology of urban soils. Whilst other techniques such as spore samples have previously also been informative (Cousins *et al.*, 2003), the current state-of-the-art is to use molecular techniques, which we shall demonstrate in Chapter Three of this thesis. Molecular studies have explored global patterns of bacteria and fungal community composition, including observations made in urban landscapes (Delgado-Baquerizo *et al.*, 2021). Global homogenization of the structure and function in the. There have also been several studies investigating the ways in which microbial communities within cities are different from those in surrounding areas, with particularly notable examples coming from Finland (Hui *et al.*, 2017) and Estonia (Tedersoo *et al.*, 2020). These studies have generally used the paired-site approach previously discussed, allowing them to cover a large geographic region. Thus far, contradictory findings about microbial responses to urban stresses have emerged. Some studies suggest that urban ecosystems contain a homogenic set of species, with generalists generally triumphing (Delgado-Baquerizo *et al.*, 2021, Abrego *et al.*, 2020). But others have identified plants parasites and AM-fungi dominating in urban soils (Donald *et al*., 2021). Patterns may also change between kingdoms; some evidence suggests that fungal diversity decreases in urban soils (Reese *et al*., 2016), whereas bacterial and protistan diversity may increase (Delgado-Baquerizo *et al*., 2021; Donald *et al*., 2021).

0.1.5 Research aims

In this thesis, we aim to explore the ways in which an 'urban syndrome' of variables shapes soil ecology and functioning in Berlin's grasslands. We achieve this by observing the soil ecosystem at three different levels of resolution, presented here in three chapters.

Our first goal, presented here in Chapter One, was to understand the ways in which soil structure and functioning is impacted by the urban syndrome. This macroscopic investigation used a combination of field observations and laboratory tests to explore the changes in physico-chemical properties of soil across an urban gradient.

The next level of resolution was a microscopic investigation of root colonisation by fungal endophytes across the same urban gradient, with particular attention being paid to the presence of AM fungi.

Finally, we used molecular techniques to explore the diversity of Fungi, Bacteria, and a Protist phylum, the Cercozoa, from the sites used in the previous studies. Within the fungi, we again paid particular attention to AM fungi, by using a primer-pair which targeted the Glomeromycota. We investigated the Cercozoa, rather than Protists as a whole, due to the existence of a primer pair that targets this highly diverse group.

We will end this thesis by taking a holistic approach, using our results to explore where our findings can explain each other. We will also highlight apparent contradictions that should be addressed in future work.

0.1.6 Own contributions

This thesis consists of three papers, for all of which I was the lead author. For Chapter One, myself, Stefan Hempel and Matthias Rillig designed the research. I conducted the fieldwork and lab analyses, and wrote the first draft of the paper. Anne Hiller and Moritz von der Lippe provided field data. All authors added to and edited the text. For Chapter Two, myself, Stefan Hempel, and Matthias Rillig designed the research. I conducted fieldwork and lab analyses, and wrote the first draft of the paper. All

authors added to and edited the text. For Chapter Three myself, Stefan Hempel, and Matthias Rillig designed the research. I conducted lab work, bioinformatics, statistics, and wrote the first draft of the manuscript. Julien Roy created the bioinformatics pipeline and provided guidance on bioinformatics and statistics. Lena Feichter carried out field work. All authors added to and edited the text. All additional text in this thesis, in the general introduction, general discussion, and general conclusion is my own work.

0.1.7 References

Abrego, N., B. Crosier, P. Somervuo, N. Ivanova, A. Abrahamyan, A. Abdi, K. Hamalainen, K. Junninen, M. Maunula, J. Purhonen and O. Ovaskainen (2020). "Fungal communities decline with urbanization-more in air than in soil." Isme Journal 14(11): 2806-2815.

Andrade, R., J. Franklin, K. L. Larson, C. M. Swan, S. B. Lerman, H. L. Bateman, P. S. Warren and A. York (2021). "Predicting the assembly of novel communities in urban ecosystems." Landscape Ecology 36(1): 1-15.

Arandjelovic, B. and D. Bogunovich (2014). "City profile: Berlin." Cities 37: 1-26.

Berthelot, C., M. Chalot, C. Leyval and D. Blaudez (2019). From Darkness to Light: Emergence of the Mysterious Dark Septate Endophytes in Plant Growth Promotion and Stress Alleviation. Endophytes for a Growing World.

B. R. Murphy, F. M. Doohan, M. J. Saunders and T. R. Hodkinson. Cambridge, Cambridge University Press: 143-164.

Berthelot, C., Chalot, M., Leyval, C., and Blaudez, D. (2019). “From Darkness to Light: Emergence of the Mysterious Dark Septate Endophytes in Plant Growth Promotion and Stress Alleviation,” in Endophytes for a Growing World. Editors B. R. Murphy, F. M. Doohan, M. J. Saunders, and T. R. Hodkinson (Cambridge: Cambridge University Press), 143–164. doi:10.1017/9781108607667.008

Chen, X. W., S. de Vries, T. Assmuth, J. Dick, T. Hermans, O. Hertel, A. Jensen, L. Jones, S. Kabisch, T. Lanki, I. Lehmann, L. Maskell, L. Norton and S. Reis (2019). "Research challenges for cultural ecosystem services and public health in (peri-)urban environments." Science of the Total Environment 651: 2118-2129.

Corradi N, Bonfante P. The arbuscular mycorrhizal symbiosis: origin and evolution of a beneficial plant infection. PLoS Pathog. 2012;8(4):e1002600. doi: 10.1371/journal.ppat.1002600. Epub 2012 Apr 19. PMID: 22532798; PMCID: PMC3330121.

Cousins, J. R., D. Hope, C. Gries and J. C. Stutz (2003). "Preliminary assessment of arbuscular mycorrhizal fungal diversity and community structure in an urban ecosystem." Mycorrhiza 13(6): 319-326.

Delgado-Baquerizo, M., D. J. Eldridge, Y. R. Liu, B. Sokoya, J. T. Wang, H. W. Hu, J. Z. He, F. Bastida, J. L. Moreno, A. R. Bamigboye, J. L. Blanco-Pastor, C. Cano-Diaz, J. G. Illan, T. P. Makhalanyane, C. Siebe, P. Trivedi, E. Zaady, J. P. Verma, L. Wang, J. Y. Wang, T. Grebenc, G. F. Penaloza-Bojaca, T. U. Nahberger, A. L. Teixido, X. Q. Zhou, M. Berdugo, J. Duran, A. Rodriguez, X. B. Zhou, F. Alfaro, S. Abades, C. Plaza, A. Rey, B. K. Singh, L. Tedersoo and N. Fierer (2021). "Global homogenization of the structure and function in the soil microbiome of urban greenspaces." Science Advances 7(28): 14.

du Toit, M. J. and S. S. Cilliers (2011). "Aspects influencing the selection of representative urbanization measures to quantify urban-rural gradients." Landscape Ecology 26(2): 169-181.

Dominati, E., Patterson, M., Mackay, A., 2010. A framework for classifying and quantifying the natural capital and ecosystem services of soils. Ecol. Econ. 69 (9), 1858–1868.

Donald, J., Murienne, J., Chave, J., Iribar, A., Louisanna, E., Manzi, S., et al. (2021). Multi-taxa environmental DNA inventories reveal distinct taxonomic and functional diversity in urban tropical forest fragments. Glob. Ecol. Conserv. 29:e01724. doi: 10.1016/j.gecco.2021.e01724

Dutta, A., Patra, A., Ghosh, S., Rakshit, A. (2022). Urban Soil: A Review on Historical Perspective. In: Rakshit, A., Ghosh, S., Vasenev, V., Pathak, H., Rajput, V.D. (eds) Soils in Urban Ecosystem. Springer, Singapore. https://doi.org/10.1007/978-981-16-8914-7_1

Ellis, E. C., N. Gauthier, K. Klein Goldewijk, R. Bliege Bird, N. Boivin, S. Díaz, D. Q. Fuller, J. L. Gill, J. O. Kaplan, N. Kingston, H. Locke, C. N. H. McMichael, D. Ranco, T. C. Rick, M. R. Shaw, L. Stephens, J.-C. Svenning and J. E. M. Watson (2021). "People have shaped most of terrestrial nature for at least 12,000 years." Proceedings of the National Academy of Sciences 118(17): e2023483118.

Galbraith, J.M (2018) Human-altered and human-transported (HAHT) soils in the U.S. soil classification system, Soil Science and Plant Nutrition, 64:2, 190-199, DOI: 10.1080/00380768.2018.1442682

Grünfeld, L., Mola, M., Wulf, M., Hempel, S., and Veresoglou, S. D. (2021). Disentangling the relative importance of spatio-temporal parameters and host specificity in shaping arbuscular mycorrhizal fungus communities in a temperate forest. Mycorrhiza 31, 589–598. doi: 10.1007/s00572-021-01041-6

Hu, X. F., Y. G. Qian, S. T. A. Pickett and W. Q. Zhou (2020). "Urban mapping needs up-to-date approaches to provide diverse perspectives of current urbanization: A novel attempt to map urban areas with nighttime light data." Landscape and Urban Planning 195: 16.

Hui, N., Jumpponen, A., Francini, G., Kotze, D. J., Liu, X. X., Romantschuk, M., et al. (2017). Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. Environ. Microbiol. 19, 1281–1295. Doi: 10.1111/1462-2920.13660

Kalandides, A. and B. Grésillon (2021). "The Ambiguities of “Sustainable” Berlin." Sustainability 13(4): 1666.

Kowarik, I. (2020). "Herbert Sukopp - an inspiring pioneer in the field of urban ecology." Urban Ecosystems 23(3): 445-455.

Li, G., G. X. Sun, Y. Ren, X. S. Luo and Y. G. Zhu (2018). "Urban soil and human health: a review." European Journal of Soil Science 69(1): 196-215.

Loki, V., B. Deak, A. B. Lukacs and V. A. Molnar (2019). "Biodiversity potential of burial places - a review on the flora and fauna of cemeteries and churchyards." Global Ecology and Conservation 18: 14.

O'Riordan, R., J. Davies, C. Stevens, J. N. Quinton and C. Boyko (2021). "The ecosystem services of urban soils: A review." Geoderma 395.

Markiewicz M, Bednarek R, Jankowski M, Świtoniak M (2013) Paleotechnosols’ of ancient

settlements in Grodno and Kałdus. Technogenic soils of Poland. Polish Society of Soil Science,

Toruń, pp 111–122

Rebele, F. (1994). "URBAN ECOLOGY AND SPECIAL FEATURES OF URBAN ECOSYSTEMS." Global Ecology and Biogeography 4(6): 173-187.

Redecker D, Kodner R, Graham LE. Glomalean fungi from the Ordovician. Science. 2000;289:1920–1921.

Reese, A. T., Savage, A., Youngsteadt, E., McGuire, K. L., Koling, A., Watkins, O., et al. (2016). Urban stress is associated with variation in microbial species composition-but not richness-in Manhattan. ISME J. 10, 751–760. doi: 10.1038/ismej.2015.152

Rillig, M. C., Allen, M. F., Klironomos, J. N., Chiariello, N. R., and Field, C. B. (1998). Plant Species-specific Changes in Root-Inhabiting Fungi in a California Annual Grassland: Responses to Elevated CO 2 and Nutrients. Oecologia 113 (2), 252–259. doi:10.1007/s004420050376

Senatsverwaltung für Stadtentwicklung und Umwelt (Ed.) Strategie Stadtlandschaft Berlin. Natürlich, Urban, Produktiv; Senatsverwaltung für Stadtentwicklung und Umwelt: Berlin, Germany, 2016.

Schulz, B. and C. Boyle (2005). "The endophytic continuum." Mycological Research 109: 661-686.

United Nations, D. o. E. a. S. A., Population Division (2018) "World Urbanization Prospects: The 2018 Revision."

Sobocká J (2004) Necrosol as a new anthropogenic soil type. Soil Anthropization VII, pp 107–112

Tedersoo, L., Anslan, S., Bahram, M., Drenkhan, R., Pritsch, K., Buegger, F., et al. (2020). Regional-scale in-depth analysis of soil fungal diversity reveals strong pH and plant species effects in Northern Europe. Front. Microbiol. 11:1953. doi:10.3389/fmicb.2020.01953

Tempelhof Project GmbH (2022) History in numbers. https://www.thf-berlin.de/standortinfos/standortgeschichte/chronik/ accessed: 15.08.2022

von der Lippe, M., S. Buchholz, A. Hiller, B. Seitz and I. Kowarik (2020). "CityScapeLab Berlin: A Research Platform for Untangling

Urbanization Effects on Biodiversity." Sustainability 12(6): 30.

Wang, B., and Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza, 16, 299–363. https://doi.org/10.1007/s00572-005-0033-6

White, Lynn Townsend, Jr. "The Historical Roots of our Ecological Crisis." Science 155 (10 March 1967): 1203-1207

Chapter One

Soil Physico-Chemical Properties Change Across an Urbanity Gradient in Berlin

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1.1 Abstract

In this study the impacts of urbanity on physical soil properties were explored by measuring water stable aggregates, combined particle size, infiltration rate and hydrophobicity across an urban gradient. The use of a gradient allowed for the relative importance of different environmental drivers to be assessed. We sampled 54 sites across Berlin and used a pre-existing database of environmental variables to extract three main axes of variation relating to urbanity, soil nutrient content, and heavy metal content. These axes, along with site age, were used to explore the drivers of changes in physical properties across an urban landscape. The percentage of water stable aggregates was found to decrease with urbanity, whilst infiltration rate was found to increase. Hydrophobicity did not appear to be influenced by urbanity but interacted with both infiltration rate and water stable aggregates. Combined particle sizes in the soil were found to increase with urbanity. Our findings provide evidence for urbanity being an important driver of variation in physico-chemical soil properties, which has implications for the provision of ecosystem services by these soils.

1.2 Introduction

Since the middle of the 20th Century, the global urban population has been rapidly increasing, from 751 million individuals in 1950 to an estimated 4.2 billion in 2018, which accounts for roughly 55% of the total global population (United Nations, 2018). It has been estimated that whilst less than 0.5% of total global land surface is covered in built-up urban developments, this small percentage represented a total of 0.5 million km2 at the turn of the last century (Goldewijk *et al.*, 2010), although estimates vary (Potere and Schneider, 2007). These urban ecosystems not only provide services to their inhabitants but are also often hotspots for global change factors such as increased temperature (Arnfield, 2003), salinisation (Equiza *et al.*, 2017), and the presence of pollutants such as heavy metals (Plyaskina and Ladonin, 2009). Hence, urban soils can provide valuable information when it comes to understanding the impacts of global change factors on soil ecosystems and their functioning, which might impact human health (Brevik and Burgess, 2014). In these systems, anthropogenic interference is commonplace; this can include the management of soils (e.g. mowing and irrigation in urban parks), transportation of soils (e.g. due to construction; Hooke, 2000), soil sealing, and the addition of waste and construction material, such as building sand (Bridges, 1991). Urban landscapes have previously been associated with generally high levels of compaction (Lehmann and Stahr, 2007), although this may only be true in localised areas of high intensity usage (Edmondson *et al.*, 2011). Geochemical cycling, hydrosystems and biodiversity have been demonstrated to change with rising urbanity (Grimm *et al.*, 2008), however, many of the underlying soil characteristics which determine ecosystem processes have not been studied. For example, whilst aggregate stability is known to be a vital component of soil structure and functioning (Bronick and Lal, 2005), its response to urbanity has not previously been investigated and it is not known whether responses are universally similar (as could be expected according to the "urban ecosystem convergence hypothesis" proposed by Pouyat *et al.*, 2003).

Urban soils provide a range of important ecosystem services themselves, such as hydrological control through infiltration, and as the substrate for plant growth (Morel *et al.*, 2015). Via this impact on local flora, they not only support food production but also the parks and green spaces which have been demonstrated to support the wellbeing of urban residents (Tzoulas *et al.*, 2007; Diaz *et al.*, 2018). Inputs of fertilizers, elevated levels of N deposition and altered irrigation, as well as the removal of organic matter, cause altered nutrient cycling compared to natural ecosystems (Lorenz and Lal, 2009), with the new equilibrium varying across climatic regions, parent materials and socioeconomic areas (Pickett *et al.*, 2001). The functioning of soil is closely related to its structure, which determines the availability of air, water, nutrients and pollutants to the microbial life which inhabits it. This relationship is of course not one sided, with soil microbes playing an important role in shaping soils through both their chemical and (in the case of filamentous fungi) physical properties. In this study, four soil physico-chemical parameters were measured across an urban gradient in Berlin. These properties were selected for their importance for soil functioning and their likelihood of showing a response to urbanity, they are: the

percentage of water stable aggregates (WSA%), combined particle size (in this case referring to the soil fractions separated by simple dry sieving, comprising separate measures of mean weight diameter, coefficient of curvature, and uniformity coefficient), water infiltration rate and hydrophobicity.

The key building blocks of soils are aggregates, the stability and structure of which determine many of the ecosystem services provided by soil (Six *et al.*, 2004; Baer and Birge, 2018). Aggregates provide a variety of niches for microbial life due to the oxygen gradients within them (Wilpiszeski *et al.*, 2019; Cui *et al.*, 2020) and impact soil hydrology through influencing infiltration and water holding capacity (Baer and Birge, 2018). Aggregates also provide surfaces for the adsorption of nutrients (e.g. Thao *et al.*, 2008) and heavy metals (Huang *et al.*,2020), thus determining their availability. The ability of aggregates to maintain their structural integrity in the face of drying, wetting (Caron *et al.*, 1996), freezing, thawing (Layton *et al.*, 1993), and rain drop impact (Ramos and Nacci, 2003) is a key factor in the prevention of slaking and maintaining soil porosity. The increased levels of disturbance associated with urban systems have also been hypothesised to disrupt aggregate formation, with one notable experimental study by Chen *et al.* (2014). Observational studies by Jim (1998a, 1998b) reported a wide variety of levels of aggregation in urban soils in Hong Kong, however, in the absence of a non-urban comparison or an urbanity gradient, the impact of urbanity remains unclear. In general, observational studies of urban aggregate presence are lacking. Here, we used mean weight diameter and WSA% to test if soil aggregation properties change in relation to urbanity.

Infiltration is a macroscopic event, but it is largely determined by microscopic factors. Soil pores and crevices provide avenues for water infiltration, whilst hydrophobic compounds coating surfaces prevent flow. The infiltration of water carries both nutrients and pollutants through the soil matrix; the impacts of infiltration rate are therefore manifold, ranging from flood management to influencing plant growth (Assouline, 2013). If the rate of infiltration is low, water supply can easily exceed it, resulting in ponding on the surface and consequently run-off or even flooding (Morbidelli *et al.*, 2018). These macro-scale services of infiltration rate have caused urban infiltration studies to generally focus on a large geographic scale where anthropogenic soil sealing (i.e. paving) is the determining factor of infiltration rate (e.g. Haase and Nuissl, 2007; Perry and Nawaz, 2008), however, it is not clear whether non-sealed areas within the urban landscape also express altered infiltration rates. These areas are likely to receive run-off from sealed areas, the implications of which are uncertain. Hypothetically these runoff events could lead to the destruction of aggregates and the loss of finer soil fractions, resulting in an increased infiltration rate. If this were to be the case, un-sealed soils may buffer the loss of infiltration potential in proximal sealed areas. Some studies suggest that urban soils express low infiltration rates due to soil compaction (Yang and Zhang, 2011), with construction and its associated heavy machinery having a particularly pronounced impact (Gregory *et al.*, 2006). However, increased compaction in urban systems is unlikely to be universal (Edmondson *et al.*, 2011) and a wide variety of other factors are thought to play a role (e.g. crust formation according to Assouline, 2004), therefore, it is difficult to predict the impacts of urbanity upon infiltration.

Soil hydrophobicity, which causes soil water repellency, is an important factor determining the infiltration rate of water into soil, thereby controlling whether precipitation can contribute to the groundwater recharge or is repelled as surface run-off. Having been largely ignored until the 1960s, this factor has gone from being considered an obscurity to being considered a ubiquitous factor in many ecosystems around the globe (DeBano, 2000; Mao *et al.*, 2019). Despite this, it is only in the past few years that the practicalities of exploring the underlying causes of hydrophobicity have been surmounted. The consensus is now that long-chain carbon molecules coat the outer layers of soil particles, creating a thin hydrophobic layer (Doerr *et al.*, 2000; Mao *et al.*, 2019). Consequently, many studies report sandy, large-particle soils as expressing the greatest levels of hydrophobicity (Savage *et al.*, 1969; McGhie and Posner, 1981; York and Canaway, 2000). However, this is not universal; in some cases, clay-rich soils also present high levels of hydrophobicity. In these situations, hydrophilic particles aggregate together, surrounded by hydrophobic particles, thus producing one large aggregate with a low surface area and a water-repellent surface (Bisdom *et al.*, 1993; Doerr *et al.*, 2000). Other research has pointed to hydrophobicity occurring in even the finest fractions of soils (de Jong *et al.*, 1999), suggesting

that fine particles of organic matter may themselves exhibit hydrophobicity. The source of hydrophobic compounds in soils appears to vary significantly, some of those identified include fires, plant roots, leaf detritus, soil microbes, and untreated wastewater (Mao *et al.*, 2019). The impacts of soil hydrophobicity are also varied, with some reporting an increase in erosion due to increased water run-off (Miyata *et al.*, 2007) and others suggesting that hydrophobicity increases aggregate resilience (Giovannini and Lucchesi, 1983; Korenkova and Matus, 2015). Hydrophobicity is known to be temporary, normally appearing after drought and lasting until a minimum wetting level has been reached (DeBano, 1971; Doerr *et al.*, 2000; Dekker *et al.*, 2001). The spatial layout of hydrophobicity also often varies in soils, with a hydrophobic surface layer concealing a hydrophilic layer beneath (Doerr *et al.*, 2000; Mao *et al.*, 2019). Hydrophobicity is a key component determining the infiltration of water and may well be influenced by urbanity; however, the total infiltration rate is also influenced by a variety of other factors, including soil structure and climatic conditions. As such, separate measures of hydrophobicity and infiltration rate were carried out in this study.

Urbanity is not a binary factor; urban systems are normally heterogenous landscapes expressing a wide variety of biotopes impacted by a variety of urban related and non-urban related variables. Urbanity itself is a qualitative measure, for which there is no universal method of quantification (Moll *et al.*, 2019). By limiting this study to grasslands and comparing their key physical soil properties to the wide array of data already gathered on the study sites, an insight into the ways in which urbanity influences Berlin's soil can be gained. A selection of these variables can also be identified which are associated with urbanity, and thus used to create a post-hoc scale of urbanity amongst sites. Thereby, the impact of urbanity on important components of soil functioning can be understood in more detail.

1.3 Methods

1.3.1 Study Site

Berlin, Germany's capital and largest city has triggered a longlasting history of urban ecological research (Kowarik, 2020). Based on this tradition, the establishment of the CityScapeLab experimental research platform (von der Lippe *et al.*, 2020) allows for the investigation of urban effects on biodiversity and ecological functioning independently from the vast heterogeneity of urban habitats. The grassland branch of the CityScapeLab consists of 56 plots (16 m2 each) situated in patches of dry grasslands across Berlin and its surrounding federal state, Brandenburg. These sites represent a spectrum of urbanity, ranging from grasslands in forested or agricultural contexts, to historical parks, to novel sites with artificial or heavily modified soils, such as road verges and vacant land (e.g. abandoned rail yards and airfields). A database of environmental parameters for these study sites was established in the summer of 2017 and consists of soil chemical data as well as habitat connectivity, site age, plant cover, and a variety of measures of urban factors, including population density, soil sealing, road density and floor area ratio (see database information below). For this study, two sites were discounted due to missing data, leaving a total of 54 sites (Figure 1.1).

The 54 study sites encompassed dry grasslands which ranged in pH from 4.1 to 7.5. Moreover, the sites represented a range of urban impact from locations where the population density was as high as 97 people per hectare within a 100 m buffer and sites where 70% of the soil within a 100 m buffer was sealed, to rural sites where both these values were 0 (see database information below). The climate in Berlin is temperate with an average precipitation of 576 mm per year and an average annual temperature of 9.9°C, although recent years have witnessed hotter, drier periods (von der Lippe *et al.*, 2020). All of Berlin's soil has a high sand content, with textures limited to sand, medium loamy sand and medium silty sand (Gerstenberg, 2017). During the latter half of the 19th century Berlin expanded rapidly due to industrialisation and its selection as the new capital of Germany. In the 20th century, Berlin experienced the destruction of one third of its housing during the second world war, followed by the creation of the Berlin wall in 1961, which cut a swathe through the city. The destruction of the Berlin wall and German reunification in 1989 and 1990 led to the creation of many brownfield sites (meaning;

sites previously built on and/or possibly polluted) and parks, as well as the abandonment of many buildings (Arandjelovic and Bogunovich, 2014). Many of the sites in this study will have been directly impacted by this tumultuous history and have either been left as brownfield sites or been turned into urban parks (e.g. Park am Nordbahnhof, a former location of the Berlin Wall).

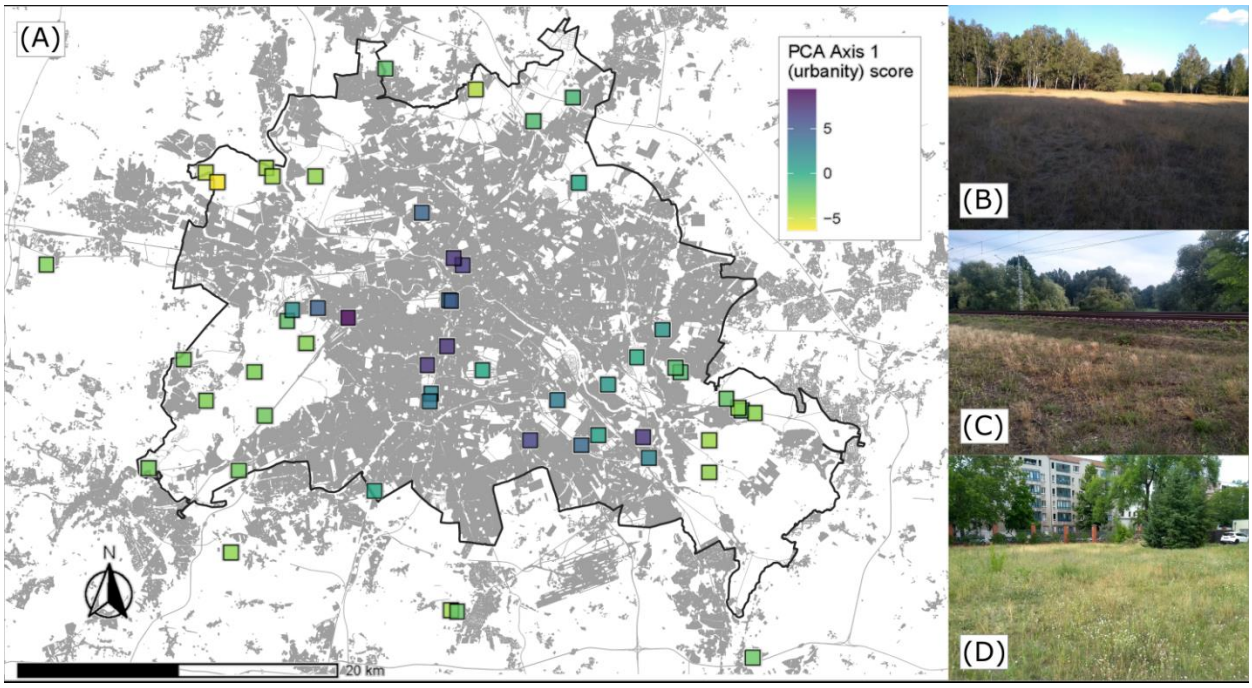

Figure 1.1 (A) A map of Berlin and the surrounding state, Brandenburg, showing the locations of the 54 CityScapeLab sites used in this investigation and their PCA axis 1 scores (see results), representing a gradient of urbanity. Grey areas denote built-on and traffic areas. Photographs are examples of grassland patches at (B) low (PCA axis 1 score: −4.71) (C) intermediate (PCA axis 1 score: 0.08) and (D) high (PCA axis 1 score: 7.4) urbanity levels.

1.3.2 In-Situ Measurement of Infiltration

Infiltration was measured in-situ using a Mini-Disk Infiltrometer (METER Group, Inc., Pullman, WA, United States). Three distinct flat areas were located within each field site and surface detritus was removed. Sampling locales were evenly spread across the study site and were selected for their ability to represent visible within-plot variation. At each of the three flat areas the infiltration rate was measured for 5 min with the Mini- Disk Infiltrometer set to a suction rate of 2 cm. Infiltration rates were then calculated using a tool provided by the Meter Group for use with the infiltrometer. The mean infiltration value was then calculated for each site, as well as standard error, which was used as a measure of within-site variation. This method was selected due to the ease with which field measurement can be taken with the Mini-Disk Infiltrometer, requiring only small amounts of water (<100 ml) and time for each replicate (Bát'ková *et al.*, 2020). All infiltration measurements and sampling were performed between June and August 2020.

1.3.3 Ex-Situ Measurements

From within each of the 4 m × 4 m CityScapeLab plots three replicates of 30–40 g of topsoil were taken and placed in falcon tubes. The sampling locales within the plot were decided upon due to their ability to represent within-plot variation. The impacts of urbanity were expected to be concentrated at the soil surface; therefore, samples were taken from the top 2–3 cm of soil using a flat-bladed wide-headed spatula. Care was taken to not compress samples during extraction. Samples were air dried in the opened falcon tubes before being stored at 4°C until use.

To achieve a measure of the percentage of water stable aggregates (WSA%), 4.0 g of soil was wet sieved using a wet sieving apparatus (Eijkelkamp, Netherlands) fitted with 0.25 mm sieves and the WSA% was calculated in accordance with Kemper and Rosenau (1986). There were three technical replicates per site, from which an average WSA% was calculated.

Combined particle size was measured through dry sieving: 8.0 g of soil were passed through a 4 mm sieve to remove excess debris before being passed through 2 mm, 1 mm, 500 µm, 100 and 50 µm sieves. The fractions held by each sieve were used to calculate the mean weight diameter (MWD), coefficient of curvature and uniformity coefficient (Samtani, 2006) for each replicate. A mean value for each measurement was then calculated for each site. MWD is often used in addition to wet sieving to measure the size of soil aggregates (van Bavel, 1950), however, here it was used to measure the size of all soil particles including sand. The coefficient of curvature is a measure of how well a soil is graded (i.e. contains a continuous range of particle sizes) and the uniformity coefficient is a measure of how similar in size the particles in the soil are. Together, these parameters can indicate if additional material has been imported to the site (e.g. larger diameter gravel for construction).

Hydrophobicity was measured using the Molarity of Ethanol Drop (MED) test, based on the protocol of Doerr (1998). Soil samples were placed into a pipetting reservoir with sloped sides to maximise available surface area for testing. Organic detritus and solid substrate particles larger than ⌀4 mm were removed and the surface was gently smoothed. ~⌀5 mm droplets of ethanol in concentrations of 0, 4, 8, 12, 16, 20, 24, 28, and 32 percent were pipetted onto the soil surface and the lowest concentration whereby the drop infiltrated within 3s was recorded. This was repeated for three technical replicates per site, from which an average ethanol concentration was calculated.

Due to all soils being dried before analysis, the levels of hydrophobicity measured may not exactly represent the levels found in-situ. However, given that sampling took place over a period of weeks pre-drying was appropriate. Hydrophobicity is generally most pronounced in surface soils, with localised areas of hydrophilic soil beneath them creating “finger flow” during wetting events, it was therefore decided to focus only on surface soil in order to sample comparable soils from each site.

1.3.4 Database of Other Variables

A database of variables (von der Lippe *et al.*, 2021) measured mostly in 2017 was used to provide information about the study sites. This database comprised of soil chemical measurements such as pH, concentrations of heavy metals (Zn, Cd, Pb, Ni, Cu), soil nutrients (N, P, K, S, organic C), water content, cation exchange capacity, electrical conductivity, as well as variables such as climate and weather measures, site connectivity, density of roads and railways, distance to roads and railways, soil sealing, population density, floor area ratio (FAR; a measure of building density) and an index of urbanity, combining FAR, soil sealing and population density. The sites were also categorised as either “old” or “new” depending on whether they existed as a grassland prior to 1940. For further information on all these parameters, please see: von der Lippe *et al.* (2020). Additionally, the distance from each site to the city centre point (Flächenschwerpunkt stone, Berlin) was calculated and included. We decided that the inclusion of the soil chemical data, which could be considered to some extent transitory, was appropriate for this study. The reason for this decision was that the main causes of change for these values would be land-use or climate change. As land use could be expected to remain relatively constant in the intervening years between sampling and change in climate should have impacted all sites relatively evenly, it was decided that the benefits of including these data outweighed any possible limitations.

1.3.5 Statistical Analyses

Due to many of the database variables being highly collinear, a PCA of variables was carried out using the ade4 package (Thioulouse *et al.*, 2018) to resolve the main axes of variation (as discussed in: du

Toit and Cilliers, 2011; McDonnell *et al.*, 2012; Moll *et al.*, 2019). Axes to keep for further inclusion in analyses were selected qualitatively, depending on whether they summarised a discernible syndrome that could be expected to influence soil properties. Kendall correlations (using R package Corrr; Kuhn *et al.*, 2020) were used to correlate the physical soil properties measured with the axis scores of the PCA. Wilcoxon rank sum tests were used to test for differences in the physical properties between age groups of sites. Physical properties were correlated with each other using Kendall correlations. Non-parametric tests were used due to infiltration rate, standard error of infiltration rate, hydrophobicity and PCA axis 1 scores being nonnormally distributed. Due to the sample size, correlations were more appropriate than nonparametric alternatives to regression models. All statistics were carried out in R (R Core Team, 2020). All plots were created using the ggplot2 package (Wickham, 2016), and aggregated using the ggpubr package (Kassambara, 2020). Figure 1.1 was created using the sf (Pebesma, 2018) and ggspatial (Dunnington, 2021) packages, with colours provided by the viridis package (Garnier *et al.*, 2021).

1.4 Results

Raw soil physico-chemical data and PCA axis scores are available via the Figshare online repository (Whitehead *et al.*, 2021).

1.4.1 PCA of Variables

A summary of all PCA variable loadings can be found in Supplementary Table S.1.1.

The first three PCA axes of variation were selected for the further analysis in this study due to the relatively clear syndromes they represented: Axis 1 comprised of mainly urban-related variables, relating to roads, railways, soil sealing, population, and distance from the city centre, and explained 24.3% of the variation seen in the data. Axis 2 explained 9.4% of the variation in the data and comprised of the soil nutrients N, S and organic C, as well as water content. Axis 3 explained only 6.9% of the variation in the data and comprised of heavy metals, particularly Ni and Cd, and site connectivity. pH was found to be a contributor to axis 3, but to a lesser extent than the heavy metals (Figure 1.2 and Supplementary Figure S.1.1). Further PCA axes were excluded due to their failure to represent interpretable environmental syndromes.

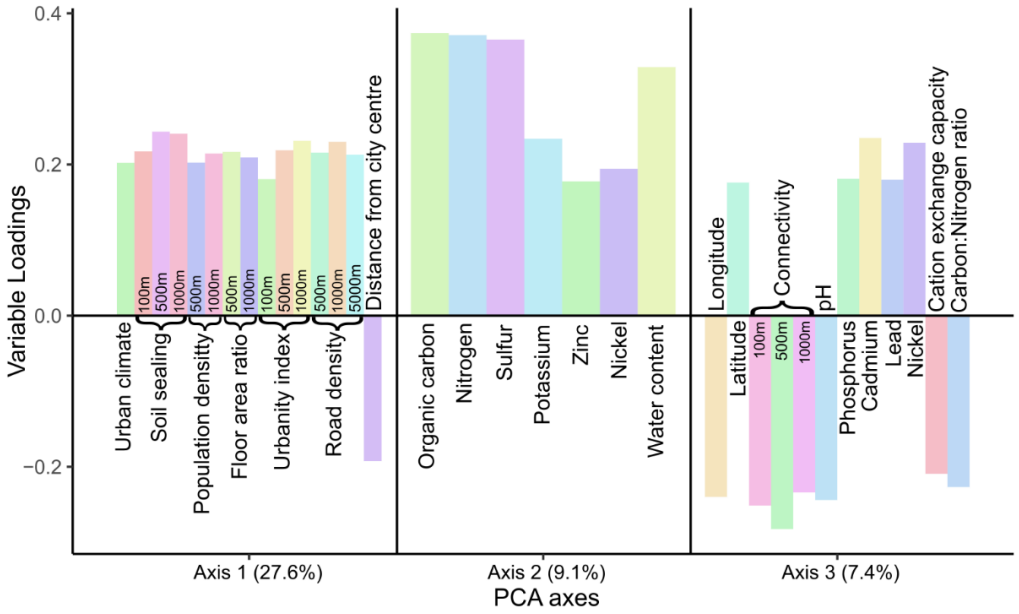

Figure 1.2 Significant variables in each of the three PCA axes; only variables with a loading of >0.175 or < −0.175 are plotted. The total amount of variation in the dataset that the axis accounts for is stated alongside the axis name. For variables which were calculated within multiple different distance buffers surrounding the study sites, the size of the distance buffer is presented on the bar. For biplots of axes see Supplementary Figure S.1.1.

It was found that axis 1 scores were significantly higher for new sites than old sites (Wilcoxon rank sum W = 623, $p < 0.001$), whilst axes 2 and 3 did not appear to be related to site age (Supplementary Figure S.1.2).

1.4.2 Effects of Environmental Axes on Soil Physico-chemical Properties

A summary of results for all response variables is presented in Supplementary Table S.1.2.

WSA% showed a negative correlation with axis 1 (Kendall's Tau = −0.20, p = 0.03) and a positive trend with axis 2 (Kendall's Tau = −0.17, p = 0.07) (Figure 1.3A). Old sites had a higher WSA% than new sites (Wilcoxon rank sum W value = 234, p = 0.04) (Figure 1.3B).

Mean weight diameter showed a positive correlation with axis 1 (Kendall's Tau = 0.23, p = 0.01) and a negative trend with axis 3 (Kendall's Tau = −0.17, p = 0.07) (Figure 1.3C). There was also a significant difference in MWD between old and new sites, with newer sites having a higher MWD (Wilcoxon rank sum W = 497, p = 0.01) (Figure 1.3D). Both the coefficient of uniformity and coefficient of curvature did not relate to any of the PCA axes.

Infiltration rate had a positive correlation with axis 1 (Kendall's Tau = 0.25, p = 0.009) and a negative correlation with axis 2 (Kendall's Tau = −0.20, p = 0.03) (Figure 1.3E). The standard error of infiltration rate also showed a negative correlation with axis 2 (Kendall's Tau = −0.20, p = 0.03). No significant difference in infiltration rate between old and new sites was observed (Figure 1.3F).

Hydrophobicity did not show clear trends with any of the PCA axes or site age.

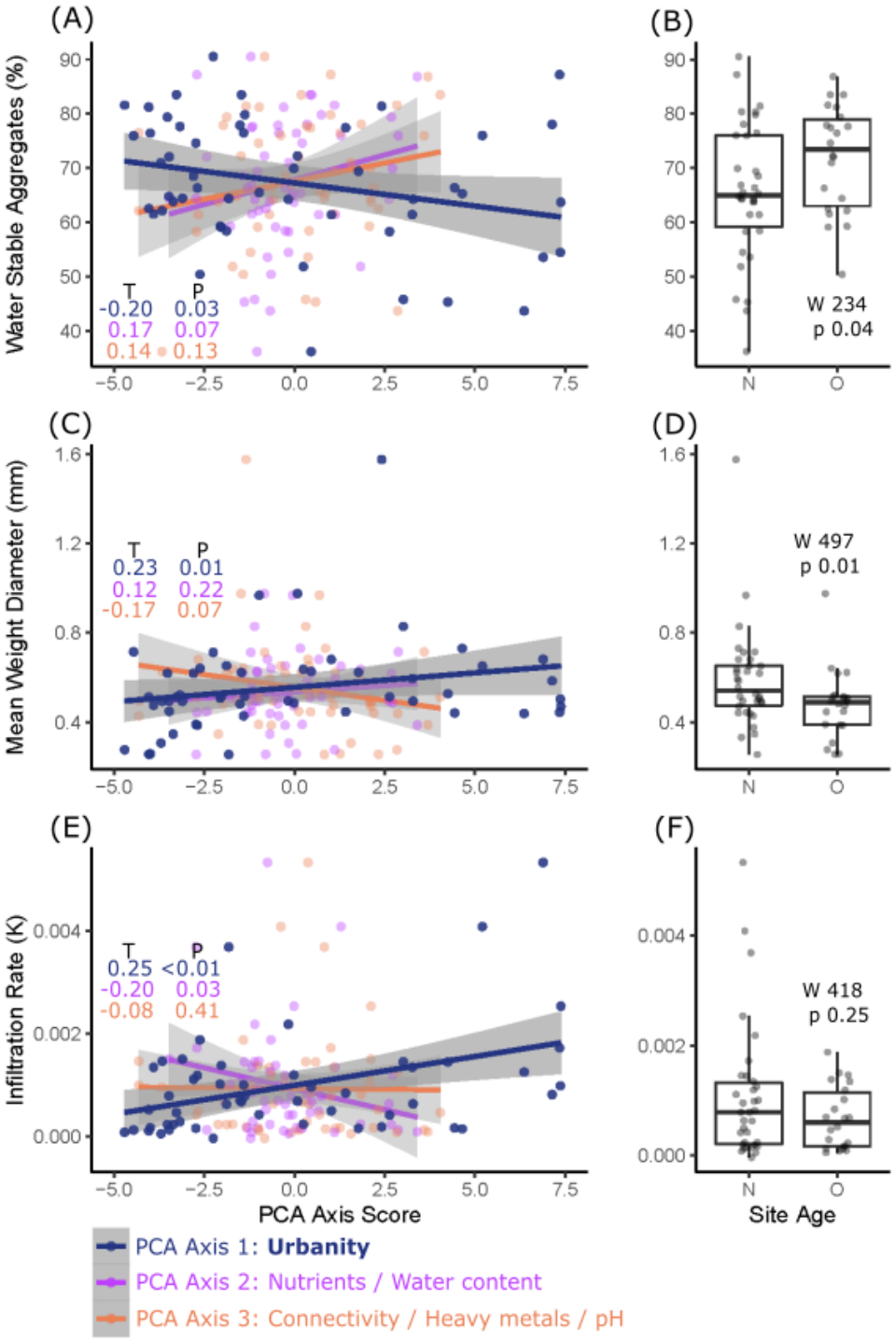

Figure 1.3 Relationships between soil parameters versus PCA axes and site age (N New, O Old). (A) WSA% vs. PCA axes (B) WSA% vs. Age (C) MWD vs. PCA axes (D)MWD vs. Age (E) Infiltration Rate vs. PCA axes (F) Infiltration Rate vs. Age. Lines shown are simple linear regression lines and grey areas represent standard error, values of Kendall's Tau and P are presented in each plot. Boxplots show median, first and third quantiles, with whiskers showing range, Wilcoxon Rank SumW and p values are presented in each plot.

1.4.3 Relationships Between Soil Properties

Average infiltration rate demonstrated a strong negative correlation with WSA% (Kendall's Tau = −0.33, p = <0.001) (Figure 1.4A) and a negative correlation with hydrophobicity (Kendall's Tau =

−0.22, p = 0.02), although this relationship appeared to be more complicated than a simple linear relationship (Figure 1.4B). There was a strong positive correlation between the WSA% and hydrophobicity (Kendall's Tau = 0.34, p < 0.001) (Figure 1.4C). It should also be noted that as the infiltration rate increased, so too did the within-site variation, as measured by the standard-error of the infiltration rate (Kendall's Tau = 0.55, p < 0.001).

We found no significant relation between the coefficient of curvature and coefficient of uniformity, meaning that there was no notable connection between the grading of the soil particles and the variety of sizes of soil particles. However, the coefficient of uniformity did show a strong positive correlation with MWD (Kendall's Tau = 0.49, p < 0.001), meaning that as average particle size got larger so too did the variety of particle sizes.

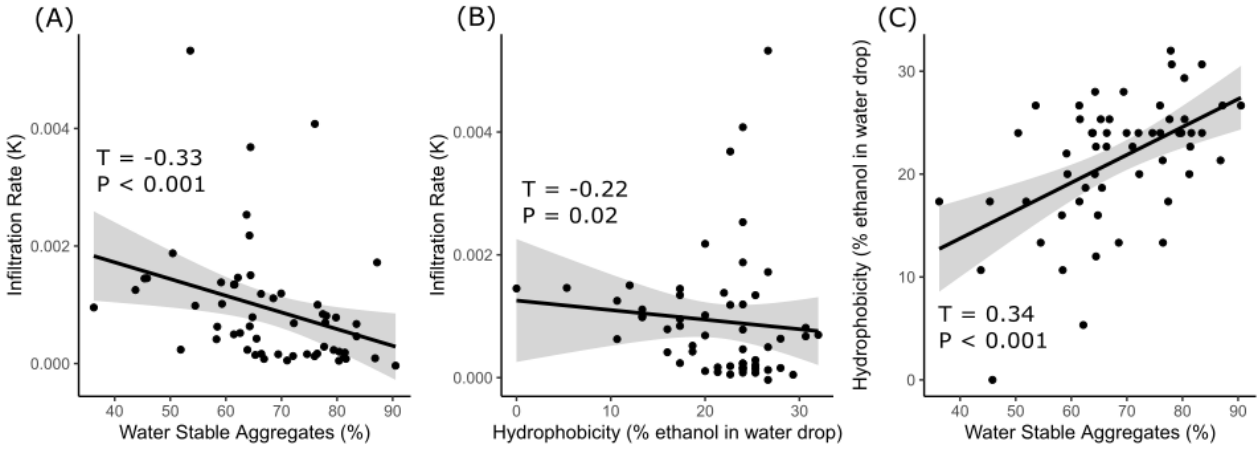

Figure 1.4 Relationships between soil properties. (A) Infiltration rate vs. WSA% (B) Infiltration rate vs. hydrophobicity (C) Hydrophobicity vs. WSA%. Lines shown are simple linear regression lines and grey areas represent standard error. Kendall's Tau and p values are presented in each plot.

1.5 Discussion

The PCA of variables led to the identification of three distinguishable main groups of explanatory variables. Axis 1 clearly related to urbanity, axis 2 related to soil nutrient content (specifically N, S and organic C) and water content, and axis 3 is related to heavy metals and site connectivity. Soil pH, often a key parameter in soil science, contributed to axis 3 but to a relatively minor extent. Electrical conductivity, a proxy for salinity, did not contribute significantly to any of the environmental axes. In urban research salinity is often a significant variable, however, in Berlin the use of salt as a de-icing agent has been banned since 2013 (Berliner Naturschutzgesetz, 2013), explaining its lack of contribution to the variance within our environmental dataset.

WSA% showed a significant trend for lower values in newer, more urban sites. Observations of urban impacts on soil structure have generally related to compaction, a reduction of WSA in relation to urbanity does not appear to have previously been reported. Due to the interest in compaction, bulk density is often the main parameter measured in studies of urban soil properties (Scharenbroch *et al.*, 2005; Hagan *et al.*, 2012; da Silva *et al.*, 2017, Nero and Anning, 2018). Despite aggregate stability being discussed in association with urban soil bulk density measures (Matziris *et al.*, 2016), direct observations of aggregate stability in relation to urbanity are lacking. Low to medium levels of aggregate stability have been recorded in an urban context before (Jim, 1998a; Jim, 1998b), but the findings were in relation to tree growth and no gradient in urbanity was reported. The use of bulk density is often chosen for its relation to abiotic ecosystem services such as infiltration, however, our findings about aggregate stability provide evidence for important biological and nutrient cycling ramifications of urbanity. A reduction in WSA% with increasing urbanity has implications for urban soil biodiversity due to the important role that bacteria and fungi (Lehmann *et al.*, 2017), in particular arbuscular

mycorrhizal fungi (Lehman *et al.*, 2020), play in aggregate formation, thus a reduction in WSA% in urban soils may be a result of reduced microbial activity. However, this relationship is not unidirectional and may not tell the full story. If WSA are lost in an urban setting due to external mechanical stresses (e.g. trampling), then a reduction in WSA% may be a cause of, rather than a symptom of, a reduction in microbial activity. The chemical concentration gradients within WSA provide niches for organisms, in particular nitrifying bacteria, which are protected within their core (Wilpiszeski *et al.*, 2019). A loss of WSA therefore heralds a loss of microbial functional diversity. Either way, their loss in an urban environment is deeply troubling and signals a disruption of nutrient cycling. WSA are also important due to their ability to prevent erosion and absorb nutrients, in a polluted environment they may also absorb heavy metals (Fan *et al.*, 2013; Xu *et al.*, 2017). The nutrient cycling function of WSA may be demonstrated by examining the relationship between WSA% and axis 2; both axes 2 and 3 appear to show a positive relationship with WSA%. This positive relationship to both axes is likely due to the increased sorption capacity of soil with a high WSA%, however, the weaker relationship with axis 3 is probably due to the compounding impact of axis 1, whereby more urban areas (which also tend to have higher heavy metal content) had lower WSA%. It seems unlikely that site connectivity, another component of axis 3, could directly impact WSA%. The increased WSA% in old sites may also be explained by an increased colonization of these soils by roots and microorganisms sensitive to disturbance; arbuscular mycorrhizal fungi, which are known to be sensitive to disturbance and land use change (Trejo *et al.*, 2016) could explain this trend through the important role they play in aggregate formation (Rillig *et al.*, 2010). Microbial life may also explain the link between high WSA% and high hydrophobicity due to the hydrophobins bacteria and fungi have been demonstrated to excrete to expediate the process of aggregation (Lehmann *et al.*, 2017; Lehmann *et al.*, 2020). Former studies have demonstrated a close link between hydrophobicity and aggregate stability (Zheng *et al.*, 2016), with research demonstrating that hydrophobicity improves aggregate stability during wetting (Vogelmann *et al.*, 2013). As such, a positive feedback loop between aggregate presence and hydrophobicity may be present. Hydrophobicity is often associated with sandy soils, where smaller surface areas are more easily coated by hydrophobic carbon-chain compounds. However, it appears that in this case hydrophobicity showed no relationship to MWD.

The findings of this study suggest that an increase in urbanity is associated with a loss in soil structure through reduced aggregate stability. It is, however, interesting to note that individual sites did not abide by this trend. Indeed, the highest levels of WSA% were seen at a surprising mix of sites; the highest was in a small grass patch between a new road development and a forest in Brandenburg, the second highest levels were observed in an abandoned underpass development in Schöneberg, a district in Southwest Berlin, and the third highest was in a grassland patch in Spandauer forest, near the Brandenburg-Berlin border. In accordance with the relationship between WSA% and axis 1, two of these sites were in comparatively rural locations, however, when the urban underpass development was sampled it was noted that moss and lichens were forming a crust and the previously cleared ground appeared to be experiencing secondary succession. Unsurprisingly all the sites with lowest WSA% were newer sites, with two of the lowest on central verges between roads. However, the very lowest WSA% was observed on parkland. This site was formerly the runway for Flugplatz Johannisthal, the second oldest airfield in the world. It seems that the remediation work that transformed this site into a non-industrial site had not yet resulted in substantial soil aggregate formation.

The positive trend between combined particle size and axis 1 is unlikely to be the result of the size of aggregates that withstood sieving, due to the negative relationship between WSA% and axis 1. The relationship between MWD and axis 1 is therefore likely due to sand and gravel particles. The cause for such a relationship could either be the removal of the finer fraction by erosion, or the addition of a larger fraction. It is possible that in urban grasslands, which experience heightened water flow because of proximal soil sealing, the finer fraction would be eroded over time. Alternatively, the importation of gravel for construction and the addition of fragments of building waste from anthropogenic activity may have supplied larger soil particles.

The positive relationship between the uniformity coefficient and MWD suggests that the addition of new, larger particle size fractions is the cause of the aforementioned increase in MWD, rather than the removal of smaller soil fractions. All three of the sites with highest MWD were found near railway lines, with the highest MWD being in Natur-Park Schöneberger Südgelände, which was previously a rail yard and is proximal to a railway still in active use. Of the other two sites, one was on the embankment of a railway and the other was in a field around 140 m away from a railway. The site with the smallest MWD was a grassland formerly under the Berlin wall, previously mentioned due to its high infiltration rate. The other two sites with the lowest MWD were both grassland patches located in the Spandauer forest. The high MWD in railway-related sites is a clear indication that the urban-related increase inMWD observed in the study may be due to the use of gravel in the construction of railways.

Infiltration rate appears to be positively linked with urbanity (axis 1) and negatively with soil nutrient content (axis 2). Urban environments are often thought to exhibit low infiltration across a large geographic area due to the creation of impervious surfaces sealing the soil, but the findings of our study suggest that there is not a simple trade-off when it comes to soil sealing and infiltration rate; a loss in infiltration through soil sealing may be buffered by an increase in infiltration rate in proximal nonsealed soils. Infiltration rate has previously been explored in urban ecosystems in relation to soil compaction (Gregory *et al.*, 2006; Yang and Zhang, 2011), which resulted in reduced infiltration. However, the findings of our study suggest that this is not the case in Berlin's grasslands. Increased infiltration in sites adjacent to sealed areas is discussed in Scalenghe and Ajmone-Marsan (2009), however, this situation does not appear to have been comprehensively studied elsewhere.

It is possible that the increase in infiltration rate we observed may be due to the loss of WSA in urban areas, a hypothesis which is supported by the negative relationship between infiltration rate and WSA%, and betweenWSA% and axis 1. The soils in Berlin contain a large sand fraction, and so a lackofWSAmay indicate the presence of very sandy soil which (not considering hydrophobicity) offers little resistance to infiltration. Infiltration rate showed its most significant response to axis 2, which was dominated by N, S, organic C, and water content. This negative relationship may simply be due to the aforementioned lack of WSA in sites with high infiltration, however, there could also be a direct effect of high levels of water flowing through the soil matrix upon nutrient content; both N and S are susceptible to leaching, and although C loss from soil is more often related to microbial activity, leaching of organic C has been demonstrated to have a detectable impact on C levels in grasslands (Kindler *et al.*, 2011).

The negative relationship between infiltration and hydrophobicity is unsurprising, as it could be expected that a major limiting factor for infiltration would be hydrophobicity. However, the absence of a relationship between hydrophobicity and axis 1 means it is unlikely that hydrophobicity explains the urban-related change in infiltration. The trend for decreasing infiltration rate with increasing hydrophobicity did, however, have some notable exceptions; three sites with both high infiltration rates and high levels of hydrophobicity were recorded. It is possible these sites were experiencing "finger flow," allowing replicates with very high rates of infiltration to compensate for those with slow rates due to spatially variable hydrophobicity, a hypothesis supported by the increase in standard error of infiltration at sites with high average infiltration rates.

The highest levels of infiltration were observed in Park am Nordbahnhof, an urban park built on land previously covered by the Berlin wall. Tiergarten (the iconic park in the centre of Berlin) and a very sandy grassland patch on the edge of Bieselheide national forest (formerly covered by the Berlin wall) also demonstrated high infiltration rates. It is interesting that these sites all expressed high infiltration rates but clearly had different management practices and site histories. One possible unifying feature of these sites could be a high level of human visitation; the former two are in popular urban parks whilst the third is sandwiched between two poorly defined pathways on the border between a suburban neighbourhood and a forest. Previous research has reported a loss of soil aggregate structure and a reduction in water infiltration rate because of cattle trampling, however, the reduction in infiltration was apparently less notable in sandy loam than in soil with a high clay content (Pietola *et al.*, 2005). Our

findings suggest that sandy soils exposed to trampling in urban parks may exhibit an increase in infiltration, perhaps due to a loss of soil structure without the substantial increase in bulk density associated with trampling by cattle. The two lowest infiltration rates were observed in grassland patches in forests surrounding Berlin, whilst the third lowest was on a patch of grassland next to Berlin's International Congress Center (the site with the highest axis 1 score), shunning the general trend of infiltration rate increasing with urbanity. In contrast to the sites expressing high levels of infiltration, these sites are all secluded; either in a rural forest or bordered by a conference centre and several busy roads. Our findings suggest that while axis 1 comprised of many urban related factors which drive infiltration rate, human visitation may also be an important driver. It seems likely that in a soil with higher clay content, this pattern could easily be reversed due to compaction at highly visited sites.

In the wider context of urban research, these findings show a mixed result in terms of supporting the "urban ecosystem convergence hypothesis" (Pouyat *et al.*, 2003). Our results imply a loss of soil structure with urbanity, as hypothesised previously by Chen *et al.* (2014). A reduction in infiltration rate is not observed, conversely to what has previously been demonstrated with compacted urban soils (Yang and Zhang, 2011) and in fact demonstrated an increase. It seems likely that the sandy nature of Berlin's soil greatly influences the impacts of urban soil degradation, resulting in increased infiltration rather than a reduction. The measurement of combined particle sizes provides additional evidence for the importance of anthropogenic artifacts in determining the structure of urban soils (Bridges *et al.*, 1991; Lehmann and Stahr, 2007; Pouyat *et al.*, 2010). Our findings demonstrate that urban soils do not necessarily converge in their structure and function, with important implications for the cycling of nutrients within them, and the ecosystem services they provide.

1.6 Conclusion

Our findings signal the important role urbanity has in determining soil structure and function. The loss in stable aggregates with urbanity will likely be associated with a reduction in microbial functional diversity and consequently a loss in nutrient cycling capacity. An increased risk of soil erosion is also likely to be present. Our observation of increased infiltration rate with urbanity suggests that urban soils have a capacity to buffer local soil sealing in terms of infiltration capacity, this may however, be because of a loss of soil structure and aggregates. Those managing urban soils should be aware that, at least in the case of sandy soils, high infiltration rates may come at the cost of a loss of the biotic functioning of soils. Therefore, a careful balancing of management activities fostering physical or biotic soil functions is required.

1.7 Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Figshare: https://doi.org/10.6084/m9.figshare.16775701, https://doi.org/10.6084/m9.figshare.16811701.

1.8 Author contributions

JW, SH, and MR designed the research. JW conducted fieldwork and lab analyses, and wrote the first draft of the paper. AH and ML provided field data. All authors added to and edited the text.

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1.11 References

Arandjelovic, B., and Bogunovich, D. (2014). City Profile: Berlin. Cities 37, 1–26. doi:10.1016/j.cities.2013.10.007

Arnfield, A. J. (2003). Two Decades of Urban Climate Research: A Review of Turbulence, Exchanges of Energy and Water, and the Urban Heat Island. Int. J. Climatol. 23 (1), 1–26. doi:10.1002/joc.859

Assouline, S. (2013). Infiltration into Soils: Conceptual Approaches and Solutions. Water Resour. Res. 49 (4), 1755–1772. doi:10.1002/wrcr.20155

Assouline, S. (2004). Rainfall-induced Soil Surface Sealing: A Critical Review of Observations, Conceptual Models, and Solutions. Vadose Zone J. 3 (2), 570–591. doi:10.2136/vzj2004.0570

Baer, S. G., and Birgé, H. E. (2018). "Soil Ecosystem Services: An Overview". Managing Soil Health for Sustainable Agriculture. Editor D. Reicosky (London: Burleigh Dodds Science Publishing Limited), 1, 1–22. doi:10.19103/as.2017.0033.02

Bát'ková, K., Mihalikova, M., and Matula, S. (2020). Hydraulic Properties of a Cultivated Soil in Temperate Continental Climate Determined by Mini Disk Infiltrometer. Water 12 (3), 21. doi:10.3390/w12030843

Bisdom, E. B. A., Dekker, L. W., and Schoute, J. F. T. (1993). Water Repellency of Sieve Fractions from Sandy Soils and Relationships with Organic Material and Soil Structure. Geoderma 56 (1-4), 105–118. doi:10.1016/b978-0-444-81490-6.50013-3

Brevik, E. C., and Burgess, L. C. (2014). The Influence of Soils on Human Health. Nat. Educ. Knowledge 5 (12), 1.

Bridges, E. M. (1991). Waste Materials in Urban Soils. Oxford: Blackwell Scientific Publications Ltd, 28–46.

Bronick, C. J., and Lal, R. (2005). Soil Structure and Management: a Review. Geoderma 124 (1-2), 3–22. doi:10.1016/j.geoderma.2004.03.005

Caron, J., Espindola, C. R., and Angers, D. A. (1996). Soil Structural Stability during Rapid Wetting: Influence of Land Use on Some Aggregate Properties. Soil Sci. Soc. America J. 60 (3), 901–908. doi:10.2136/sssaj1996.03615995006000030032x

Chen, Y., Day, S. D., Wick, A. F., and McGuire, K. J. (2014). Influence of Urban Land Development and Subsequent Soil Rehabilitation on Soil Aggregates, Carbon, and Hydraulic Conductivity. Sci. Total Environ. 494-495, 329–336.doi:10.1016/j.scitotenv.2014.06.099

Cui, H., Ou, Y., Lv, D.,Wang, L. X., Liang, A. Z., Yan, B. X., *et al.* (2020). Aggregate related Microbial Communities and Nutrient Stoichiometry under Different Croplands. Ecol. Process. 9 (1), 9. doi:10.1186/s13717-020-00239-4

de Jonge, L. W., Jacobsen, O. H., and Moldrup, P. (1999). Soil Water Repellency: Effects of Water Content, Temperature, and Particle Size. Soil Sci. Soc. America J. 63 (3), 437–442. doi:10.2136/sssaj1999.03615995006300030003x

DeBano, L. F. (1971). The Effect of Hydrophobic Substances on Water Movement in Soil during Infiltration. Soil Sci. Soc. America J. 35 (2), 340–343. doi:10.2136/sssaj1971.03615995003500020044x

DeBano, L. F. (2000). The Role of Fire and Soil Heating on Water Repellency in Wildland Environments: a Review. J. Hydrol. 231-232, 195–206. doi:10.1016/s0022-1694(00)00194-3

Dekker, L. W., Doerr, S. H., Oostindie, K., Ziogas, A. K., and Ritsema, C. J. (2001).Water Repellency and Critical SoilWater Content in a Dune Sand. Soil Sci. Soc.Am. J. 65 (6), 1667–1674. doi:10.2136/sssaj2001.1667

Díaz, S., Pascual, U., Stenseke, M., Martín-López, B., Watson, R. T., Molnár, Z., *et al.* (2018). Assessing Nature's Contributions to People. Science 359 (6373), 270–272. doi:10.1126/science.aap8826

Doerr, S. H. (1998). On Standardizing the ‚water Drop Penetration Time' and the ‚molarity of an Ethanol Droplet' Techniques to Classify Soil Hydrophobicity: A Case Study Using Medium Textured Soils. Earth Surf. Process. Landforms 23 (7), 663–668. doi:10.1002/(sici)1096-9837(199807)23:7<663:aidesp909>3.0.co;2-6

Doerr, S. H., Shakesby, R. A., and Walsh, R. P. D. (2000). Soil Water Repellency: its Causes, Characteristics and Hydro-Geomorphological Significance. Earth-Science Rev. 51 (1-4), 33–65. doi:10.1016/s0012-8252(00)00011-8

du Toit, M. J., and Cilliers, S. S. (2011). Aspects Influencing the Selection of Representative Urbanization Measures to Quantify Urban-Rural Gradients. Landscape Ecol. 26 (2), 169–181. doi:10.1007/s10980-010-9560-4

Dunnington, D. (2021). Ggspatial: Spatial Data Framework for Ggplot2. R package version 1.1.5. Available at: https://CRAN.R-project.org/package_ggspatial.

Edmondson, J. L., Davies, Z. G., McCormack, S. A., Gaston, K. J., and Leake, J. R. (2011). Are Soils in Urban Ecosystems Compacted? A Citywide Analysis. Biol. Lett. 7 (5), 771–774. doi:10.1098/rsbl.2011.0260

Equiza, M. A., Calvo-Polanco, M., Cirelli, D., Señorans, J., Wartenbe, M., Saunders, C., *et al.* (2017). Long-term Impact of Road Salt (NaCl) on Soil and Urban Trees in Edmonton, Canada. Urban For. Urban Green. 21, 16–28. doi:10.1016/j.ufug.2016.11.003

Fan, J., Ding, W., and Ziadi, N. (2013). Thirty-Year Manuring and Fertilization Effects on Heavy Metals in Black Soil and Soil Aggregates in Northeastern China. Commun. Soil Sci. Plant Anal. 44 (7), 1224–1241. doi:10.1080/00103624.2012.756002

Garnier, S., Ross, N., Rudis, R., Camargo, P. A., Sciaini, M., and Scherer, C. (2021). Viridis - Colorblind-Friendly Color Maps for R. R package version 0.6.1. Available online at: https://sjmgarnier.github.io/viridis/.

Gerstenberg, J. (2017). 01.06.1 Bodenarten. Available online at: https://www.berlin.de/umweltatlas/boden/bodenkundliche-kennwerte/2015/karten/artikel. 948554.php (Accessed 06 18, 2021).

Giovannini, G., and Lucchesi, S. (1983). Effect of Fire on Hydrophobic and Cementing Substances of Soil Aggregates1. Soil Sci. 136 (4), 231–236. doi:10.1097/00010694-198310000-00006

Gregory, J. H., Dukes, M. D., Jones, P. H., and Miller, G. L. (2006). Effect of Urban Soil Compaction on Infiltration Rate. J. Soil Water Conservation 61 (3), 117–124.

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., *et al.* (2008). Global Change and the Ecology of Cities. Science 319 (5864), 756–760. doi:10.1126/science.1150195

Haase, D., and Nuissl, H. (2007). Does Urban Sprawl Drive Changes in the Water Balance and Policy? The Case of Leipzig (Germany) 1870–2003. Landscape Urban Plann. 80 (1), 1–13. doi:10.1016/j.landurbplan.2006.03.011

Hagan, D., Dobbs, C., Timilsina, N., Escobedo, F., Toor, G. S., and Andreu, M. (2012). Anthropogenic Effects on the Physical and Chemical Properties of Subtropical Coastal Urban Soils. Soil Use Manage. 28 (1), 78–88. doi:10.1111/j.1475-2743.2011.00379.x

Hooke, R. L. (2000). On the History of Humans as Geomorphic Agents. Geology 28 (9), 843–846. doi:10.1130/0091-7613(2000)028<0843:othoha>2.3.co;2

Huang, B., Yuan, Z., Li, D., Zheng, M., Nie, X., and Liao, Y. (2020). Effects of Soil Particle Size on the Adsorption, Distribution, and Migration Behaviors of Heavy Metal(loid)s in Soil: a Review. Environ. Sci. Process. Impacts 22 (8),1596–1615. doi:10.1039/d0em00189a

Jim, C. Y. (1998b). Physical and Chemical Properties of a Hong Kong Roadside Soil in Relation to Urban Tree Growth. Urban Ecosyst. 2 (2), 171–181. doi:10.1023/ a:1009585700191

Jim, C. Y. (1998a). Urban Soil Characteristics and Limitations for Landscape Planting in Hong Kong. Landscape Urban Plann. 40 (4), 235–249. doi:10.1016/ s0169-2046(97)00117-5

Kassambara, A. (2020). Ggpubr: ',ggplot2' Based Publication Ready Plots. R package version 0.4.0. Available online at: https://CRAN.R-project.org/package_ggpubr.

Kemper, W. D., and Rosenau, R. C. (1986). Aggregate Stability and Size Distribution. Methods Soil Anal. 175, 425–442. doi:10.2136/ sssabookser5.1.2ed.c17

Kindler, R., Siemens, J., Kaiser, K.,Walmsley, D. C., Bernhofer, C., Buchmann, N., *et al.* (2011). Dissolved Carbon Leaching from Soil Is a Crucial Component of the Net Ecosystem Carbon Balance. Glob. Change Biol. 17 (2), 1167–1185. doi:10.1111/j.1365-2486.2010.02282.x

Klein Goldewijk, K., Beusen, A., and Janssen, P. (2010). Long-term Dynamic Modeling of Global Population and Built-Up Area in a Spatially Explicit Way: HYDE 3.1. The Holocene 20 (4), 565–573. doi:10.1177/0959683609356587

Korenkova, L., and Matus, P. (2015). Role of Water Repellency in Aggregate Stability of Cultivated Soils under Simulated Raindrop Impact. Eurasian Soil Sci. 48 (7), 754–758. doi:10.1134/s1064229315070054

Kowarik, I. (2020). Herbert Sukopp - an Inspiring pioneer in the Field of Urban Ecology. Urban Ecosyst. 23 (3), 445–455. doi:10.1007/s11252-020-00983-7

Kuhn, M., Jackson, S., and Cimentada, J. (2020). Corrr: Correlations in R. R package version 0.4.3. Available online at: https://CRAN.R-project.org/package_corrr.

Layton, J. B., Skidmore, E. L., and Thompson, C. A. (1993). Winter-associated Changes in Dry-Soil Aggregation as Influenced by Management. Soil Sci. Soc. America J. 57 (6), 1568–1572. doi:10.2136/sssaj1993.03615995005700060029x

Lehmann, A., Zheng, W., Ryo, M., Soutschek, K., Roy, J., Rongstock, R., *et al.* (2020). Fungal Traits Important for Soil Aggregation. Front. Microbiol. 10, 2904. doi:10.3389/fmicb.2019.02904

Lehmann, A., and Stahr, K. (2007). Nature and Significance of Anthropogenic Urban Soils. J. Soils Sediments 7 (4), 247–260. doi:10.1065/jss2007.06.235

Lehmann, A., Zheng, W., and Rillig, M. C. (2017). Soil Biota Contributions to Soil Aggregation. Nat. Ecol. Evol. 1 (12), 1828–1835. doi:10.1038/s41559-017-0344-y

Lorenz, K., and Lal, R. (2009). Biogeochemical C and N Cycles in Urban Soils. Environ. Int. 35 (1), 1–8. doi:10.1016/j.envint.2008.05.006

Mao, J., Nierop, K. G. J., Dekker, S. C., Dekker, L. W., and Chen, B. (2019). Understanding the Mechanisms of Soil Water Repellency from Nanoscale to Ecosystem Scale: a Review. J. Soils Sediments 19 (1), 171–185. doi:10.1007/s11368-018-2195-9

Matziris, E., Stefanou, S., Papazafeiriou, A. Z., Seilopoulos, D., and Papaioannou, A. (2016). Impacts of Human Activities on Soil Physical Properties of Urban green Areas: a Case Study in Thessaloniki City, Greece. Carpathian J. Earth Environ. Sci. 11 (2), 381–394.

McDonnell, M. J., Hahs, A. K., and Pickett, S. T. A. (2012). Exposing an Urban Ecology Straw Man: Critique of Ramalho and Hobbs. Trends Ecol. Evol. 27 (5), 255–256. doi:10.1016/j.tree.2012.01.009

McGhie, D., and Posner, A. (1981). The Effect of Plant Top Material on the Water Repellence of Fired Sands and Water Repellent Soils. Aust. J. Agric. Res. 32 (4), 609–620. doi:10.1071/ar9810609

Miyata, S., Kosugi, K. i., Gomi, T., Onda, Y., and Mizuyama, T. (2007). Surface Runoff as Affected by Soil Water Repellency in a Japanese cypress forest. Hydrol. Process. 21 (17), 2365–2376. doi:10.1002/hyp.6749

Moll, R. J., Cepek, J.D., Lorch, P. D.,Dennis, P.M., Tans, E., Robison, T., *et al.* (2019). What Does Urbanization Actually Mean? A Framework for Urban Metrics in Wildlife Research. J. Appl. Ecol. 56 (5), 1289–1300. doi:10.1111/1365-2664.13358

Morbidelli, R., Corradini, C., Saltalippi, C., Flammini, A., Dari, J., and Govindaraju, R. S. (2018). Rainfall Infiltration Modeling: A Review. Water 10 (12), 20. doi:10.3390/w10121873

Morel, J. L., Chenu, C., and Lorenz, K. (2015). Ecosystem Services provided by Soils of Urban, Industrial, Traffic, Mining, and Military Areas (SUITMAs). J. Soils Sediments 15 (8), 1659–1666. doi:10.1007/s11368-014-0926-0

Naturschutzgesetz, B. (2013). Section 39: Streusalzverbot. Permanent Link. Available online at: https://gesetze.berlin.de/perma?d_jlr-NatSchGBE2013pP39.

Nero, B. F., and Anning, A. K. (2018). Variations in Soil Characteristics Among Urban Green Spaces in Kumasi, Ghana. Environ. Earth Sci. 77 (8). doi:10.1007/s12665-018-7441-3

Pebesma, E. (2018). Simple Features for R: Standardized Support for Spatial Vector Data. R. J. 10, 439–446. doi:10.32614/rj-2018-009

Perry, T., and Nawaz, R. (2008). An Investigation into the Extent and Impacts of Hard Surfacing of Domestic Gardens in an Area of Leeds, United Kingdom. Landscape Urban Plann. 86 (1), 1–13. doi:10.1016/j.landurbplan.2007.12.004

Pickett, S.T.A., Cadenasso, M. L.,Grove, J.M.,Nilon, C. H., Pouyat, R. V., Zipperer, W. C., *et al.* (2001). Urban Ecological Systems: Linking Terrestrial Ecological, Physical, and Socioeconomic Components of Metropolitan Areas. Annu. Rev. Ecol. Syst. 32, 127–157. doi:10.1146/annurev.ecolsys.32.081501.114012

Pietola, L., Horn, R., and Yli-Halla,M. (2005). Effects of Trampling by Cattle on the Hydraulic and Mechanical Properties of Soil. Soil Tillage Res. 82 (1), 99–108. doi:10.1016/j.still.2004.08.004

Plyaskina, O. V., and Ladonin, D. V. (2009). Heavy Metal Pollution of Urban Soils. Eurasian Soil Sc. 42 (7), 816–823. doi:10.1134/s1064229309070138

Potere, D., and Schneider, A. (2007). A Critical Look at Representations of Urban Areas in Global Maps. GeoJournal 69 (1), 55–80. doi:10.1007/s10708-007-9102-z

Pouyat, R. V., Russell-Anelli, J., Yesilonis, I. D., and Groffman, P. M. (2003). “Soil Carbon in Urban forest Ecosystems,” in The Potential of U.S. forest Soils to Sequester Carbon and Mitigate the Greenhouse Effect. Editors J. M. Kimble, L. S. Heath, R. A. Birdsey, and R. Lal (U.S.: CRC Press), 347–362.

Pouyat, R. V., Szlavecz, K., Yesilonis, I. D., Groffman, P. M., and Schwarz, K. (2010). Chemical, Physical, and Biological Characteristics of Urban Soils. Madison, WI: Urban Ecosystem Ecology, 119–152.

R Core Team (2020). R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing.

Ramos, M. C., Nacci, S., and Pla, I. (2003). Effect of Raindrop Impact and its Relationship with Aggregate Stability to Different Disaggregation Forces. Catena 53 (4), 365–376. doi:10.1016/s0341-8162(03)00086-9

Rillig, M. C., Mardatin, N. F., Leifheit, E. F., and Antunes, P. M. (2010). Mycelium of Arbuscular Mycorrhizal Fungi Increases Soil Water Repellency and Is Sufficient to Maintain Water-Stable Soil Aggregates. Soil Biol. Biochem. 42 (7), 1189–1191. doi:10.1016/j.soilbio.2010.03.027

Samtani, N. C. (2006). Soils and Foundations Reference Manual, 1. Washington D.C.: National Highway Institute, US Department of Transportation.

Savage, S. M.,Martin, J. P., and Letey, J. (1969). Contribution of Some Soil Fungi to Natural and Heat-Induced Water Repellency in Sand. Soil Sci. Soc. Am. J. 33 (3), 405–409. doi:10.2136/sssaj1969.03615995003300030021x

Scalenghe, R., and Marsan, F. A. (2009). The Anthropogenic Sealing of Soils in Urban Areas. Landscape Urban Plann. 90, 1–10. doi:10.1016/j.landurbplan.2008.10.011

Scharenbroch, B. C., Lloyd, J. E., and Johnson-Maynard, J. L. (2005). Distinguishing Urban Soils with Physical, Chemical, and Biological Properties. Pedobiologia 49 (4), 283–296. doi:10.1016/j.pedobi.2004.12.002

Silva,A.M. d.,Urban, R. C.,Manfré,L.A.,Brossard, M., andMoreira, M. Z. (2017). Soil Quality Attributes Related to Urbanization in BrazilianWatershed. J. Environ. Eng. Landscape Manage. 25 (4), 317–328. doi:10.3846/16486897.2017.1296451

Six, J., Bossuyt, H., Degryze, S., and Denef, K. (2004). A History of Research on the Link between (Micro)aggregates, Soil Biota, and Soil Organic Matter Dynamics. Soil Tillage Res. 79 (1), 7–31. doi:10.1016/j.still.2004.03.008

Thao, H. T. B., George, T., Yamakawa, T., and Widowati, L. R. (2008). Effects of Soil Aggregate Size on Phosphorus Extractability and Uptake by rice (Oryza sativaL.) and Corn (Zea maysL.) in Two Ultisols from the Philippines. Soil Sci. Plant Nutr. 54 (1), 148–158. doi:10.1111/j.1747-0765.2007.00220.x

Thioulouse, J., Dray, S., Dufour, A., Siberchicot, A., Jombart, T., and Pavoine, S. (2018). Multivariate Analysis of Ecological Data with Ade4. Springer. Trejo, D., Barois, I., and Sangabriel-Conde, W. (2016). Disturbance and Land Use Effect on Functional Diversity of the Arbuscular Mycorrhizal Fungi. Agroforest Syst. 90 (2), 265–279. doi:10.1007/s10457-015-9852-4

Tzoulas, K., Korpela, K., Venn, S., Yli-Pelkonen, V., Kaźmierczak, A., Niemela, J., *et al.* (2007). Promoting Ecosystem and Human Health in Urban Areas Using Green Infrastructure: A Literature Review. Landscape Urban Plann. 81 (3), 167–178. doi:10.1016/j.landurbplan.2007.02.001

United Nations (2018). 2018 Revision of World Urbanization Prospects. Available at: https://www.un.org/development/desa/publications/2018-revision-ofworld-urbanization-prospects.html (Accessed November 20, 2021)

van Bavel, C. H. M. (1950). Mean Weight-Diameter of Soil Aggregates as a Statistical Index of Aggregation. Soil Sci. Soc. America J. 14 (C), 20–23. doi:10.2136/sssaj1950.036159950014000c0005x

Vogelmann, E. S., Reichert, J. M., Prevedello, J., Awe, G. O., and Mataix-Solera, J. (2013). Can Occurrence of Soil Hydrophobicity Promote the Increase of Aggregates Stability. Catena 110, 24–31. doi:10.1016/j.catena.2013.06.009

von der Lippe, M., Buchholz, S., Hiller, A., Seitz, B., and Kowarik, I. (2020). CityScapeLab Berlin: A Research Platform for Untangling Urbanization Effects on Biodiversity. Sustainability 12 (6), 30. doi:10.3390/su12062565

von der Lippe, M., Fiechter, L., Hiller, A., and Whitehead, J. (2021). Data from: Environmental Data Used for PCA, and Site Age. Figshare. doi:10.6084/m9.figshare.16811701

Whitehead, J., Hempel, S., Hiller, A., von der Lippe, M., and Rillig, M. C. (2021). Data from: Soil Physico-Chemical Data and PCA axis Scores. Figshare. doi:10.6084/m9.figshare.16775701

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag.

Wilpiszeski, R. L., Aufrecht, J. A., Retterer, S. T., Sullivan, M. B., Graham, D. E., Pierce, E. M., *et al.* (2019)

Xu, L., Cui, H., Zheng, X., Zhou, J., Zhang, W., Liang, J., *et al.* (2017). Changes in the Heavy Metal Distributions in Whole Soil and Aggregates Affected by the Application of Alkaline Materials and Phytoremediation. RSC Adv. 7 (65), 41033–41042. doi:10.1039/c7ra05670b

Yang, J.-L., and Zhang, G.-L. (2011). Water Infiltration in Urban Soils and its Effects on the Quantity and Quality of Runoff. J. Soils Sediments 11 (5), 751–761. doi:10.1007/s11368-011-0356-1

York, C. A., and Canaway, P. M. (2000). Water Repellent Soils as They Occur on UK golf Greens. J. Hydrol. 231-232, 126–133. doi:10.1016/s0022-1694(00)00189-x

Chapter Two

Non-Mycorrhizal Fungal Presence Within Roots Increases Across an Urban Gradient in Berlin, Germany

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2.1 Abstract

Symbioses between plants and fungi are important in both promoting plant fitness and maintaining soil structure. The ways in which these relationships change across an urban gradient is subject to debate. Here we measured root colonisation including the presence of arbuscular mycorrhizal fungi, non-mycorrhizal fungi, and root hair presence. We found no evidence of changes in levels of arbuscular mycorrhizal fungal colonisation across an urban gradient, colonisation levels being driven instead by plant community. However, we did observe an increase in non-mycorrhizal fungal colonisation in association with increasing urbanity. Additionally, we observed an urban-related increase in root hair presence. Using principal component analysis we were able to provide strong evidence for these patterns being driven by an "urban syndrome", rather than soil chemistry. Our findings have important implications for the wider understanding of abiotic stresses on fungal endophyte presence and shed light on the impacts of urbanity upon plant roots.

2.2 Introduction

We live in the midst of the anthropocene, an age defined by the profound impact of humanity upon the Earth. Urban centres are the pinnacles of human impact upon the planet and contain a concentration of global-change factors not seen elsewhere (heavy metal pollutants, salinity, nitrogen deposition, heat islands and altered rainfall, to name but a few; Ziska *et al*., 2003; Carreiro and Tripler, 2005). These landscapes are by no means solely human domains and their ecology has been the target for several decades of research, largely due to the wide array of ecosystem services that are provided by their non-human components (Luederitz *et al*., 2015). A general rule appears to be that urbanisation results in the loss of animal diversity and can either increase or decrease plant diversity depending on the intensity of urbanisation (McKinney, 2008). However, the basis of all plant, and consequently animal, diversity is the soil. Urban soils have variously been described as compressed (Yang and Zhang, 2011), polluted (Hanfi *et al*., 2020) and lacking aggregates (Whitehead *et al*., 2021), expressing increased pH (Asabere *et al*., 2018) and salinity (Dmuchowski *et al*., 2021). As such, the ability of symbiotic fungi to buffer the negative impacts of urbanity upon plant hosts makes them a vital component of maintaining urban greenspaces. Urban ecosystems have also been described as hotspots for biological invasions (Gaertner *et al*., 2017). Fungal symbionts play a variety of roles in plant invasions, with some evidence suggesting that invasive plants are more likely to be facultatively symbiotic than native competitors, or at least not dependent on specific symbionts (Vogelsang and Bever, 2009; Hempel *et al*., 2013) and plants limited to specific symbionts appear to have their invasive potential constrained (Catford *et al*., 2009). Additionally, some symbioses are thought to help native plants maintain a competitive advantage over invasive species (Pringle *et al*., 2009). These interactions appear to be spatially variable, with an apparent split between North America and Europe (Pringle *et al*., 2009).

One of the most globally significant groups of plant symbionts are the arbuscular mycorrhizal (AM) fungi, which have been demonstrated to not only boost plant fitness (Begum *et al*., 2019) but also play a key role in the formation and stabilisation of soil aggregates (Piotrowski *et al*., 2004; Leifheit *et al*., 2014; Morris *et al*., 2019). Given the importance of AM fungal symbionts, a substantial amount of effort has been put into understanding the factors that influence AM fungi-plant symbioses. Many of these factors are also known to be impacted by urbanisation. For example; elevated nitrogen (Lovett *et al*., 2000) and phosphorus (Zhang *et al*., 2001; Liu *et al*., 2016) concentrations have been demonstrated to both occur in urban ecosystems and result in a down-regulation of AM fungal symbioses by plants (Bonneau *et al*., 2013; Nouri *et al*., 2014). Alterations in soil pH have also been observed to alter AM fungal colonisation, with acidity appearing to limit AM fungal colonisation (Clark, 1997; Coughlan *et al*., 2000). Despite the influence of acid rain, the overwhelming levels of alkaline compounds provided

by concrete dust generally result in a net-alkaline pH for urban soils (Newbound *et al*., 2010). This elevated pH reduces the availability of heavy metals and thus partially counteracts the high levels of heavy-metal contamination often observed at hotspots of anthropogenic activity (Xian and Shokohifard, 1989). AM fungal colonisation has been reported as being reduced in heavy-metal contaminated soils (Ferrol *et al*., 2016), although the role of heavy metals in the plant-AM fungi-soil system is likely to be complex, depending on the species involved. Indeed, some AM fungal species confer greater heavy metal tolerance to plants, whilst others can result in plants accumulating heavy metals (Ferrol *et al*., 2016). Previous research has sought to explore the ways in which urban systems shape AM fungal diversity. Cousins *et al*. (2003) investigated AM fungal spore diversity across different land uses in Arizona and observed a shift in community composition between urban sites and agricultural sites. Research carried out in the same study system found that AM fungal colonisation of roots was significantly lower in urban desert preserves than in rural desert (Ontiveros-Valencia, 2009). A very thorough study on the same subject by Bainard *et al*. (2011) also found a similar pattern in Ontario. They lent significant weight to their conclusions by limiting their study to 26 tree species, of which 11 showed a significant reduction in colonisation in urban sites compared to rural sites. Wiseman and Wells (2005) also found a significantly higher level of AM fungal colonisation in Acer rubrum in forested sites compared to those in developed areas. However, a study in Michigan has reported no change in AM fungal colonisation rate in saplings across a gradient of urbanity (Tonn and Ibáñez, 2017).

AM fungi are, however, not the only fungi which colonise roots. There are a wide range of other fungal symbioses that occur, including ecto- and ericoid-mycorrhiza and an array of non-mycorrhizal symbioses, the most abundant of which are the dark septate endophytes. The position of these non-mycorrhizal fungi on the "parasitism-mutualism continuum" (Mandyam and Jumpponen, 2015) is varied and often hard to discern, with some evidence for it changing according to the species, or even life stage, of the host plant involved (Berthelot *et al*., 2019). The benefits to plants of these mutualistic non-mycorrhizal symbioses range from increased heavy metal tolerance to accelerated growth rate (Mandyam and Jumpponen, 2005). The position of a particular symbiosis on this continuum may also be impacted by the environment (Berthelot *et al*., 2017), but this is debated (Kia *et al*., 2018). Colonisation rates do, however, appear to vary significantly in response to environmental conditions (Rillig *et al*., 1998) and to be most common in abiotically stressed environments (Mandyam and Jumpponen, 2005).

Here, we carried out an assessment of AM fungal and non-AM fungal colonisation from grassland sites across an urbanity gradient in Berlin, Germany. Through limiting the study to one habitat type and using Principal Component Analysis to extract three major axes of variation from a substantial database of environmental data, we were able to isolate the impacts of an urban 'syndrome', here referred to as urbanity, upon root colonisation and morphology. By using a gradient of sites rather than a paired-sample design we were able to use linear statistical models and have a greater degree of control over covarying environmental variables.

2.3 Methods

Berlin is a temperate central European city with around 3.5 million inhabitants. It is surrounded by the significantly more rural state of Brandenburg. Berlin's soil texture ranges from sand to sandy loam with a pH range of 4.1–7.5. Fifty-four grassland sites distributed across Berlin and into Brandenburg were sampled between 30th June and 13 August 2020 for this study. These 16 m2 sites were part of the CityScapeLabs research platform, a network of grassland sites situated along an urbanrural gradient (von der Lippe *et al*., 2020). Root samples were taken from three locales within each site. Sampling locales were selected which reflected the diversity of plants present in each site. A random selection of roots was taken from each locale. Samples were stored in paper bags before being dried at 60°C

overnight. Samples were subsequently stored at 4°C until processing. Only roots that were springy and had an intact cortex and tissue structure were selected for further analysis. Samples were bleached for 30 min in KOH, washed, acidified with 1% HCL for 12 min, and stained using 0.05% Trypan Blue in lactoglycerol for 40 min (Boedijn, 1956). Bleaching, acidification and staining were all performed in a 90° C water bath. Roots were subsequently mounted on slides in lactoglycerol.

AM fungal colonisation was quantified under ×200 magnification in accordance with McGonigle *et al*. (1990). At ~100 intersects per slide any AM fungal structure was recorded in a binary fashion (presence/absence), with additional binary counts of arbuscules, AM fungal vesicles and AM fungal coils. Non-AM fungal structures and root hairs were also recorded in the same way; we counted as non-AM fungal structures any fungal presence that was not attributable to AM fungi. Count data were used to calculate the percentage of presence for each variable.

Principal Component Analysis (PCA), performed using the ade4 package (Thioulouse *et al*., 2018) in R (R Core Team, 2021), was used to extract three main axes of variation from a preexisting database of environmental data for the sampled sites (von der Lippe *et al*., 2020). These three axes of data were found to relate to three ecologically relevant syndromes. The PCA axis scores, the age of sites (a binary variable representing pre-vs. post1945 establishment as a grassland), and the day of the year that sampling took place (to control for seasonal effects) were used to create 10 multiple linear regression models to explain each colonisation/root trait. Akaike model selection, performed using the AICcmodavg package (Mazerolle, 2020) in R, was then used to select the most appropriate model, taking into consideration the percentage of cumulative model weight. For a list of models included please see Supplementary Table S1.

A pre-existing dataset of plant communities was used to explore the impact of plant community composition on the traits measured in this study. This dataset consisted of the percentage-cover of 233 grassland plant species within each study site. Plant communities were plotted using non-metric multidimensional scaling (NMDS) with the root responses and the three PCA environmental axes plotted as contour lines, this was achieved using the vegan package (Oksanen *et al*., 2020) in R. These data were used to qualitatively assess any possible relationships between plant community, environmental variation and root traits. Responses which demonstrated relationships with plant community were selected for further investigation. A series of multiple linear regression models were created which included the axis scores of the correlating NMDS axis, which functioned as a proxy for plant community. Akaike model selection was then used to select the most appropriate multiple linear regression model.

2.4 Results

The PCA of environmental variables created three main axes of variation that could be categorised as 1) urbanity (Figure 2.1), 2) soil chemistry and water content, and 3) site connectivity and pH. For a full list of variable loadings see Supplementary Table S1. Akaike model selection found that the most appropriate model for each of the response variables varied. For the percentage of AM fungal presence, arbuscules, vesicles (log transformed) and coils (log transformed) a multiple linear regression model containing PCA axes one and two was found to be appropriate. However, for the percentage of non-AM fungal presence and root hairs a single linear regression using only PCA axis 1 as a predictor was a more appropriate model. For the full output of Akaike model selection please see Supplementary Table S3.

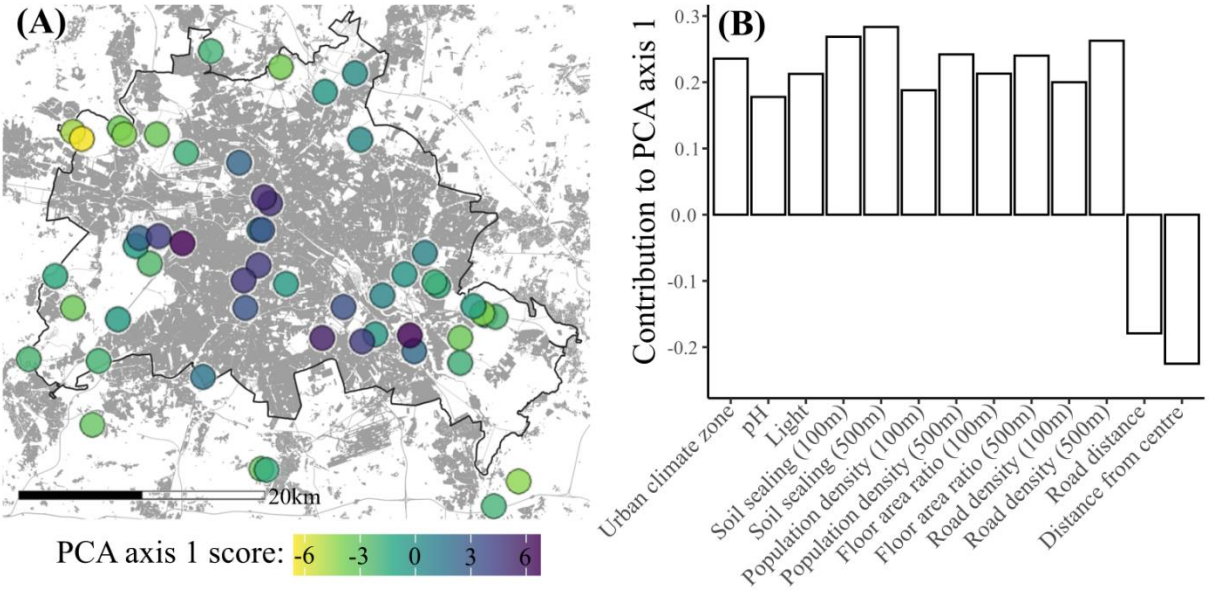

Figure 2.1 (A) Map of site locations. The colour of the point shows PCA axis one score, representing urbanity. The black line shows the border of Berlin and grey areas denote built up areas. (B) Major contributing variables to PCA axis 1, which can be categorised as an urban syndrome. For plots of all three of the main PCA axes please see Supplementary Figure S.2.1, with biplots in Supplementary Figure S.2.2.

We found that only two variables exhibited strong evidence for being driven by the environmental parameters included in their selected linear regression models. These variables were the presence of non-AM fungi and root hairs. Both of these responses showed a positive correlation with PCA axis one scores, meaning values increased at more urban sites (Figure 2.2). For the results of regression analyses please see Supplementary Table S.2.4.

Through plotting plant communities using NMDS (Supplementary Figure S.2.4), it was clear that plant community composition had a relationship with the levels of AM fungal, arbuscule and root hair presence. Plant communities did not appear to clearly shift with any of the environmental PCA axes although a trend with PCA axis 3, representing site connectivity, could be seen. Multiple linear regressions demonstrated that for AM fungi and arbuscules, presence was driven by plant community, whilst for root hairs the separate impacts of PCA axis one and plant community could not easily be disentangled, although there did not appear to be a significant interaction (Supplementary Table S.2.6).

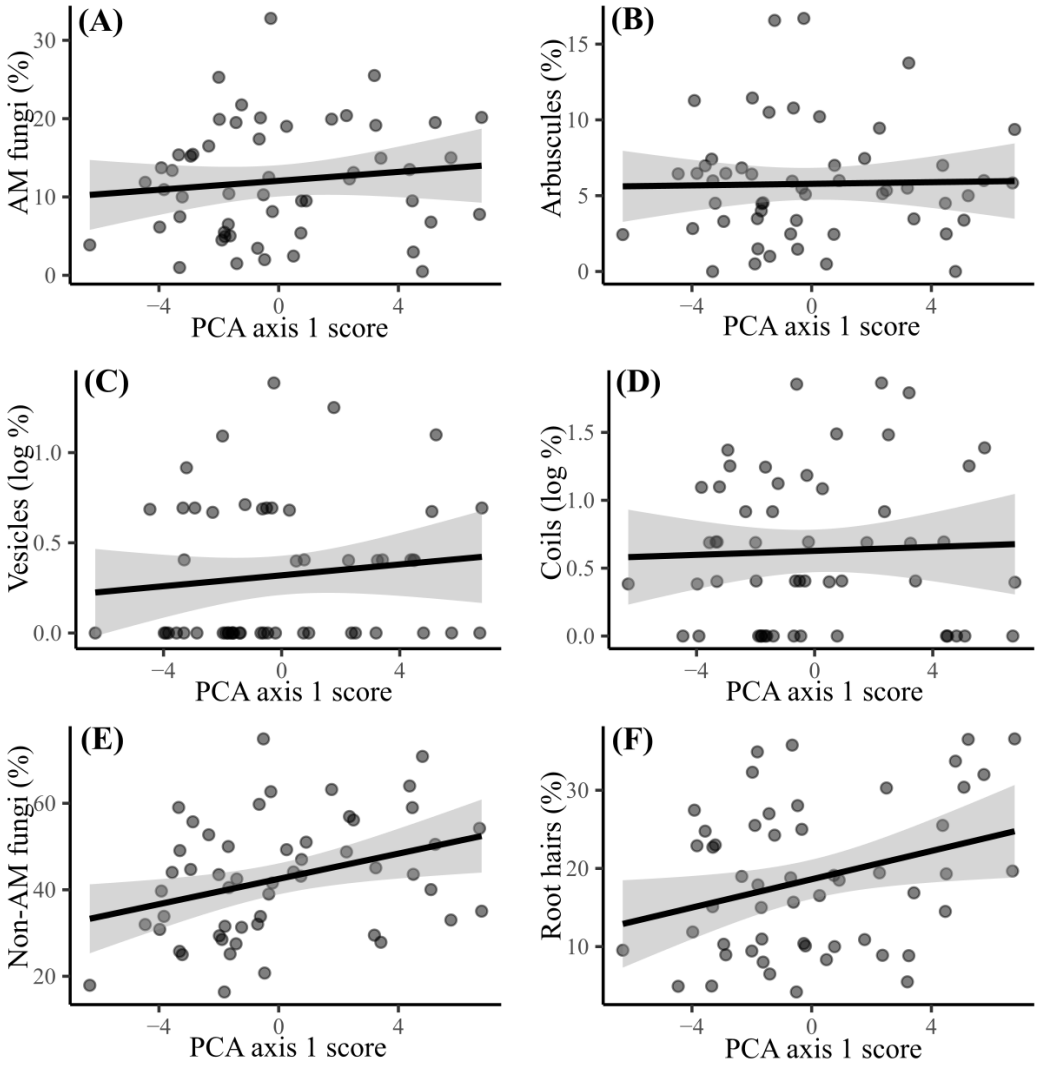

Figure 2.2 The correlation between PCA axis one score (representing urbanity) and the percentage of root locations containing (A) AM fungi; R2 = 0.016, F = 0.821, p = 0.369. (B) Arbuscules; R2 = 0.001, F = 0.026, p = 0.873. (C) Vesicles (log transformed); R2 = 0.015, F = 0.787, p = 0.379. (D) Coils (log transformed); R2 = 0.002, F = 0.088, p = 0.768. (E) Non-AM fungi; R2 = 0.117, F = 6.763, p = 0.012. (F) Root hairs; R2 = 0.094, F = 5.283, p = 0.026. Shaded areas represent 95% confidence intervals. For scatter plots of responses to all three PCA axes, see Supplementary Figure S.2.3.

2.5 Discussion

We found strong evidence for AM fungi-plant interactions, including the presence of arbuscules, being resilient to the effects of urbanity, being shaped instead by plant community. Contrastingly, our results provide strong evidence for non-AM fungal interactions with plant roots increasing with urbanity. Specifically, our study suggests that this change is due to an intrinsic element of an urban syndrome, rather than just soil chemistry. Functional roles of these non-mycorrhizal fungi within the roots may have been varied. The non-AM fungal structures observed could have belonged to parasitic fungi, nonmycorrhizal mutualists, or AM fungi that were not exhibiting AM characteristics (Orchard *et al.*, 2017). It is unlikely that they resulted from ectomycorrhiza, due to the absence of any distinguishable ectomycorrhizal structures being observed, or ericoid mycorrhiza, given the rarity of ericoid plants within Berlin's grasslands. If the increase in non-AM fungi was due to a reduction in AM structures

being exhibited by AM fungi, we would also expect to see a proportional reduction in observed AM structures, which we did not.

The direction of the relationship between observed non-AM fungal presence and urbanity is rather surprising, because almost all generally-accepted urban-related variables are thought to result in reduced levels of fungal activity. Indeed, many environmental parameters often thought indicative of urban systems were found to be contributors to PCA axes 2 and 3, rather than being collinear with variables such as population density and soil sealing in PCA axis 1. Heavy metals and increased pH (although pH also made a small contribution to PCA axis 1) are two such parameters, both of which are thought to negatively impact fungi in urban systems (Newbound *et al*., 2010). There are only a small number of urban-related parameters which may be expected to increase fungal activity, of these, the heat island effect is the only one that is represented in our dataset. The presence of an 'urban climate zone' parameter within PCA axis one makes temperature a strong candidate for being the cause of increased fungal activity. Additional factors not taken into account in this study such as the presence of animal faeces and remains have previously been demonstrated to increase fungal presence (Sagara, 1995; Watling, 1997; Newbound *et al*., 2010). Within our dataset graveyards accounted for some of the sites with highest PCA axis one scores, however, the sampling locations within these were not directly associated with graves and in one case was in a section of the graveyard that had been cleared for the creation of the Berlin wall. Site usage, such as dog walking, could also potentially be an explanation for altered fungal presence. However, the unimportance of soil chemistry in our study suggests that soil fertilisation via faecal deposition or decomposition in graveyards is an unlikely causal factor.

Through our use of PCA we were able to separate urbanity from soil chemistry. Our study provides evidence that we cannot solely rely on soil chemistry research to explain plant-fungal interactions in urban systems. Previous research from the same study system has suggested that urban soils have lower levels of aggregate stability (Whitehead *et al*., 2021), our current study suggests that a lack of AM fungi, previously described as key causal agents in aggregate formation (Lehmann *et al*., 2020), is likely not the leading cause of the reduction in aggregate stability in this system.

The increase in root hairs with increasing urbanity is a novel result which lacks any obvious explanation. It is surprising that such a trait, so clearly linked to the acquisition of nutrients, correlates with the PCA axis representing urbanity rather than that which represents soil chemistry. The trade-offs involved in root morphology are driven by a complex economy of carbon partitioning, growth rate and mycorrhizal colonisation (Bergmann *et al*., 2020). Within our study system however, there is no apparent trade-off between root hair presence and AM fungal colonisation, indeed, the only observable trend in fungal activity is collinear with root hair presence. One possible explanation for a change in root hair presence is a change in plant community, evidence for such an explanation can be seen in the NMDS plots in Supplementary Figure S.2.4. However, whilst plant community does indeed appear to have a significant role in determining root hair presence, the lack of any interaction between plant community and PCA axis one suggests that this is likely not the decisive link between root hair presence and urbanity, although it cannot be ruled out. Our findings suggest that an intrinsic element of urbanity has ecologically significant impacts on fungal presence in plant roots. This increase in non-AM fungal colonisation may be as a result of higher parasite load or an up-regulation of non-mycorrhizal mutualisms. We also found a general lack of significant response in AM fungi colonisation to urbanity, supporting the previous observations of Tonn and Ibáñez (2017). Our results for AM fungal colonisation share the lack of response to urbanity seen in ectomycorrhizal symbioses (Hui *et al*., 2017). The increase in root hair presence with urbanity is also interesting as there did not appear to be any trade-off with fungal colonisation.

2.6 Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Figshare: https://doi. org/10.6084/m9.figshare.19248926.v1 and https://doi.org/10. 6084/m9.figshare.19276481.v1.

2.7 Author contributions

JW, SH, and MR designed the research. JW conducted fieldwork and lab analyses, and wrote the first draft of the paper. All authors added to and edited the text.

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2.10 References

Asabere, S. B., Zeppenfeld, T., Nketia, K. A., and Sauer, D. (2018). Urbanization Leads to Increases in pH, Carbonate, and Soil Organic Matter Stocks of Arable Soils of Kumasi, Ghana (West Africa). Front. Environ. Sci. 6, 17. doi:10.3389/ fenvs.2018.00119

Bainard, L. D., Klironomos, J. N., and Gordon, A. M. (2011). The Mycorrhizal Status and Colonization of 26 Tree Species Growing in Urban and Rural Environments. Mycorrhiza 21 (2), 91–96. doi:10.1007/s00572-010-0314-6

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., *et al*. (2019). Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. Front. Plant Sci. 10, 1068. doi:10. 3389/fpls.2019.01068

Bergmann, J., Weigelt, A., van Der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., *et al*. (2020). The Fungal Collaboration Gradient Dominates the Root Economics Space in Plants. Sci. Adv. 6 (27), 9. doi:10. 1126/sciadv.aba3756

Berthelot, C., Blaudez, D., and Leyval, C. (2017). Differential Growth Promotion of poplar and Birch Inoculated with Three Dark Septate Endophytes in Two Trace Element-Contaminated Soils. Int. J. Phytoremed. 19 (12), 1118–1125. doi:10. 1080/15226514.2017.1328392

Berthelot, C., Chalot, M., Leyval, C., and Blaudez, D. (2019). "From Darkness to Light: Emergence of the Mysterious Dark Septate Endophytes in Plant Growth Promotion and Stress Alleviation," in Endophytes for a Growing World. Editors B. R. Murphy, F. M. Doohan, M. J. Saunders, and T. R. Hodkinson (Cambridge: Cambridge University Press), 143–164. doi:10.1017/9781108607667.008

Boedijn, K. B. (1956). Trypan Blue as a Stain for Fungi. Stain Techn. 31 (3), 115–116. doi:10.3109/10520295609113788

Bonneau, L., Huguet, S., Wipf, D., Pauly, N., and Truong, H. N. (2013). Combined Phosphate and Nitrogen Limitation Generates a Nutrient Stress Transcriptome Favorable for Arbuscular Mycorrhizal Symbiosis in M Edicago Truncatula. New Phytol. 199 (1), 188–202. doi:10.1111/nph.12234

Carreiro, M. M., and Tripler, C. E. (2005). Forest Remnants along Urban-Rural Gradients: Examining Their Potential for Global Change Research. Ecosystems 8 (5), 568–582. doi:10.1007/s10021-003-0172-6

Catford, J. A., Jansson, R., and Nilsson, C. (2009). Reducing Redundancy in Invasion Ecology by Integrating Hypotheses into a Single Theoretical Framework. Divers. Distributions 15 (1), 22–40. doi:10.1111/j.1472-4642. 2008.00521.x

Clark, R. B. (1997). Arbuscular Mycorrhizal Adaptation, Spore Germination, Root Colonization, and Host Plant Growth and mineral Acquisition at Low pH. Plant Soil 192 (1), 15–22. doi:10.1023/a:1004218915413

Coughlan, A. P., Dalpé, Y., Lapointe, L., and Piché, Y. (2000). Soil pH-Induced Changes in Root Colonization, Diversity, and Reproduction of Symbiotic Arbuscular Mycorrhizal Fungi from Healthy and Declining maple Forests. Can. J. For. Res. 30 (10), 1543–1554. doi:10.1139/x00-090

Cousins, J. R., Hope, D., Gries, C., and Stutz, J. C. (2003). Preliminary Assessment of Arbuscular Mycorrhizal Fungal Diversity and Community Structure in an Urban Ecosystem. Mycorrhiza 13 (6), 319–326. doi:10.1007/s00572-003-0239-4

Dmuchowski, W., Baczewska-Dabrowska, A., Gozdowski, D., Bragoszewska, P., Gworek, B., Suwara, I., *et al*. (2021). Effect of Salt Stress in Urban Conditions on Two Acer Species with Different Sensitivity. PeerJ 9, 26. doi:10.7717/peerj. 10577

Ferrol, N., Tamayo, E., and Vargas, P. (2016). The Heavy Metal Paradox in Arbuscular Mycorrhizas: from Mechanisms to Biotechnological Applications. J. Exp. Bot. 67 (22), 6253–6265. doi:10.1093/jxb/erw403

Gaertner, M., Wilson, J. R. U., Cadotte, M. W., MacIvor, J. S., Zenni, R. D., and Richardson, D. M. (2017). Non-native Species in Urban Environments: Patterns, Processes, Impacts and Challenges. Biol. Invasions 19 (12), 3461–3469. doi:10.1007/s10530-017-1598-7

Hanfi, M. Y., Mostafa, M. Y. A., and Zhukovsky, M. V. (2020). Heavy Metal Contamination in Urban Surface Sediments: Sources, Distribution, Contamination Control, and Remediation. Environ. Monit. Assess. 192 (1), 32. doi:10.1007/s10661-019-7947-5

Hempel, S., Götzenberger, L., Kühn, I., Michalski, S. G., Rillig, M. C., Zobel, M., *et al*. (2013). Mycorrhizas in the Central European flora: Relationships with Plant Life History Traits and Ecology. Ecology 94 (6), 1389–1399. doi:10.1890/ 12-1700.1

Hui, N., Jumpponen, A., Francini, G., Kotze, D. J., Liu, X., Romantschuk, M., *et al*. (2017). Soil Microbial Communities Are Shaped by Vegetation Type and Park Age in Cities under Cold Climate. Environ. Microbiol. 19 (3), 1281–1295. doi:10.1111/1462-2920.13660

Kia, S. H., Jurkechova, M., Glynou, K., Piepenbring, M., and Maciá-Vicente, J. G. (2018). The Effects of Fungal Root Endophytes on Plant Growth Are Stable along Gradients of Abiotic Habitat Conditions. FEMS Microbiol. Ecol. 94 (2), 10. doi:10.1093/femsec/fix162

Lehmann, A., Zheng, W., Ryo, M., Soutschek, K., Roy, J., Rongstock, R., *et al*. (2020). Fungal Traits Important for Soil Aggregation. Front. Microbiol. 10, 2904. doi:10.3389/fmicb.2019.02904

Leifheit, E. F., Veresoglou, S. D., Lehmann, A.,Morris, E. K., and Rillig,M. C. (2014).Multiple Factors Influence the Role of Arbuscular Mycorrhizal Fungi in Soil Aggregation-A MetaAnalysis. Plant Soil 374 (1-2), 523–537. doi:10.1007/s11104-013-1899-2

Liu, W., Zhang, Y., Jiang, S., Deng, Y., Christie, P., Murray, P. J., *et al*. (2016). Arbuscular Mycorrhizal Fungi in Soil and Roots Respond Differently to Phosphorus Inputs in an Intensively Managed Calcareous Agricultural Soil. Sci. Rep. 6, 24902. doi:10.1038/srep24902

Lovett, G. M., Traynor, M. M., Pouyat, R. V., Carreiro, M. M., Zhu, W.-X., and Baxter, J. W. (2000). Atmospheric Deposition to Oak Forests along an Urban−Rural Gradient. Environ. Sci. Technol. 34 (20), 4294–4300. doi:10.1021/es001077q

Luederitz, C., Brink, E., Gralla, F., Hermelingmeier, V., Meyer, M., Niven, L., *et al*. (2015). A Review of Urban Ecosystem Services: Six Key Challenges for Future Research. Ecosystem Serv. 14, 98–112. doi:10.1016/j.ecoser.2015.05.001

Mandyam, K., and Jumpponen, A. (2005). Seeking the Elusive Function of the Root-Colonising Dark Septate Endophytic Fungi. Stud. Mycol. 53, 173–189. doi:10.3114/sim.53.1.173

Mandyam, K. G., and Jumpponen, A. (2015). Mutualism-parasitism Paradigm Synthesized from Results of Root-Endophyte Models. Front. Microbiol. 5, 776. doi:10.3389/fmicb.2014.00776

Mazerolle, M. J. (2020). AICcmodavg: Model Selection and Multimodel Inference Based on (Q)AIC(c). R Package Version 2.3-1. Available at: https://cran.rproject.org/package=AICcmodavg (Accessed January 28, 2022).

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., and Swan, J. A. (1990). A New Method Which Gives an Objective Measure of Colonization of Roots by Vesicular-Arbuscular Mycorrhizal Fungi. New Phytol. 115 (3), 495–501. doi:10.1111/j.1469-8137.1990.tb00476.x

McKinney, M. L. (2008). Effects of Urbanization on Species Richness: A Review of Plants and Animals. Urban Ecosyst. 11 (2), 161–176. doi:10.1007/s11252-007-0045-4

Morris, E. K., Morris, D. J. P., Vogt, S., Gleber, S.-C., Bigalke, M., Wilcke, W., *et al*. (2019). Visualizing the Dynamics of Soil Aggregation as Affected by Arbuscular Mycorrhizal Fungi. ISME J. 13 (7), 1639–1646. doi:10.1038/s41396-019-0369-0

Newbound, M., McCarthy, M. A., and Lebel, T. (2010). Fungi and the Urban Environment: A Review. Landscape Urban Plann. 96 (3), 138–145. doi:10.1016/ j.landurbplan.2010.04.005

Nouri, E., Breuillin-Sessoms, F., Feller, U., and Reinhardt, D. (2014). Phosphorus and Nitrogen Regulate Arbuscular Mycorrhizal Symbiosis in Petunia Hybrida. Plos One 9 (3), 14. doi:10.1371/journal.pone.0090841

Oksanen, J., Guillaume, B. F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., *et al*. (2020). Vegan: Community Ecology Package. R Package Version 2.5-7. Available at: https://CRAN.R-project.org/package=vegan (Accessed August 18, 2021).

Ontiveros-Valencia, A. (2009). Arbuscular Mycorrhizal and Dark Septate Endophytic Fungi and Urban Preserves and Surrounding Sonoran Desert. Master's Thesis. Tempe (AZ): University of Arizona.

Orchard, S., Standish, R. J., Dickie, I. A., Renton, M., Walker, C., Moot, D., *et al*. (2017). Fine Root Endophytes under Scrutiny: a Review of the Literature on Arbuscule Producing Fungi Recently Suggested to Belong to the Mucoromycotina. Mycorrhiza 27 (7), 619–638. doi:10.1007/s00572-017-0782-z

Piotrowski, J. S., Denich, T., Klironomos, J. N., Graham, J. M., and Rillig, M. C. (2004). The Effects of Arbuscular Mycorrhizas on Soil Aggregation Depend on the Interaction between Plant and Fungal Species. New Phytol. 164 (2), 365–373. doi:10.1111/j.1469-8137.2004.01181.x

Pringle, A., Bever, J. D., Gardes, M., Parrent, J. L., Rillig, M. C., and Klironomos, J. N. (2009). Mycorrhizal Symbioses and Plant Invasions. Annu. Rev. Ecol. Evol. Syst. 40, 699–715. doi:10.1146/annurev.ecolsys.39.110707.173454

R Core Team (2021). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.Rproject.org/.

Rillig, M. C., Allen, M. F., Klironomos, J. N., Chiariello, N. R., and Field, C. B. (1998). Plant Species-specific Changes in Root-Inhabiting Fungi in a California Annual Grassland: Responses to Elevated CO 2 and Nutrients. Oecologia 113 (2), 252–259. doi:10.1007/s004420050376

Sagara, N. (1995). Association of Ectomycorrhizal Fungi with Decomposed Animal Wastes in forest Habitats - a Cleaning Symbiosis. Can. J. Botany 73, S1423–S1433. doi:10.1139/b95-406

Tonn, N., and Ibáñez, I. (2017). Plant-mycorrhizal Fungi Associations along an Urbanization Gradient: Implications for Tree Seedling Survival. Urban Ecosyst. 20 (4), 823–837. doi:10.1007/s11252-016-0630-5

Thioulouse, J., Dray, S., Dufour, A., Siberchicot, A., Jombart, T., and Pavoine, S. (2018). Multivariate Analysis of Ecological Data with ade4. Springer. doi:10.1007/978-1-4939-8850-1

Vogelsang, K. M., and Bever, J. D. (2009). Mycorrhizal Densities Decline in Association with Nonnative Plants and Contribute to Plant Invasion. Ecology 90 (2), 399–407. doi:10.1890/07-2144.1

von der Lippe, M., Buchholz, S., Hiller, A., Seitz, B., and Kowarik, I. (2020). CityScapeLab Berlin: A Research Platform for Untangling Urbanization Effects on Biodiversity. Sustainability 12 (6), 30. doi:10.3390/su12062565

Watling, R. (1997). Pulling the Threads Together: Habitat Diversity. Biodiver. Conserv. 6 (5), 753–763. doi:10.1023/a:1018374404998

Whitehead, J., Hempel, S., Hiller, A., von der Lippe, M., and Rillig, M. C. (2021). Soil Physico-Chemical Properties Change across an Urbanity Gradient in Berlin. Front. Environ. Sci. 9 (516), 765696. doi:10.3389/fenvs.2021.765696

Wiseman, P. E., and Wells, C. (2005). Soil Inoculum Potential and Arbuscular Ycorrhizal Colonization of Acer Rubrum in Forested and Developed Landscapes. J. Arboric. 31 (6), 296–302. doi:10.48044/jauf.2005.038

Xian, X. F., and Shokohifard, G. I. (1989). Effect of pH on Chemical Forms and Plant Availability of Cadmium, Zinc, and lead in Polluted Soils. Water Air Soil Pollut. 45 (3-4), 265–273. doi:10.1007/bf00283457

Yang, J.-L., and Zhang, G.-L. (2011). Water Infiltration in Urban Soils and its Effects on the Quantity and Quality of Runoff. J. Soils Sediments 11 (5), 751–761. doi:10.1007/s11368-011-0356-1

Zhang, G.-L., Burghardt, W., Lu, Y., and Gong, Z.-T. (2001). Phosphorusenriched Soils of Urban and Suburban Nanjing and Their Effect on Groundwater Phosphorus. J. Plant Nutr. Soil Sci. 164 (3), 295–301. doi:10.1002/1522-2624(200106)164:33.0.co;2-t

Ziska, L. H., Gebhard, D. E., Frenz, D. E., Faulkner, S., Singer, B. D., and Straka, J. G. (2003). Cities as Harbingers of Climate Change: Common Ragweed, Urbanization, and Public Health. J. Allergy Clin. Immunol. 111 (2), 290–295. doi:10.1067/mai.2003.53

Chapter Three

Soil microbial communities shift along an urban gradient in Berlin, Germany

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3.1 Abstract

The microbial communities inhabiting urban soils determine the functioning of these soils, in regards to their ability to cycle nutrients and support plant communities. In an increasingly urbanized world these properties are of the utmost importance, and the microbial communities responsible are worthy of exploration. We used 53 grassland sites spread across Berlin to describe and explain the impacts of urbanity and other environmental parameters upon the diversity and community composition of four microbial groups. These groups were (i) the Fungi, with a separate dataset for (ii) the Glomeromycota, (iii) the Bacteria, and (iv) the protist phylum Cercozoa. We found that urbanity had distinct impacts on fungal richness, which tended to increase. Geographic distance between sites and soil chemistry, in addition to urbanity, drove microbial community composition, with site connectivity being important for Glomeromycotan communities, potentially due to plant host communities. Our findings suggest that many microbial species are well adapted to urban soils, as supported by an increase in diversity being a far more common result of urbanity than the reverse. However, we also found distinctly separate distributions of operational taxonomic unit (OTU)s from the same species, shedding doubt of the reliability of indicator species, and the use of taxonomy to draw conclusion on functionality. Our observational study employed an extensive set of sites across an urbanity gradient, in the region of the German capital, to produce a rich microbial dataset; as such it can serve as a blueprint for other such investigations.

3.2 Introduction

Recent research suggests that humans have heavily impacted the majority of the planet's ecosystems for at least 12,000 years (Ellis *et al*., 2021). The pinnacle of human impact occurs within urban landscapes. Definitions of urbanity vary between countries, but in 2014 one estimate of the total land surface covered by urban development was between 2 and 3% (Liu *et al*., 2014). According to the United Nations, the current trend for urban expansion is likely to result in 68% of people living in urban centers by 2050 (UNDESA Population Division, 2018).

Urban ecosystems contain a unique array of anthropogenic factors, including many relating to global change. For example; summer temperatures in Berlin, Germany, have been reported as reaching ~10°C higher in highly sealed areas compared to less sealed areas (Dugord, 2013). This situation, known as the urban heat island effect, is expected to become increasingly significant for northern European cities where urban planning has generally not taken high temperatures into account (Ward *et al*., 2016). Exploring the impacts of these increased temperatures can give advance warning of what to expect from future global temperature spikes. Increased salinity has also been reported in urban landscapes due to irrigation (Ganjegunte *et al*., 2017) and road de-icing salt, which in some cases reaches levels toxic for land plants (Cunningham *et al*., 2008). Nitrogen deposition is pervasive in urban ecosystems, although accurately quantifying levels is difficult (Decina *et al*., 2020). Heavy metal accumulation has also been recorded in many urban ecosystems as a result of anthropogenic activity (e.g., Facchinelli *et al*., 2001; Lee *et al*., 2006). However, the impact of heavy metals in urban soils is likely to be buffered: heavy metals are known to demonstrate substantially reduced availability with increasing pH, which has been shown to occur in urban ecosystems due to deposition of alkaline ash and construction dust (Newbound *et al*., 2010). Urban systems are also likely to experience day-today, localized disturbances due to activities such as dog-walking and jogging, the levels of which may determine their impact upon biodiversity. It is possible that anthropogenic activities may even increase biotic diversity according to the intermediate disturbance hypothesis (Connell, 1978).

The relationships between urban systems and biodiversity are still being explored. It appears that different organism groups respond in distinct manners. A general rule of thumb appears to be that in

exceptionally urban areas the richness of plants and animals is reduced, whilst at more "standard" urban locations levels of plant richness are increased in comparison to nonurban locations (due in large part to the presence of non-native species), whilst animal richness decreases (McKinney, 2008). Some species of animals (e.g., the house mouse, *Mus musculus*) have been described as living a commensal, or even "anthrodependent," life with humans which results in a global distribution across urban biomes (Johnson and Munshi-South, 2017). Research in this regard for soil microorganisms is somewhat patchy, most urban soil research having focused on ecosystem services (O'Riordan *et al*., 2021).

Studies of urban soil microbial diversity have thus far found differing patterns between fungal and bacterial responses to urbanity. Evidence suggests that fungal diversity tends to decrease in urban areas, compared to nearby "natural" locations (Abrego *et al*., 2020; Tedersoo *et al*., 2020; Donald *et al*., 2021), with this pattern even extending to fungal richness being lower in road medians than in urban parks (Reese *et al*., 2016). Bacterial communities, on the other hand, appear to increase in diversity in urban soils (Delgado-Baquerizo *et al*., 2021; Donald *et al*., 2021), with some evidence that protists express the same pattern (Delgado-Baquerizo *et al*., 2021). However, community composition is also a key component of microbial ecology. Some evidence suggests that globally, urban fungal communities may homogenize and become more similar to one another (Delgado-Baquerizo *et al*., 2021) with fungal groups favored by urban systems being plant parasites and arbuscular mycorrhizal fungi (AMF) (Donald *et al*., 2021). Alternatively, urban soils may favor generalists (Abrego *et al*., 2020). Other patterns observed include a reduced abundance of ectomycorrhizal fungi (Delgado-Baquerizo *et al*., 2021) and shifts in AMF community composition (Cousins *et al*., 2003). Bacterial nitrifiers and plant parasites have been reported as showing increased abundance in urban soils (Donald *et al*., 2021), with a fast-growing lifestyle being favored (Delgado-Baquerizo *et al*., 2021).

The general list of factors governing microbial biogeographic patterns can be classified into selection, drift, dispersal, and mutation (Vellend, 2010; Hanson *et al*., 2012). Within urban systems it is as-yet unclear whether selection favors specialists or generalists, and it is also unclear whether dispersal is more heavily constrained by habitat fragmentation, or promoted through transport infrastructure. Highly urban environments may also include niches not seen elsewhere, or the exclusion of normally dominant species due to unfavourable growing conditions. Here, we examined the diversity of soil microorganisms across the urban landscape of Berlin, one of the biggest cities of Europe, with the goal of understanding whether urban soil ecology is shaped by soil chemistry or an intrinsic element of the urban syndrome. Our investigation spanned three kingdoms, namely, Bacteria, Fungi, and Protists. Within the Fungi, we included an additional focus on the plant symbionts, the AMF (phylum Glomeromycota). Within the Protists, we limited our study to the highly diverse phylum Cercozoa (Öztoprak *et al*., 2020).

Previous research from the same study system as that of this study has observed a change in soil physico-chemical properties in response to an "urban syndrome" of variables (Whitehead *et al*., 2021). These changes included a reduction in aggregate stability and an increase in water infiltration rate. Both fungi and bacteria influence soil physico-chemical properties through secreting compounds which result in hydrophobicity, thus increasing water run-off potential but also promoting aggregate stability (Mataix-Solera and Doerr, 2004; Epstein *et al*., 2011). Filamentous fungal hyphae also physically aid in aggregate development and function as translocation routes for unicellular microorganisms (Wick *et al*., 2007). We decided to investigate Glomeromycotan diversity at a high degree of precision within the fungal kingdom due the pivotal role this group plays in ecosystem functioning, including aggregate formation and stability (Rillig and Mummey, 2006; Leifheit *et al*., 2014). In addition, AMF colonize 75% of all land plants, playing a vital role in plant fitness (Treseder and Cross, 2006; Brundrett and Tedersoo, 2018). Plants themselves experience varying levels of dispersal limitation and shifts in community assemblage in urban ecosystems depending on their own dispersal traits (Schleicher *et al*.,

2011). It is possible that this may provide a top-down control on Glomeromycotan diversity through host selection (Grünfeld *et al*., 2021). The possibility of this is, however, subject to debate (Horn *et al*., 2017). Previous research, also from the study system used here, has demonstrated an increase in non-mycorrhizal root colonization in response to the urban syndrome, but a resilience of AMF colonization rates (Whitehead *et al*., 2022). It is as-yet unclear whether there is a similar resilience in Glomeromycotan community composition.

Despite biogeography having been born from the study of protists (Schewiakoff, 1893), it is only in recent years that the importance of protists within soil ecosystems has been truly recognized (Geisen *et al*., 2018). Containing a degree of diversity previously labeled "near-imponderable" (Foissner, 1999), protists have been described as "puppet-masters" that exhibit top down control on the soil microbiome (Gao *et al*., 2019). Given the dearth of previous research into urban protist communities and the difficulty entailed in accurately presenting their diversity, we have limited our investigation to the Cercozoa. This highly diverse group of flagellate protists have been demonstrated to influence bacterial communities through selective predation (Glücksman *et al*., 2010) and are possible to target through the use of a primer pair (Fiore-Donno *et al*., 2018).

In this study, we examined shifts in soil microbial communities across the urban landscape of Berlin, with the aim of understanding if intrinsically urban variables drive microbial community richness and composition.

3.3 Materials and methods

3.3.1 Study site

Our study was conducted in Berlin, Germany. The soil textures of Berlin are limited to sand, medium loamy sand and medium silty sand, with a pH range of 4.1–7.5. The yearly average temperature is 9.9°C, with an average yearly rainfall of 976 mm, although recent years have contained periods of reduced rainfall and higher temperatures (von der Lippe *et al*., 2020). In order to make our results internally comparable we limited our study to dry grasslands. For this study we sampled 53 4x4 m grassland plots, all of which were part of the CityScapeLabs research platform (von der Lippe *et al*., 2020). A wealth of data is available for these sites, discussed below in the section "Database of environmental data."

Spread across Berlin and into its surrounding federal state, Brandenburg, our study sites represent a gradient of urban locations, consisting of parks, graveyards, forest clearings, road and rail embankments, and derelict land.

3.3.2 Collection and extraction

The 53 dry grassland plots from the CityScapeLabs research platform used for this study were sampled during the summer of 2017. Fifteen evenly space replicates of 30 cm deep soil cores were taken from each site using a soil-corer and were homogenized in the field in plastic bags, before being divided into three 1 ml Eppendorf tubes. These samples were handled using sterile gloves to prevent contamination, and were temporarily placed in a cooler in the field before being stored in a -20°C freezer. Soil DNA was extracted using the DNeasy PowerSoil Pro Kit (Qiagen, Venlo, Netherlands) following the user instructions.

3.3.3 Amplification and sequencing

Four taxonomic groups were targeted using specific polymerase chain reaction (PCR) primer pairs. Fungi were identified through the sequencing of the ITS2 region using primers fITS7/ITS4 (Ihrmark *et al*., 2012). The Glomeromycota were identified through the sequencing of the LSU region using a nested PCR protocol with AMF-specific primers (Krüger *et al*., 2009) and LR2rev/LR3 (Horn *et al*., 2014). Bacteria ere identified by sequencing the 16S region using primers Eub_338f/Eub_518r (Ghyselinck *et al*., 2013), and the Protistan division Cercozoa were identified by sequencing the 18S region using primers S963R_Cerco/S947R_Cerco (Fiore-Donno *et al*., 2018). Following PCR, amplification was checked via gel electrophoresis. Samples were purified using solid phase reversible immobilization (SPRI) magnetic beads, indexed, and pooled. DNA concentrations were quantified using first a Qubit and then an Agilent TapeStation. 2*300 bp paired-end Illumina MiSeq sequencing was carried out at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv).

3.3.4 Bioinformatics and data preparation

Sequencing results were passed through a bioinformatics pipeline in R (R Core Team, 2020) using DADA2 (Callahan *et al*., 2016), ShortRead (Morgan *et al*., 2009), and Biostrings (Pagès *et al*., 2021). The DADA2 pipeline produced 100% similarity operational taxonomic unit (OTUs), however, for a more realistic representation of fungal diversity (Roy *et al*., 2019; Estensmo *et al*., 2021), fungal and Glomeromycotan OTUs were reclustered into 97% OTUs. Taxonomic assignments were carried out using the UNITE fungal database for fungal and Glomeromycotan datasets (Nilsson *et al*., 2019) using the assignTaxonomy() function within DADA2. Glomeromycotan assignments were compared using Blast (Park *et al*., 2012) to the Krüger *et al*. (2012) and NCBI databases (NCBI Resource Coordinators, 2016). Bacterial taxonomies were assigned using the Genome Taxonomy Database (Parks *et al*., 2021) and the Cercozoan taxonomies were assigned using the PR2 database (Guillou *et al*., 2013). Only assignments with bootstrapped probability values of 100% were kept. Each dataset had some samples where only low numbers of reads were present, these sites were removed leaving the total number of sites represented in each dataset as: Fungi, n = 51; Glomeromycota, n = 52; Bacteria, n = 48; Cercozoa, n = 52. The datasets were then normalized using rarefaction to the lowest remaining read count, resulting in read counts in every site of: Fungi, n = 30010; Glomeromycota, n = 16974; Bacteria, n = 20115; Cercozoa, n = 10944. This data was used for all of the following analysis, except for the non-metric multidimensional scaling (NMDS), for which Hellinger-transformed data was used.

3.3.5 Database of environmental data

A database of environmental data was established for these sites in 2017 as part of the CityScapeLabs research platform (von der Lippe *et al*., 2020). For this study a selection of 45 variables were used, of which 44 were continuous variables and one was categorical, denoting whether sites existed as grasslands prior to 1945, or were only established post-1945. Continuous variables related to site connectivity, site size, slope, plant and litter cover, various measures relating to urbanity, including population density, road density and proximity, railway density and proximity, soil sealing, and distance to the official city center (Flächenschwerpunkt Berlin). Soil chemical properties included N, S, P, K, organic C, and the heavy metals cadmium (Cd), copper (Cu), lead (Pb), Nickel (Ni), and Zinc (Zn), as well as pH, cation exchange capacity and water content. Latitude and longitude were also included. The soil chemical parameters were properties measured in samples taken concurrently to those used in this study. In order to be able to practically use this dataset outliers were removed and the variables were collapsed into 3 main axes of variation via principal component analysis (PCA), a technique pioneered in urban ecology by du Toit and Cilliers (2011), for which we used the dudi_pca() function in the ade4 package (Thioulouse *et al*., 2018). The axes chosen for inclusion in data analysis were selected

qualitatively by whether they reflected plausible environmental parameters/syndromes (e.g., urbanity, soil chemistry, see Section "Results").

3.3.6 Data analysis

All data analyses were carried out in R (R Core Team, 2020). In order to explore potential drivers of OTU richness, multiple linear regressions were used to examine relationships between OTU richness and each of the three PCA axes, with site age included as an additional predictor. To understand the roles of these variables in shaping community composition, Permutational multivariate analysis of variance (PERMANOVA) of each community data frame was also performed using the same list of predictors, using the adonis() function in vegan (Oksanen *et al*., 2020).

PERMANOVA models exploring total variance (i.e., where the total variation explained by each predictor, ignoring predictor overlap, was reported) and single predictor-unique variance were performed, both in models including and excluding site age. To visualize community composition, NMDS was performed upon community data using the metaMDS() function in the vegan package with PCA axis scores overlaid using the ordisurf() function. To complement this analysis and explore the relative importance of geographic distance upon community composition, distance-decay analysis was performed using both simple and partial Mantel tests (in the vegan package). To do this, Bray–curtis community distances were correlated with both Euclidean distances between PCA axis scores and geographic distances, which had been calculated using the geosphere package (Hijmans, 2019). Beta diversity partitioning was performed using the "betapart" package (Baselga and Orme, 2012) to reveal the relative importance of species turnover and nestedness.

Heatmaps of the top 20 most abundant OTUs within each dataset were created using the vegan, reshape2 (Wickham, 2007), tidyr (Wickham, 2021), and viridis (Garnier *et al*., 2021) packages. In order to identify taxonomic groups which showed responses to our environmental axes, we used Kendall correlations to select groups which expressed significant correlations in richness with any of the three PCA axes, using the corrr package (Kuhn *et al*., 2020). In order to ascertain which environmental parameter was most important in driving the richness of these taxonomic groups, hierarchical partitioning was performed using the package heir.part (Nally and Walsh, 2004). Additionally, indicator species analysis was carried out by splitting the sites into two levels according to their PCA axis 1 score. In order to do this, the 18 sites with the lowest PCA axis 1 scores were categorized as being the most urban. OTUs which were both statistically likely to be indicator species of these highly urban sites, and were assigned at the species level, were recorded. This analysis was carried out using the indicspecies package (De Caceres and Legendre, 2009).

3.4 Results

3.4.1 Principal component analysis of environmental data

Using PCA we extracted three axes of environmental variation for the study sites (Figure 3.1; for biplots of PCA axes see Supplementary Figures S.3.1, S.3.2, and for a list of variable loadings see Supplementary Table S.3.1). PCA axis 1 (21.2% of total variance) reflected a syndrome of urban variables, providing a gradient of "urbanity" across sites, with low scores representing more urban sites. This "urban syndrome" consisted of soil sealing, urban climate zone, floor area ratio (FAR, a parameter reflecting building development), road density and distance from the city center. PCA axis 2 (10.8% of total variance) reflected a gradient of soil chemical properties including nutrients and heavy metals. Sites with high axis 2 scores generally had high levels of metals such as Cadmium and Zinc, and high levels of Nitrogen. PCA axis 3 (7.9%) reflected a more complex gradient, separating sites according to

a collection of variables including some clearly urban-related variables such as site connectivity, and the density of roads and railways. Also included in PCA axis 3 was the size of the grassland patch, and some chemical variables such as C:N ratio and cation exchange capacity.

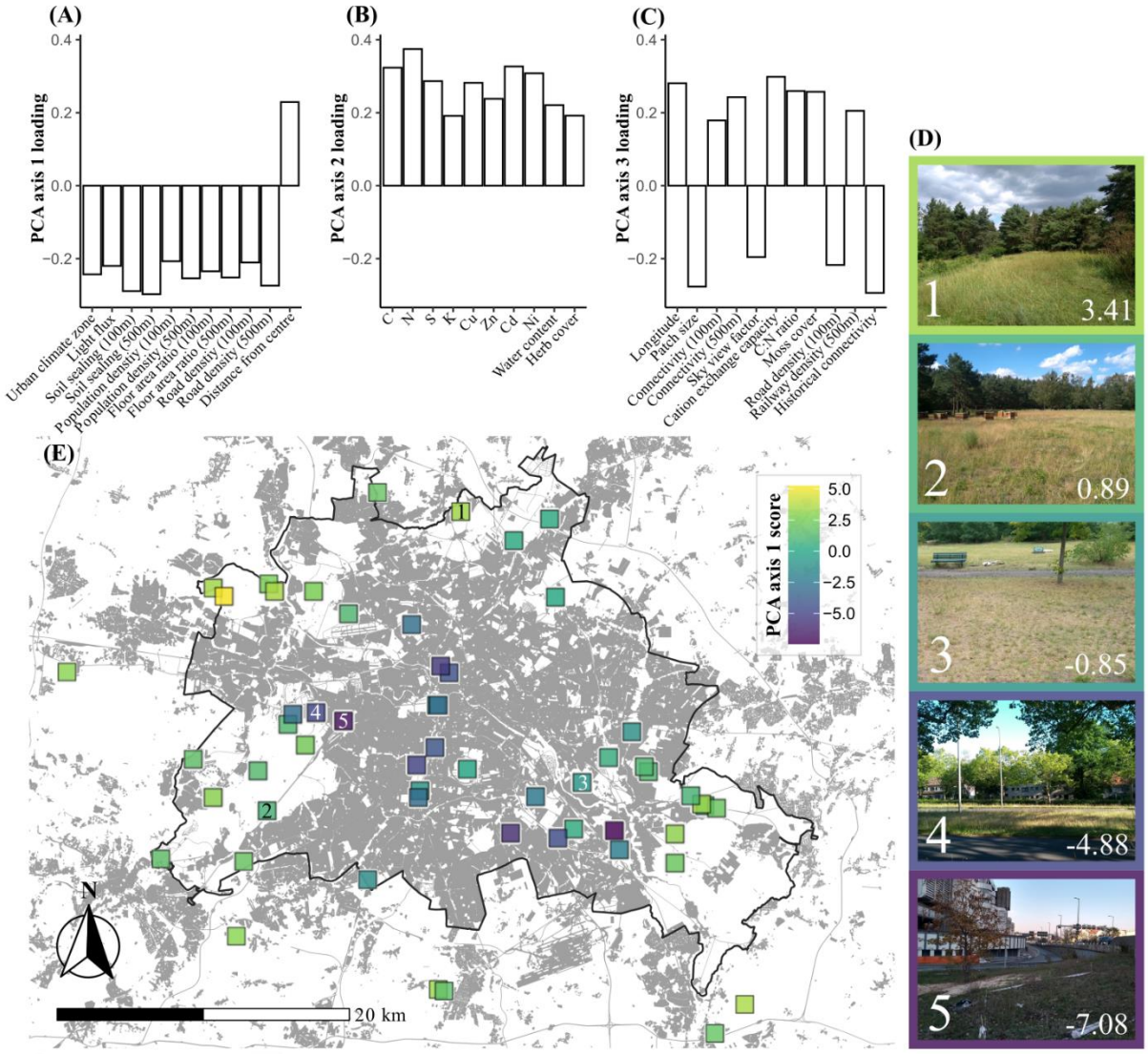

Figure 3.1 Environmental gradients across grasslands in Berlin. (A) Environmental variable loadings within principal component analysis (PCA) axis 1 (21.2% of variance), which can be summarized as being representative of urbanity. (B) Variable loadings for PCA axis 2 (10.8%), which can be summarized as being representative of soil chemistry. (C) Variable loadings for PCA axis 3 (7.9%), which contains an amalgamation of variables, but with patch size, sky view factor, connectivity, and historical connectivity suggesting that this axis could be seen as something of a vignette of physical site characteristics. (D) Examples of sites with differing PCA axis 1 scores, which are denoted in the bottom right hand corner of each image. Numbers in the bottom left hand corners denote locations on the map. (E) A map of Berlin with the locations of the sites included in this study. Each site is shaded by its PCA axis 1 score, representing urbanity. Darker coloration indicates more urban sites. The black line represents the border between Berlin and its neighboring federal state, Brandenburg. Areas shaded gray represent built-on land, originally plotted by Anne Hiller (Technisches Universität Berlin), using data from the Landesamt für Umwelt Brandenburg (2009) and Senate Department for Urban Development and Housing (2014).

Principal component analysis axis 1 scores were found to vary between sites established as grasslands before 1945 and those established since (Supplementary Figure S.3.3). This collinearity was taken into account in further statistical analyses.

3.4.2 Total community composition

A table showing a breakdown of OTU richness for each taxonomic group can be seen in Supplementary Table 2, presented as pie charts in Supplementary Figure S.3.4 and as area plots in Supplementary Figure S.3.5.

The fungal dataset contained 1530510 reads. Reads had a mean length of 286 bases, with a standard deviation of 57. The most abundant phyla present were the Ascomycota (65% of reads), Basidiomycota (23%), Mortierellomycota (6%), Glomeromycota (1%), and the Chytridiomycota (1%). 1% of reads were split between 10 low-abundance phyla (see Supplementary Figure S.3.4A). 3% of reads were unassigned at the phylum level.

The Glomeromycota-specific dataset contained 882648 reads. Reads had a mean length of 382 bases, with a standard deviation of 38. All four orders of the Glomeromycota were present; the Glomerales (54% of reads), the Diversisporales (43%), the Archaeosporales (3%), and Paraglomerales (<1%).

The bacterial dataset contained 965520 reads. The mean read length was 254 bases, with a standard deviation of 14. The most abundant phyla present were the Proteobacteria (18% of reads), Actinobacteriota (14%), Acidobacteriota (14%), Verrucomicrobiota (9%), Bacteroidota (8%), Planctomycetota (5%), and Patescibacteria (1%). 12% of reads were split between 38 low-abundance phyla (see Supplementary Figure S.3.4C). 9% or reads were unassigned at the phylum level.

The Cercozoan dataset contained 569088 reads. The mean read length was 314 bases, with a standard deviation of 14. The most abundant orders present were the Glissomonadida (38% of reads), Cercomonadida (21%), Cryomonadida (15%), Euglyphida (10%), Limnofilida (1%), and Spongomonadida (2%). 5% of reads were split between 14 low-abundance orders (see Supplementary Figure S.3.4D). 7% of reads were unassigned at the order level.

3.4.3 Drivers of microbial richness

There was strong evidence that fungal OTU richness increased with urbanity (PCA axis 1 coefficient = -16.30, $p = 0.005$), and with higher concentrations of nutrients and metal elements (PCA axis 2 coefficient = 15.46, $p = 0.048$), but weak evidence that richness was higher in older sites (coefficient = -68.63, $p = 0.07$) (Figure 3.2). Glomeromycotan OTU richness was also greater in sites with higher nutrient and heavy metal content (coefficient = 2.61, $p = 0.007$). There was a weak trend for bacterial OTU richness being higher in post-1945 sites than pre-1945 sites ($F = 3.50$, $p = 0.068$), although no relationship was seen with any of the other environmental parameters. Cercozoan OTU richness did not correlate with any of the environmental parameters or site age.

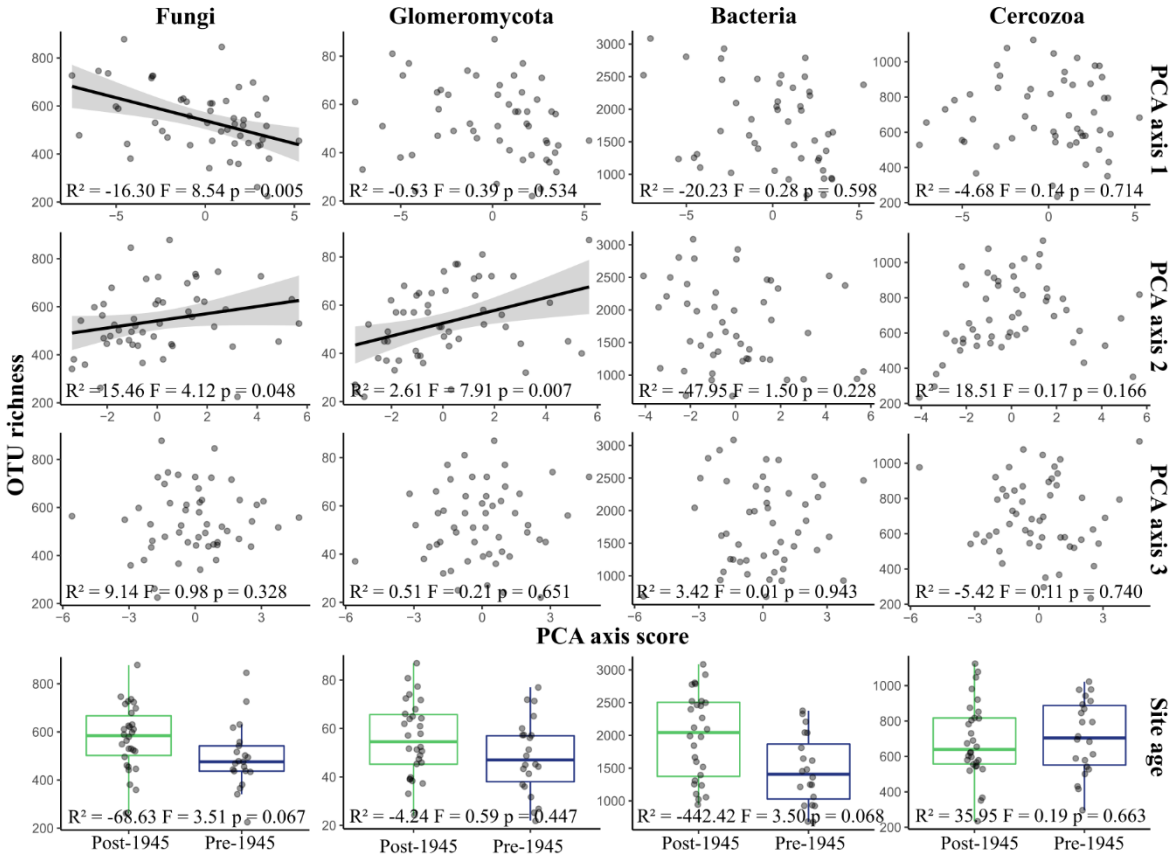

Figure 3.2 Variation of OTU richness in taxonomic groups across environmental gradients. The figure displays a graphical representation of the results of multiple linear regressions of OTU richness within each taxonomic group. The regression model was Site age + PCA axis 1 + PCA axis 2 + PCA axis 3. Each plot shows richness plotted against site scores for the three PCA axes, representing environmental syndromes, and site age. Please note that lower scores for PCA axis 1 represent more urban sites. Lines are plotted on correlations for which $p \leq 0.05$; these are linear model regression lines, with gray areas representing 95% confidence areas. Boxplots show the mean, 25th, and 75th percentiles, with whiskers extending to the range, excluding outliers. Statistical results are reported at the bottom of each plot.

3.4.4 Drivers of microbial community composition

PERMANOVA revealed strong evidence for the community composition of all taxonomic groups being driven by both urbanity and soil chemistry (PCA axes 1 and 2; see Figure 3.3 and Supplementary Table S.3.3). We also found strong evidence for PCA axis 3 (site connectivity and miscellaneous variables) driving Glomeromycotan community composition. There was also weak evidence for the community composition for the other three taxonomic groups being driven by PCA axis 3 (Supplementary Table 3). We found no evidence for the age of sites influencing community composition in any of the taxonomic groups. Represented via NMDS, it is clear how communities segregate differently across all three environmental PCA axes, with this pattern being most linear for PCA axes 2 and 3 (Figure 3.3).

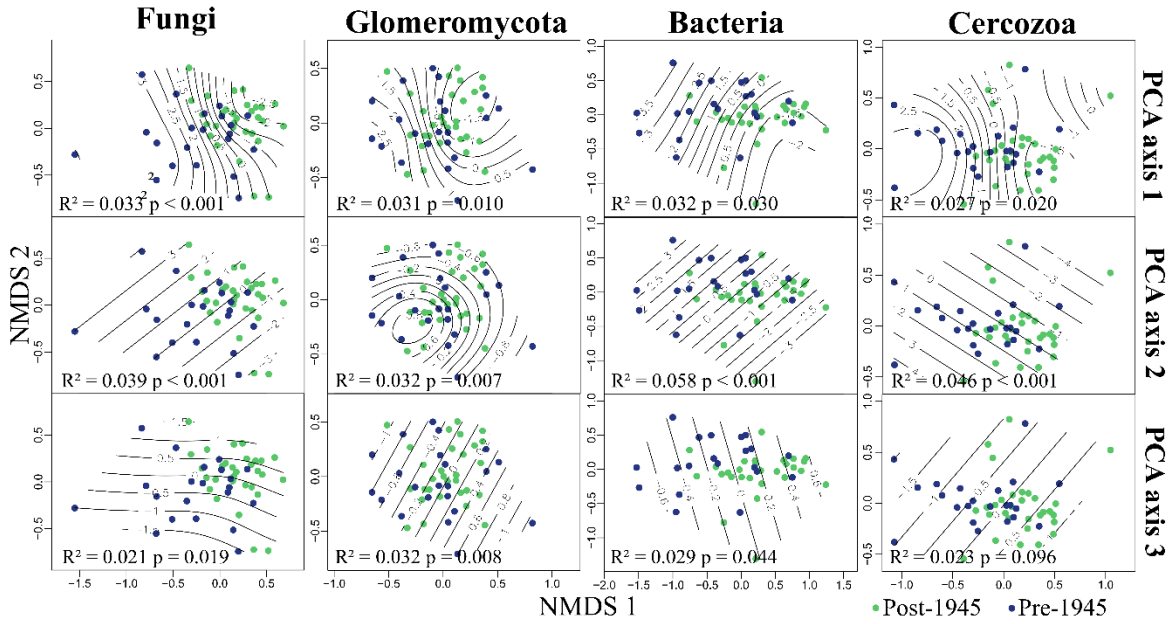

Figure 3.3 Grassland microbial communities in Berlin, plotted in relation to environmental variation. Non-metric multidimensional scaling (NMDS) plots showing fungal (2D stress = 0.16), Glomeromycotan (2D stress = 0.27), bacterial (2D stress = 0.12), and Cercozoan (2D stress = 0.13) communities segregated by environmental principal component analysis (PCA) axis scores. Reported in each plot are the statistical outputs of marginal PERMANOVA models. Sites are colored by age, the PERMANOVA results for site age are: Fungi, R2 = 0.021, p = 0.216; Glomeromycota, R2 = 0.020, p = 0.345; Bacteria, R2 = 0.017, p = 0.577; Cercozoa, R2 = 0.019, p = 0.341.

Distance-decay analysis provided strong evidence for fungal, Glomeromycotan and bacterial community diversity changing with geographic distance, with this trend being particularly strong for the fungi and Glomeromycota (Figure 3.4). Considering that PCA axis 1 included one geographic parameter (distance from city center) it is notable that for one dataset, the Glomeromycota, PCA axis 1 was not seen to correlate significantly with diversity whilst geographic distance did.

Beta diversity partitioning revealed that for all organism groups, species turnover accounted for the vast majority of beta diversity (Table 3.1).

Table 3.1 Results of beta diversity partitioning for each organism group.

	Fungi	**Glomeromycota**	**Bacteria**	**Cercozoa**
Species turnover	0.952	0.928	0.952	0.942
Nestedness	0.009	0.019	0.013	0.014
Total beta diversity	0.961	0.947	0.964	0.956

Species turnover was measured as Simpson dissimilarity, nestedness as the nestedness-resultant fraction of Sørensen dissimilarity. Total beta diversity was measured as Sørensen dissimilarity.

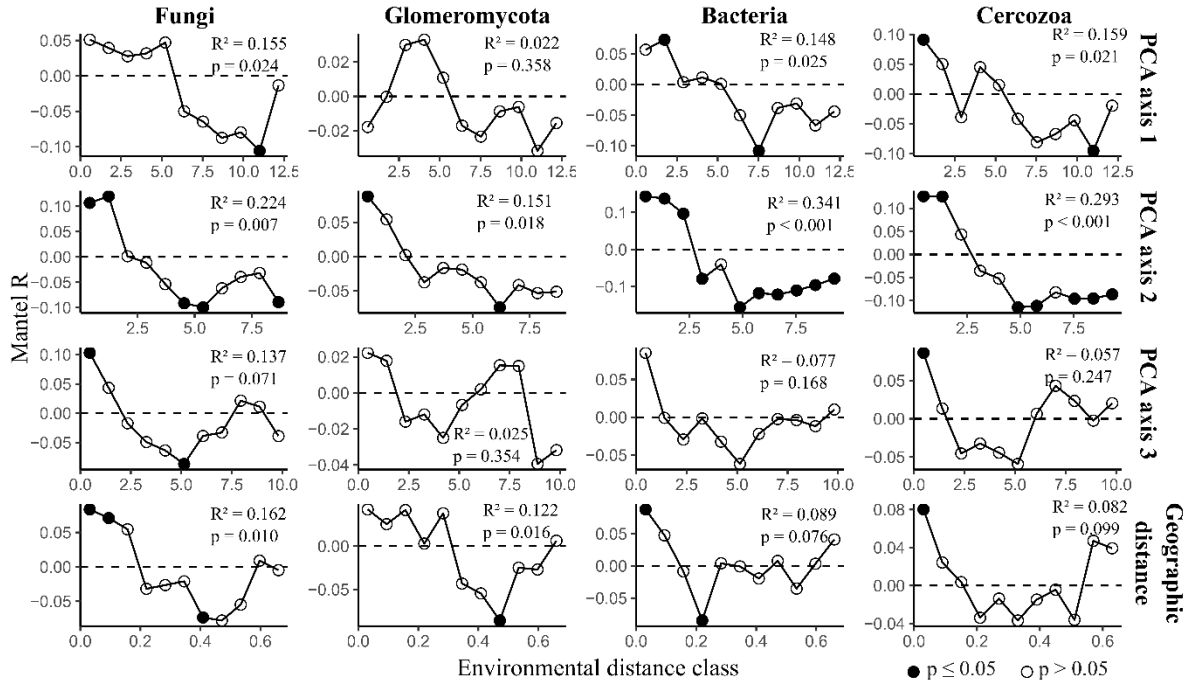

Figure 3.4 Distance-decay analysis along environmental gradients across grasslands in Berlin. The figure displays mantel correlograms showing community dissimilarity correlated with environmental and geographic distance. Black points indicate strong evidence for differences between distance matrices (p 0.05). The statistical outputs of simple Mantel tests are presented in each plot. The full outputs of simple and partial Mantel tests are reported in Supplementary Table 4.

3.4.5 Taxonomic breakdown of diversity

The richness of multiple taxonomic groups from each dataset were found to correlate significantly with one or more PCA axes according to Kendall correlations. Using hierarchical partitioning to explore the relative importance of each environmental axis to these taxonomic groups (Table 3.2), we found evidence that within the fungal dataset, PCA axis 1 was the most significant driver of richness for four phyla, including the Ascomycota (of which it explained 86.3% of variance) and Glomeromycota (85.8%), and the OTUs which were unassigned at the phyla level (80.4%). For all three of these groups, richness was higher at more urban sites (Supplementary Table 5). PCA axis 2 was found to be the most important driver of richness for three fungal phyla, all of which positively correlated with soil nutrient/heavy metal content, including the Mortierellomycota (75.5%). PCA axis 3 was found to be the most important driver of richness for only one phylum, the Basidiomycota (43.6%), which correlated positively with this environmental axis representing site connectivity. This was the only time PCA axis 3 was identified as the most important driver of richness within any of the datasets. Within the Glomeromycotan dataset, PCA axis 1 was found the be the key driver of richness for the Archaeosporales (72.2%), for whom richness was higher in urban sites, and PCA axis 2 was found to be the key driver of richness for the other two groups identified, the Diversisporales (85.7%) and Glomerales (70.9%), the richness of both of which increased with soil nutrient/heavy metal content. Of the nineteen bacterial phyla identified as correlating with an environmental PCA axis, PCA axis 1 was the most significant driver of urbanity for thirteen, with the richness of the remaining six driven by PCA axis 2. Nine Cercozoan orders were identified as correlating with PCA axes, for three of whom PCA axis 1 was the key driver, with the richness of the remaining six orders driven by PCA axis 2. For a complete list of the hierarchical partitioning results of all taxonomic groups please see Supplementary Table 5.

Table 3.2 Microbial taxonomic groups for which urbanity (principal component analysis (PCA) axis 1) was identified as the key explanatory variable for shifts in OTU richness.

Fungi (phyla)	Glomeromycota (orders)	Bacteria (phyla)	Cercozoa (orders)
Ascomycota (↑)	Archaeosporales (↑)	Actinobacteriota (↑)	Cercozoa_XX (↑)
Chytridiomycota (↑)		Bacteroidota (↑)	Filosa-Imbricatea_X (↑)
Glomeromycota (↑)		Bdellovibrionota_B (↑)	Plasmodiophorida (↑)
Olpidiomycota (↑)		Chloroflexota_A (↑)	
Unassigned at phyla level (↑)		Eremiobacterota (↓)	
		FCPU426 (↓)	
		Firmicutes_B (↑)	
		Gemmatimonadota (↑)	
		Methylomirabilota (↑)	
		Myxococcota (↑)	
		Nitrospirota (↑)	
		Proteobacteria (↑)	
		Sumerlaeota (↑)	

These groups were identified through hierarchical partitioning of all groups for which Kendall correlations provided strong evidence for environmental variation driving richness. Arrows show whether OTU richness increased (↑) or decreased (↓) in response to increasing urbanity levels (decreasing PCA axis 1 score). Results of hierarchical partitioning for all highlighted taxonomic groups is presented in Supplementary Table 5.

One hundred and thirty OTUs within the fungal dataset were identified as being likely urban indicator species ($p < 0.05$) for the eighteen most urban sites (denoted by them having the lowest PCA axis 1 scores), of these OTUs, twenty-seven were identified at the species level. We found evidence for *Gibberella tricincta* being a likely indicator species ($p = 0.047$) as well as being in the top 20 most abundant OTUs. However, our heatmap demonstrated an uneven distribution between urban sites, with a peak of abundance in only one site (Figure 3.5). Also appearing in the list of potential indicator species, and in the top 20 OTUs, were two different OTUs representing *Mortierella alpina* ($p = 0.017$ and 0.029). Within the heatmap these OTUs demonstrated different dispersal between sites and it is

likely these OTUs therefore represented separate strains. Within the Glomeromycotan dataset three OTUs were likely indicator species, of which only *Scutellospora calospora* (p = 0.032) was identified at species level. This was also the most abundant Glomeromycotan OTU present in the dataset (Figure 3.5). Within the bacterial dataset 339 OTUs were identified as likely indicator species, of which 127 were identified at the species level. One hundred and three Cercozoan OTUs were identified as likely indicator species, of which eleven were assigned at the species level. *Paracercomonas compacta* (p = 0.022) and *Neocercomonas jutlandica* (p = 0.047) were both highly abundant OTUs and potential indicator species, despite an apparently relatively wide dispersal (Figure 3.5). For a list of likely urban indicator species see Supplementary Table 6.

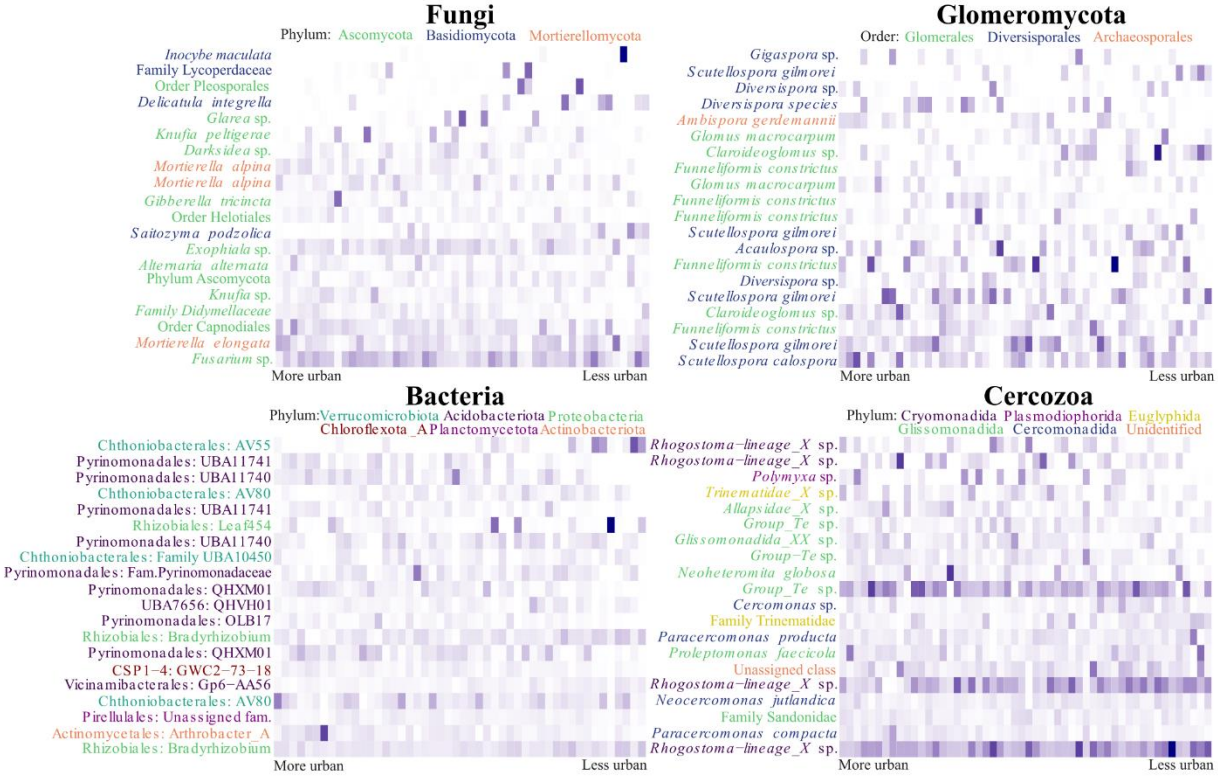

Figure 3.5 Turnover of the most abundant microbial OTUs across the urbanity gradient in Berlin. The figure displays heatmaps of the top 20 most abundant OTUs within each organism group, arranged across the axis in order of principal component analysis (PCA) axis 1 score. For the fungal and Glomeromycotan OTUs, binomial annotations are included when identified. Otherwise, the highest taxonomic level attributed to the OTU is used. Labels are colored according to phylum in the fungal heatmap and according to order in the Glomeromycotan heatmap. The bacterial heatmap is annotated with the order and genus assigned to OTUs, or family if the genus was unassigned. Labels are colored according to phylum. OTUs in the Cercozoan heatmap are labeled with their binomial name, or the highest taxonomic level assigned. Labels are colored according to order.

3.5 Discussion

The results of this study indicate that within an urban landscape, soil microbial communities are shaped not only by soil chemistry, but also by factors intrinsically linked to urbanity itself, indeed, total species richness appears to increase with urbanization. Changes in community composition and richness due to urbanization appear to vary between microbial organism groups.

It is important to note that whilst we pick out some individual species for discussion here, these species are selected due to their iconic nature in the literature; the wealth of data available from this study makes it infeasible to comprehensively cover all species.

We found strong evidence for positive relationships between urbanity and the diversity of many microbial phyla, across multiple kingdoms. This pattern was especially clear in the Fungal kingdom, where it can be seen at a higher taxonomic level than in the other organism groups we studied. The response of fungal community richness to urbanity can therefore be compared to that of plant communities, which have also been shown to increase in richness in response to urbanity (McKinney, 2008). Our results contrast with previous observations of fungal richness not changing with urbanity, and bacterial and Cercozoan richness increasing (Delgado-Baquerizo *et al*., 2021). Other studies have even reported decreases in fungal richness with urbanity (Abrego *et al*., 2020).

Our study provides evidence that soil microbial communities in urban landscapes are not solely driven by soil chemistry, although this certainly plays a role. Our PCA axis 2, representing soil chemistry, had varying impacts depending on the organism group in question. We found evidence of fungal richness increasing alongside increasing nutrient/heavy metal content. This was also true for the Glomeromycota, in accordance with Tipton *et al*. (2019), who had previously described the abundance of Glomeromycota in an urban study as being driven by soil chemistry. Despite an absence of this trend in richness in the bacterial and Cercozoan datasets, PERMANOVA and mantel tests both demonstrated strong evidence for soil chemistry shaping all microbial communities. Interestingly, the results of Mantel tests suggested an especially strong impact of soil chemistry on the bacteria and Cercozoa. The small physical size of these organism groups may inhibit their ability to maintain homeostasis in response to a chemical gradient (Luan *et al*., 2020), and thus explain this trend.

Within the Glomeromycota, we found evidence for urbanity driving an increase in the richness of the Archeosporales (ancestral AMF, sensitive to drought; Canarini *et al*., 2021), whereas the Diversisporales (thought to be vulnerable to heat or drought, and favor fertile soils; Xiang *et al*., 2016; Alguacil *et al*., 2021) and Glomerales (indicators of drought; Canarini *et al*., 2021) increased in richness in response to increasing soil chemical concentrations.

Glomus macrocarpum was present twice within the top 20 most abundant Glomeromycotan OTUs in our study, showing two different patterns of distribution. This was presumably due to two sub-species having differing environmental preferences. It is notable that one subspecies appeared to show highest abundance in the rural and urban areas, whilst the other showed its highest abundance in sites of intermediate urbanity. Interestingly, this species has previously been described as both sensitive (Oehl *et al*., 2010; Carrenho and Gomes-da-Costa, 2011) and resilient (Sousa *et al*., 2013) to disturbance. Our observations reaffirm the importance of asking questions about the correct taxonomic resolution for studies such as this (Roy *et al*., 2019), and provides strong support for van der Heijden *et al*.'s (2004) suggestion that inferring traits based on taxonomy alone, particularly at the species level, is problematic. In addition, we identified *S. calospora* as a likely indicator species for high urbanity, whilst this species has previously been described as sensitive to disturbance (Gupta *et al*., 2018) and as a generalist (Oehl *et al*., 2010).

Another notable way in which our study compares with existing literature is in the case of *Mortierella elongata*. This fungus has previously been identified as a globally abundant indicator species of urban green spaces (Delgado-Baquerizo *et al*., 2021). Although we did not identify this species as an indicator species of high urbanity, it was the second most abundant OTU within our fungal dataset. It appeared across most sites, with no clear trend relating to urbanity. It should of course be noted that, according to other, less precise, classifications of urbanity used elsewhere, all of our sites could be classed as urban or peri-urban. In addition, all sampling occurred during the summer, and consequently seasonal variation was not taken into account.

One previous study, in the same system as this one, reported an increase in non-mycorrhizal colonization of plant roots in response to urbanity, whilst AMF colonization was not affected (Whitehead *et al*., 2022). Our observation of an increase in richness of the Ascomycota in response to urbanity, supports the hypothesis that these non-mycorrhizal endophytes were Ascomycota dark septate endophytes. Previous research has suggested that globally, this phylum increases in abundance at urban locations (Delgado-Baquerizo *et al*., 2021). Whitehead *et al*. (2022) found that AMF colonization rates in these sites were likely driven by plant community composition, which was in turn driven by a syndrome of parameters very similar to the PCA axis 3 seen in this current study. Given the clear linearity seen in the NMDS plot between Glomeromycotan community, and PCA axis 3, we think it is likely that shifts in the Glomeromycotan community were driven by plant community composition. Indeed, the impacts of plants upon microbial soil communities have been shown to be extensive (Philippot *et al*., 2013), and it is likely that the shifts in microbial communities we observed in relation to environmental parameters were in some ways mediated by plants.

Our findings of increases in richness of abundant bacterial phyla such as Proteobacteria and Bacteroidota, and less abundant phyla, such as Nitrospira and Gemmatimonadota, in response to urbanity, mimic findings from previous studies (Wang *et al*., 2018; Delgado-Baquerizo *et al*., 2021; Stephanou *et al*., 2021). We also observed urbanity increasing the richness of the Actinobacteria, previously shown to be a significant phylum in cities across China (Xu *et al*., 2014). The only two taxa for whom we found evidence of a decrease in richness alongside urbanity were the bacterial phyla Eremiobacterota (thought to be acidophilic; Ji *et al*., 2021) and FCPU426 (potentially associated with cellulose degradation; Doud *et al*., 2020). It should be noted that for both of these phyla, the relative proportion of variance explained by axis 1 was <50%, suggesting a combination of factors driving this pattern. Urban-related increases in soil pH and decreases in organic matter could explain the reduction in the richness of these phyla.

Cercozoan biogeographical studies are rare in comparison to those of the other groups in this study. However, the lack of similarities between the Cercozoan NMDS plots and those of the other organism groups suggests that within our study the Cercozoa are unlikely to have exhibited top-down control of other soil microbial communities, as previously suggested by Gao *et al*. (2019). PERMANOVA demonstrated strong evidence for both urbanity and soil chemistry driving Cercozoan community composition with a weak trend for the miscellaneous, but largely connectivity-related, variables of PCA axis 3 also playing a role. Of the three Cercozoan orders we identified as having their richness driven by urbanity, only one, the Plasmodiophorida (a group of plant pathogenic slime molds; Gould, 2009) is well described in terrestrial ecosystems.

One OTU from within this order, of the genus Polymyxa (root parasites and vectors for viral parasites, such as Beet Necrotic Yellow Vein Virus; Keskin, 1964), was within our top 20 most abundant Cercozoan OTUs. Whilst the OTU was not clearly distributed across the urbanity gradient, it was, however, most abundant in sites of intermediate and high urbanity. These observations again highlight the role plant diversity is likely to have played in mediating the relationship between urbanity and microbial community assemblage.

The age of sites did not significantly influence the community composition of any of our datasets, despite the collinearity between site age and urbanity. We did, however, observe a weak trend for fungal and bacterial OTU richness decreasing with site age. This finding contrasts those of Hui *et al*. (2017), who found the reverse in a previous study. This differing result is potentially due to different locations (Berlin vs. Finland) and study sites (varied grasslands vs. lawns in parks). We identified the highest richness of Ascomycota and Nitrospirota in more urban, newer sites, whereas Hui *et al*. (2017) found these groups to be of highest abundance in older sites.

The impacts of urban landscapes upon organism dispersal are uncertain; both dispersal limitation due to habitat fragmentation and increased dispersal due to roads and railways, or even human movements, could be expected to shape microbial communities. Whilst our study does not investigate this topic in depth, our Mantel correlograms provide evidence for soil microbial communities shifting across the urban landscape, with these changes particularly pronounced in fungal, including Glomeromycotan, communities. These shifts were less pronounced for the bacterial and Cercozoan communities, perhaps due to increased dispersal capacity as a result of their small size (Luan *et al*., 2020). Within urban landscapes, geographic distance is not a linear measure of habitat dissimilarity and has a non-linear relationship with urban-related variables. This can be seen in the shapes of the Mantel correlograms, whereby sites on opposite sides of the city share more similar communities than to those in the center.

3.6 Conclusion

Within Berlin's grasslands, urbanity increases the richness of many taxonomic groups across multiple microbial kingdoms, and, alongside other more traditionally recognized environmental features, such as soil chemistry, shapes community composition. Urbanity appears to have a diversifying impact upon fungal communities as a whole, and upon many individual fungal and bacterial phyla, and a few Cercozoan orders. However, the urban Cercozoan community appears to respond less strongly to environmental parameters than other microbial groups. Whilst we did identify individual species that demonstrated particularly strong responses to urbanity, we also found differing responses within the same species, and would therefore urge caution when making functional assessments based on taxonomic findings. Despite this, within the Cercozoa, we noted a high abundance of potentially parasitic species. Whether this is indicative of a general trend deserves further study.

3.7 Data availability statement

Datasets of OTU sequences, abundances, and taxonomic assignments are available at: https://doi.org/10.6084/m9.figshare.20089817. The environmental metadata is available at: https://doi.org/10.6084/m9.figshare.20088632 and https://doi.org/10.6084/m9.figshare.20088707. PCA axis scores for sites are available at: https://doi.org/10.6084/m9.figshare.20088719. Raw sequences are available from the NCBI, accession number PRJNA862455.

3.8 Author contributions

JW, SH, and MR designed the research. JW conducted lab work, bioinformatics, statistics, and wrote first draft of the manuscript. JR created the bioinformatics pipeline and provided guidance on bioinformatics and statistics. All authors added to and edited the text.

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3.11 References

Abrego, N., Crosier, B., Somervuo, P., Ivanova, N., Abrahamyan, A., Abdi, A., *et al*. (2020). Fungal communities decline with urbanization-more in air than in soil. ISME J. 14, 2806–2815. doi: 10.1038/s41396-020-0732-1

Alguacil, M. D. M., Schlaeppi, K., López-García, Á, van der Heijden, M. G. A., and Querejeta, J. I. (2021). Contrasting responses of arbuscular mycorrhizal fungal families to simulated climate warming and drying in a Semiarid Shrubland. Microb. Ecol. Epub ahead of print. doi: 10.1007/s00248-021-01886-6

Baselga, A., and Orme, C. D. L. (2012). betapart: An R package for the study of beta diversity. Methods Ecol. Evol. 3, 808–812. doi: 10.1111/j.2041-210X.2012.00224.x

Brundrett, M. C., and Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. New Phytol. 220, 1108–1115. doi: 10.1111/nph.14976

Callahan, B., McMurdie, P., Rosen, M., Han, A., Johnson, A., and Holmes, S. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C. W., Zezula, D., *et al*. (2021). Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. Nat. Commun. 12:5308. doi: 10.1038/s41467-021-25675-4

Carrenho, R., and Gomes-da-Costa, S. M. (2011). Environmental degradation impact on native communities of arbuscular mycorrhizal fungi in an urban fragment of semideciduous plateau forest. Acta Bot. Brasilica 25, 373–379.

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs. Science 199, 1302–1310. doi: 10.1126/science.199.4335.1302

Cousins, J. R., Hope, D., Gries, C., and Stutz, J. C. (2003). Preliminary assessment of arbuscular mycorrhizal fungal diversity and community structure in an urban ecosystem. Mycorrhiza 13, 319–326. doi: 10.1007/s00572-003-0239-4

Cunningham, M. A., Snyder, E., Yonkin, D., Ross, M., and Elsen, T. (2008). Accumulation of deicing salts in soils in an urban environment. Urban Ecosyst. 11, 17–31. doi: 10.1007/s11252-007-0031-x

De Caceres, M., and Legendre, P. (2009). Associations between species and groups of sites: Indices and statistical inference. Ecology 90, 3566–3574. doi: 10.1890/08-1823.1

Decina, S. M., Hutyra, L. R., and Templer, P. H. (2020). Hotspots of nitrogen deposition in the world's urban areas: A global data synthesis. Front. Ecol. Environ. 18:92–100. doi: 10.1002/fee.2143

Delgado-Baquerizo, M., Eldridge, D. J., Liu, Y. R., Sokoya, B., Wang, J. T., Hu, H. W., *et al*. (2021). Global homogenization of the structure and function in the abg5809

Donald, J., Murienne, J., Chave, J., Iribar, A., Louisanna, E., Manzi, S., *et al*. (2021). Multi-taxa environmental DNA inventories reveal distinct taxonomic and functional diversity in urban tropical forest fragments. Glob. Ecol. Conserv. 29:e01724. doi: 10.1016/j.gecco.2021.e01724

Doud, D. F. R., Bowers, R. M., Schulz, F., De Raad, M., Deng, K., Tarver, A., *et al*. (2020). Function-driven single-cell genomics uncovers cellulose-degrading bacteria from the rare biosphere. ISME J. 14, 659–675. doi: 10.1038/s41396-019-0557-y

du Toit, M. J., and Cilliers, S. S. (2011). Aspects influencing the selection of representative urbanization measures to quantify urban-rural gradients. Landsc. Ecol. 26, 169–181.

Dugord, P. A. (2013). Assessing the influence of land use patterns on urban climate and city-dwellers' vulnerability toward heat stress. Master's thesis. Berlin: Technische Universität Berlin.

Ellis, E. C., Gauthier, N., Klein Goldewijk, K., Bliege Bird, R., Boivin, N., Díaz, S., *et al*. (2021). People have shaped most of terrestrial nature for at least 12,000 years. Proc. Natl. Acad. Sci.U.S.A. 118:e2023483118. doi: 10.1073/pnas.2023483118

Epstein, A. K., Pokroy, B., Seminara, A., and Aizenberg, J. (2011). Bacterial biofilm shows persistent resistance to liquid wetting and gas penetration. Proc. Natl. Acad. Sci. U.S.A. 108, 995–1000.

Estensmo, E. L. F., Maurice, S., Morgado, L., Martin-Sanchez, P. M., Skrede, I., and Kauserud, H. (2021). The influence of intraspecific sequence variation during DNA metabarcoding: A case study of eleven fungal species. Mol. Ecol. Resour. 21, 1141–1148. doi: 10.1111/1755-0998.13329

Facchinelli, A., Sacchi, E., and Mallen, L. (2001). Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. Environ. Pollut. 114, 313–324. doi: 10.1016/s0269-7491(00)00243-8

Fiore-Donno, A. M., Rixen, C., Rippin, M., Glaser, K., Samolov, E., Karsten, U., *et al*. (2018). New barcoded primers for efficient retrieval of cercozoan sequences in high-throughput environmental diversity surveys, with emphasis on worldwide biological soil crusts. Mol. Ecol. Resour. 18, 229–239. doi: 10.1111/1755-0998.12729

Foissner, W. (1999). Protist diversity: Estimates of the near-imponderable. Protist 150, 363–368. doi: 10.1016/S1434-4610(99)70037-4

Ganjegunte, G. K., Clark, J. A., Sallenave, R., Sevostianova, E., Serena, M., Alvarez, G., *et al*. (2017). Soil salinity of an urban park after long-term irrigation with saline ground water. Agron. J. 109, 3011–3018. doi: 10.2134/agronj2017.06.0369

Gao, Z. L., Karlsson, I., Geisen, S., Kowalchuk, G., and Jousset, A. (2019). Protists: Puppet masters of the rhizosphere microbiome. Trends Plant Sci. 24, 165–176. doi: 10.1016/j.tplants.2018.10.011

Garnier, S., Ross, N., Rudis, R., Camargo, P. A., Sciaini, M., and Scherer, C. (2021). viridis–colorblind-friendly color maps for R. Available online at: https://sjmgarnier.github.io/viridis/

Geisen, S., Mitchell, E. A. D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F., *et al*. (2018). Soil protists: A fertile frontier in soil biology research. FEMS Microbiol. Rev. 42, 293–323. doi: 10.1093/femsre/fuy006

Ghyselinck, J., Pfeiffer, S., Heylen, K., Sessitsch, A., and De Vos, P. (2013). The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. PLoS One 8:14. doi: 10.1371/journal.pone.0071360

Glücksman, E., Bell, T., Griffiths, R. I., and Bass, D. (2010). Closely related protist strains have different grazing impacts on natural bacterial communities. Environ. Microbiol. 12, 3105–3113. doi: 10.1111/j.1462-2920.2010.02283.x

Gould, A. B. (2009). "Fungi: Plant pathogenic," in Encyclopedia of microbiology, 3rd Edn, ed. M. Schaechter (Oxford: Academic Press), 457–477.

Grünfeld, L., Mola, M., Wulf, M., Hempel, S., and Veresoglou, S. D. (2021).Disentangling the relative importance of spatio-temporal parameters and host specificity in shaping arbuscular mycorrhizal fungus communities in a temperate forest. Mycorrhiza 31, 589–598. doi: 10.1007/s00572-021-01041-6

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., *et al*. (2013). The Protist ribosomal reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604. doi: 10.1093/nar/gks1160

Gupta, M. M., Gupta, A., and Kumar, P. (2018). Urbanization and biodiversity of arbuscular mycorrhizal fungi: The case study of Delhi, India. Rev. Biol. Trop. 66, 1547–1558. doi: 10.15517/rbt.v66i4.33216

Hanson, C. A., Fuhrman, J. A., Horner-Devine, M. C., and Martiny, J. B. H. (2012). Beyond biogeographic patterns: Processes shaping the microbial landscape. Nat. Rev. Microbiol. 10, 497–506. doi: 10.1038/nrmicro2795

Hijmans, R. J. (2019). geosphere: Spherical trigonometry. Available online at https://github.com/rspatial/geosphere/

Horn, S., Caruso, T., Verbruggen, E., Rillig, M. C., and Hempel, S. (2014). Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. ISME J. 8, 2231–2242.

Horn, S., Hempel, S., Verbruggen, E., Rillig, M. C., and Caruso, T. (2017). Linking the community structure of arbuscular mycorrhizal fungi and plants: A story of interdependence? ISME J. 11, 1400–1411. doi: 10.1038/ismej.2017.5

Hui, N., Jumpponen, A., Francini, G., Kotze, D. J., Liu, X. X., Romantschuk, M., *et al*. (2017). Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. Environ. Microbiol. 19, 1281–1295. doi: 10.1111/1462-2920.13660

Ihrmark, K., Bodeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., *et al*. (2012). New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. Fems Microbiol. Ecol. 82, 666–677. doi: 10.1111/j.1574-6941.2012.01437.x

Ji, M., Williams, T. J., Montgomery, K., Wong, H. L., Zaugg, J., Berengut, J. F., *et al*. (2021). Candidatus Eremiobacterota, a metabolically and phylogenetically diverse terrestrial phylum with acid-tolerant adaptations. ISME J. 15, 2692–2707. doi: 10.1038/s41396-021-00944-8

Johnson, M. T., and Munshi-South, J. (2017). Evolution of life in urban environments. Science 358:eaam8327.

Keskin, B. (1964). Polymyxa betae n.sp., ein Parasit in den Wurzeln von Beta vulgaris Tournefort, besonders während der Jugendentwicklung der Zuckerrübe. Arch. Mikrobiol. 49, 348–374.

Krüger, M., Krüger, C., Walker, C., Stockinger, H., and Schussler, A. (2012). Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytol. 193, 970–984. doi: 10.1111/j.1469-8137.2011.03962.x

Krüger, M., Stockinger, H., Krüger, C., and Schüßler, A. (2009). DNA-based species level detection of Glomeromycota: One PCR primer set for all arbuscular mycorrhizal fungi. New Phytol. 183, 212–223. doi: 10.1111/j.1469-8137.2009.02835.x

Kuhn, M., Jackson, S., and Cimentada, J. (2020). Correlations in R. Available online at https://corrr.tidymodels.org/

Landesamt für Umwelt Brandenburg (2009). Flächendeckende Biotop- und Landnutzungskartierung (BTLN) im Land Brandenburg—CIR-Biotoptypen 2009. Potsdam: Landesamt für Umwelt Brandenburg.

Lee, C. S., Li, X., Shi, W., Cheung, S. C., and Thornton, I. (2006). Metal contamination in urban, suburban, and country park soils of Hong Kong: A study based on GIS and multivariate statistics. Sci. Total Environ. 356, 45–61. doi: 10.1016/j.scitotenv.2005.03.024

Leifheit, E. F., Veresoglou, S. D., Lehmann, A. E., Morris, K., and Rillig, M. C. (2014). Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation—a meta-analysis. Plant Soil 374, 523–537. doi: 10.1007/s11104-013-1899-2

Liu, Z. F., He, C. Y., Zhou, Y. Y., and Wu, J. G. (2014). How much of the world's land has been urbanized, really? A hierarchical framework for avoiding confusion. Landsc. Ecol. 29, 763–771.

Luan, L., Jiang, Y., Cheng, M., Dini-Andreote, F., Sui, Y., Xu, Q., *et al*. (2020). Organism body size structures the soil microbial and nematode community assembly at a continental and global scale. Nat. Commun. 11:6406. doi: 10.1038/s41467-020-20271-4

Mataix-Solera, J., and Doerr, S. H. (2004). Hydrophobicity and aggregate stability in calcareous topsoils from fire-affected pine forests in southeastern Spain. Geoderma 118, 77–88. doi: 10.1016/S0016-7061(03)00185-X

McKinney, M. L. (2008). Effects of urbanization on species richness: A review of plants and animals. Urban Ecosyst. 11, 161–176. doi: 10.1016/j.envint.2015.09.013

Morgan, M., Anders, S., Lawrence, M., Aboyoun, P., Pagès, H., and Gentleman, R. (2009). ShortRead: A Bioconductor package for input, quality assessment and exploration of high-throughput sequence data. Bioinformatics 25, 2607–2608. doi: 10.1093/bioinformatics/btp450

Nally, R. M., and Walsh, C. J. (2004). Hierarchical partitioning public-domainsoftware. Biodivers. Conserv. 13, 659–660. doi: 10.1371/journal.pone.0011698

NCBI Resource Coordinators (2016). Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 44, D7–D19.

Newbound, M., McCarthy, M. A., and Lebel, T. (2010). Fungi and the urban environment: A review. Landsc. Urban Plann. 96, 138–145.

Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., *et al*. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47, D259–D264. doi: 10.1093/nar/gky1022

O'Riordan, R., Davies, J., Stevens, C., Quinton, J. N., and Boyko, C. (2021). The ecosystem services of urban soils: A review. Geoderma 395:115076.

Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bösch, R., Van Der Heijden, M., *et al*. (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biol. Biochem. 42, 724–738. doi: 10.1111/1574-6941.12420

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., *et al*. (2020). vegan: Community ecology package.

Öztoprak, H., Walden, S., Heger, T., Bonkowski, M., and Dumack, K. (2020). What drives the diversity of the most abundant terrestrial cercozoan family (Rhogostomidae, Cercozoa, Rhizaria)? Microorganisms 8:17. doi: 10.3390/microorganisms8081123

Pagès, H., Aboyoun, P., Gentleman, R., and DebRoy, S. (2021). Biostrings: Efficient manipulation of biological strings. Available online at: https://bioconductor.org/packages/

Park, Y., Sheetlin, S., Ma, N., Madden, T. L., and Spouge, J. L. (2012). New finite-size correction for local alignment score distributions.BMC Res. Notes 5:286. doi: 10.1186/1756-0500-5-286

Parks, D. H., Chuvochina, M., Rinke, C., Mussig, A. J., Chaumeil, P. A., and Hugenholtz, P. (2021). GTDB: An ongoing census of bacterial and archaeal diversity through a phylogenetically consistent, rank normalized and complete genome-based taxonomy. Nucleic Acids Res. 50, D785–D794. doi: 10.1093/nar/gkab776

Philippot, L., Raaijmakers, J., Lemanceau, P., and van der Putten, W. H. (2013). Going back to the roots: The microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799. doi: 10.1038/nrmicro3109

R Core Team (2020). R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Reese, A. T., Savage, A., Youngsteadt, E., McGuire, K. L., Koling, A., Watkins, O., *et al*. (2016). Urban stress is associated with variation in microbial species composition-but not richness-in Manhattan. ISME J. 10, 751–760. doi: 10.1038/ismej.2015.152

Rillig, M. C., and Mummey, D. L. (2006). Mycorrhizas and soil structure. New Phytol. 171, 41–53.

Roy, J., Mazel, F., Sosa-Hernandez, M. A., Duenas, J. F., Hempel, S., Zinger, L., *et al.* (2019). The relative importance of ecological drivers of arbuscular mycorrhizal fungal distribution varies with taxon phylogenetic resolution. New Phytol. 224, 936–948. doi: 10.1111/nph.16080

Schewiakoff, W. (1893). Über die geographische Verbreitung der Süßwasserprotozoen. Mem. Acad. Imp. Sci. St. Petersb. 41, 1–201.

Schleicher, A., Biedermann, R., and Kleyer, M. (2011). Dispersal traits determine plant response to habitat connectivity in an urban landscape. Landsc. Ecol. 26, 529–540. doi: 10.1007/s10980-011-9579-1

Senate Department for Urban Development and Housing (2014). Berlin environmental atlas. 05.08 Biotope types. Available online: https: //www.stadtentwicklung.berlin.de/umwelt/umweltatlas/ek508.htm (accessed July 9, 2021).

Sousa, C. D., Menezes, R. S. C., Sampaio, E., Lima, F. D., Oehl, F., andMaia, L. C. (2013). Arbuscular mycorrhizal fungi within agroforestry and traditional land use systems in semi-arid Northeast Brazil. Acta Sci. Agron. 35, 307–314.

Stephanou, C., Omirou, M., Philippot, L., Zissimos, A. M. I., Christoforou, C., Trajanoski, S., *et al*. (2021). Land use in urban areas impacts the composition of soil bacterial communities involved in nitrogen cycling. A case study from Lefkosia (Nicosia) Cyprus. Sci. Rep. 11:12. doi: 10.1038/s41598-021-87 623-y

Tedersoo, L., Anslan, S., Bahram, M., Drenkhan, R., Pritsch, K., Buegger, F., *et al*. (2020). Regional-scale in-depth analysis of soil fungal diversity reveals strong pH and plant species effects in Northern Europe. Front. Microbiol. 11:1953. doi: 10.3389/fmicb.2020.01953

Thioulouse, J., Dray, S., Dufour, A., Siberchicot, A., Jombart, T., and Pavoine, S. (2018). Multivariate analysis of ecological data with Ade4. New York, NY: Springer.

Tipton, A. G., Middleton, E. L., Spollen, W. G., and Galen, C. (2019). Anthropogenic and soil environmental drivers of arbuscular mycorrhizal community composition differ between grassland ecosystems. Botany 97, 85–99.

Treseder, K. K., and Cross, A. (2006). Global distributions of arbuscular mycorrhizal fungi. Ecosystems 9, 305–316.

UNDESA, Population Division (2018). World urbanization prospects: The 2018 revision. New York, NY: UNDESA.

van der Heijden, M. G. A., Scheublin, T. R., and Brader, A. (2004). Taxonomic and functional diversity in arbuscular mycorrhizal fungi – is there any relationship? New Phytol. 164, 201–204. doi: 10.1111/j.1469-8137.2004.01205.x

Vellend, M. (2010). Conceptual synthesis in community ecology. Q. Rev. Biol. 85, 183–206. doi: 10.1086/652373

von der Lippe, M., Buchholz, S., Hiller, A., Seitz, B., and Kowarik, I. (2020). CityScapeLab Berlin: A research platform for untangling urbanization effects on biodiversity. Sustainability 12:30.

Wang, H. T., Cheng, M. Y., Dsouza, M., Weisenhorn, P., Zheng, T. L., and Gilbert, J. A. (2018). Soil bacterial diversity is associated with human population density in urban greenspaces. Environ. Sci. Technol. 52, 5115–5124. doi: 10.1021/acs.est.7b06417

Ward, K., Lauf, S., Kleinschmit, B., and Endlicher, W. (2016). Heat waves and urban heat islands in Europe: A review of relevant drivers. Sci. Total Environ. 569–570, 527–539. doi: 10.1016/j.scitotenv.2016.06.119

Whitehead, J., Hempel, S., and Rillig, M. C. (2022). Non-mycorrhizal fungal presence within roots increases across an urban gradient in Berlin, Germany. Front. Environ. Sci. 10:888310. doi: 10.3389/fenvs.2022.888310

Whitehead, J., Hempel, S., Hiller, A., von der Lippe, M., and Rillig, M. C. (2021). Soil physico-chemical properties change across an urbanity gradient in Berlin. Front. Environ. Sci. 9:765696. doi: 10.3389/fenvs.2021.765696

Wick, L. Y., Remer, R., Wurz, B., Reichenbach, J., Braun, S., Scharfer, F., *et al*. (2007). Effect of fungal hyphae on the access of bacteria to phenanthrene in soil. Environ. Sci. Technol. 41, 500–505. doi: 10.1021/es061407s

Wickham, H. (2007). Reshaping data with the reshape package. J. Statist. Softw. 21, 1–20.

Wickham, H. (2021). tidyr: Tidy messy data. Available online at: https://tidyr.tidyverse.org/

Xiang, X., Gibbons, S. M., He, J., Wang, C., He, D., Li, Q., *et al*. (2016). Rapid response of arbuscular mycorrhizal fungal communities to short-term fertilization in an alpine grassland on the Qinghai-Tibet Plateau. PeerJ 4:e2226. doi: 10.7717/peerj.2226

Xu, H. J., Li, S., Su, J. Q., Nie, S. A., Gibson, V., Li, H., *et al*. (2014). Does urbanization shape bacterial community composition in urban park soils? A case study in 16 representative Chinese cities based on the pyrosequencing method. Fems Microbiol. Ecol. 87, 182–192. doi: 10.1111/1574-6941.12215

4.2 General Discussion

In this thesis we have explored Berlin's soil at three different resolutions (Figure 4.1). Whilst we have gone some way to explore the interactions between these different resolutions within each chapter, we shall here take the opportunity to dive further into these connections. The link between the physico-chemical properties of soil and the microbiome that inhabits it is a bi-directional one. In other words; microbial soil properties shape communities, and communities shape soil properties.

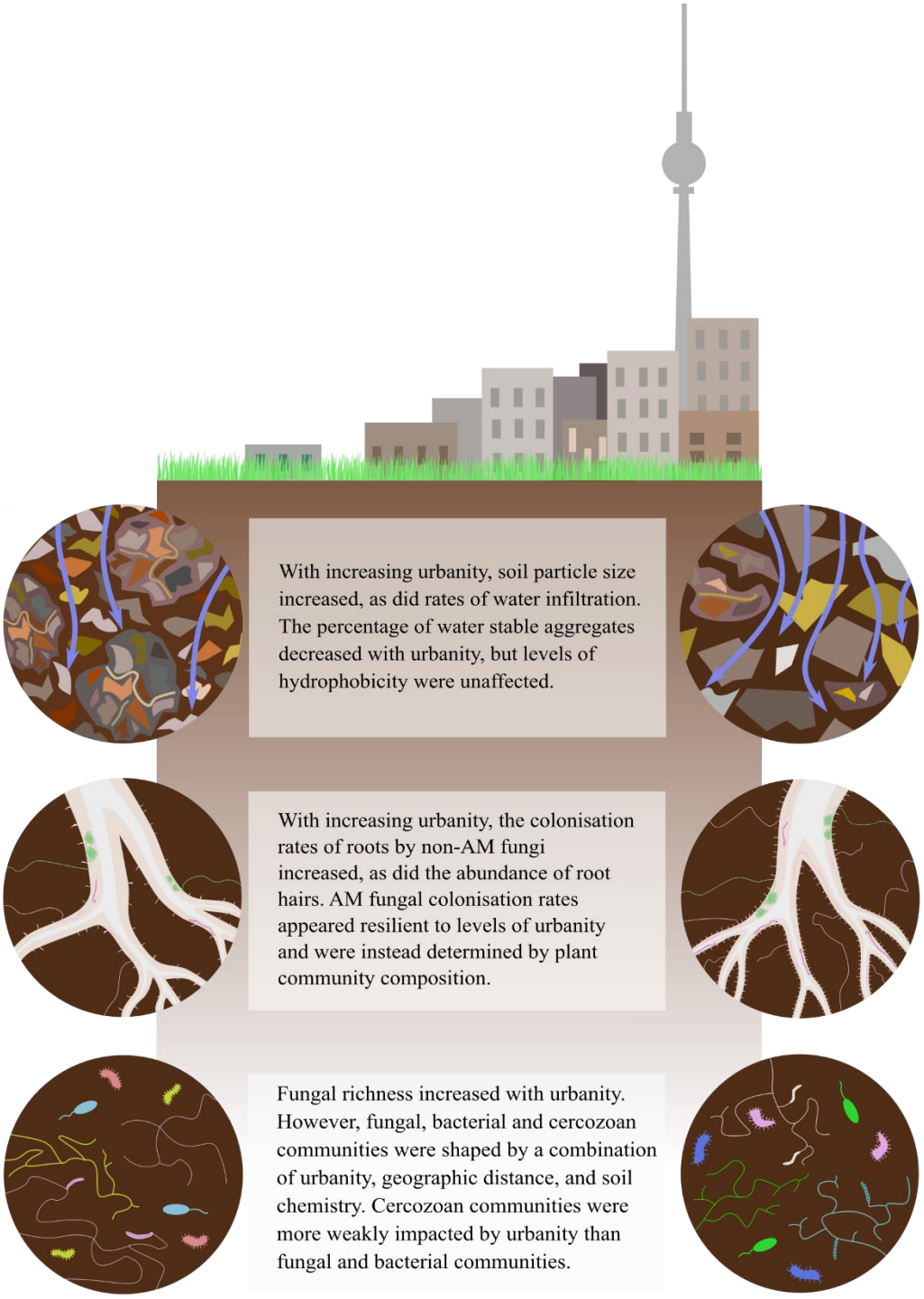


Figure 4.1. A visual summary of the key findings of this thesis.

4.2.1 Soil physico-chemical properties and root colonisation

Filamentous fungal root endophytes heavily impact the physical properties of soil; their mycelium spreads into the soil matrix, where their physical and chemical properties promote the formation of aggregates. This is achieved through being a physical basis for aggregates to form around (Morris *et al.*, 2019), and, particularly in the case of AM fungi, providing hydrophobic exudates which promote aggregate stability (Lehmann *et al*., 2017; Lehmann *et al*., 2020). These compounds, sometimes termed hydrophobins, create a hydrophobic layer encapsulating soil aggregates and preventing the entry of water which may otherwise destroy them. Under normal conditions, these compounds are removed by the flow of water through the soil matrix, however, in times of drought they can accumulate. This accumulation of hydrophobins is the reason that soils experiencing drought become hydrophobic.

Interestingly, we observed a decrease in the proportion of stable aggregates in highly urban soils, but no change in either root colonisation rates by AM fungi or in hydrophobicity. Additionally, we observed an increase in non-AM root endophyte colonisation rates. Our studies therefore suggest that an absence of root endophytes was unlikely to be the cause of the altered aggregate stability we observed. We also observed increased water infiltration rates in areas with high urbanity scores, although any direct causal link between this variable and non-AM fungal colonisation rates seems tenuous at best. If, however, we change the perspective from which we are viewing the endophyte-soil relationship, and include the plant host, we may hypothesise about potential causes for this pattern being mediated through plants. For example, when we consider the most urban soils, we observe that they have low aggregate stability, their particle sizes are large, and water infiltrates very rapidly. In addition, these soils are likely to experience high levels of disturbance. We may therefore consider these as challenging soils for plants to grow in. Plants at these sites may therefore experience stresses not seen in their more rural counterparts, increasing their susceptibility to infection by fungal pathogens. Indeed, as we discuss in Chapters Two and Three, it is likely that the endophytes we observed in Chapter Two are parasites, capitalising on reduced plant fitness to colonise roots in highly urban soils. As such, we can hypothesise that in this system soil properties drive root colonisation rates, rather than vice versa.

4.2.2 Soil physico-chemical properties and microbial diversity

One of the most fundamental ways in which soil physico-chemical properties influence soil microbial communities is through the presence of aggregates. Aggregates provide concentration gradients of nutrients and oxygen; this, in addition to providing a substantial amount of physical surface area and pore space, results in a wide variety of ecological niches for microbial life to inhabit. When aggregates are lost from soils, so too are these niches (Wilpiszeski *et al*., 2019; Cui *et al*., 2020). It is therefore highly interesting that between our studies, we observed a somewhat contradictory pattern regarding aggregate stability and microbial diversity.

Before we begin interrogating this apparent contradiction, we should first draw attention to the fact that we did not record the total microbial community sizes at sites, rather, we used relative measures of richness. In addition, we must also consider that three years elapsed between the sampling undertaken for environmental DNA extraction and the sampling undertaken for assessing physico-chemical properties. It is possible, albeit somewhat unlikely, that significant changes in soil structure or local climate in this period may render comparisons between these studies extraneous.

Despite a reduction in aggregates, we observed increases in richness of fungi in the most urban sites. However, we did not observe such a change in the bacterial or cercozoan community richness. Whilst we do not know how the total community size changed, it seems that diversity was not negatively impacted by the loss of aggregates that we observed. In fact, within our entire study, only two bacterial phyla showed a reduction in diversity in response to urbanity. Evidently, within Berlin's grasslands, the role of aggregates in maintaining diversity is superseded by another variable.

Disturbance is one potential factor that may explain both the lack of stable aggregates and the general increase in diversity we observed in highly urban areas. It is possible that the human and animal visitation commonly experienced by urban grasslands, and their historical tendency to be redeveloped, could retard the formation of stable aggregates and maintain, or even promote, diversity through the intermediate disturbance hypothesis (Connell, 1978).

4.2.3 Root colonisation and microbial diversity

One of the major open questions at the end of Chapter Two, where we investigated the root colonisation of roots from across an urban gradient, was whether the increase in non-AM fungi we observed in highly urban grasslands was due to an abundance of pathogens or mutualists. This is not an easy question to answer. Even if we had used molecular techniques to identify the fungi in question, this would not necessarily have provided a categorical answer to this question due to the transitory nature of these categories. Symbioses between plants and fungi can move along the "parasitism-mutualism gradient" throughout the lifespan of the plant, and depending on environmental conditions (Schulz and Boyle, 2005). As such, there is uncertainty as to which microbial taxa can be labelled as "plant parasites" or "plant mutualist" and new relationships can evolve rapidly (Drew *et al.*, 2021). Whilst certain genera have been labelled as belonging to one of these categories, it is also important to note that even different subspecies of the same bacterial or fungal species can demonstrate markedly different life strategies. We demonstrate this in Chapter Three, where we observed different subspecies showing different site abundances, presumably due to environmental preferences. Indeed, many parasitic relationships have arisen from formerly mutualistic ones, meaning that close relatives can have differing relationships with plants. All of these factors conspire to make taxonomy-based assertions about functional shifts in microbial communities shaky-at-best.

It is, therefore, with several major caveats that we seek to draw connections between our colonisation study from Chapter Two, and our molecular study from Chapter Three. Of particular importance is to acknowledge the three years that elapsed between the sampling for these studies. In addition, seasonal differences have previously been shown to impact both soil community composition and root colonisation rates. Whilst all sampling occurred during summer, finer-scale seasonal differences may have impacted the results.

Taking these caveats into account, and observing the wider literature surrounding urban symbioses (Delgado-Baquerizo *et al*., 2021), it seems likely that an increase in parasitism was the cause for the increase in non-AM fungal plant endophytes we observed in Chapter Two. Previous research has shown that under stress, plants are more vulnerable to parasitic attack (Pandey *et al.*, 2015). The best evidence from our study for an increased abundance of plant pathogens in urban soils actually came not in the fungi, but in the cercozoa. Here, we observed some well-studied plant parasites showing trends for high abundance in the most urban grasslands. There is, unfortunately, one more caveat which we must consider. This caveat is particularly relevant for highly diverse and poorly understood groups, such as the cercozoa. This is investigation bias; one of the most important ways that cercozoan species impact human society is through the presence of the plant pathogens within this group, although the protists as a whole have been historically difficult to study, due to the difficulty entailed in culturing them. As such, despite many other ecological roles, particularly as bacterivores, it is the plant pathogens which have been of most interest to researchers. There is, therefore, a bias in the literature towards pathogens and parasites, and so when we attempt to draw conclusions from taxonomic assignments, particularly in large datasets where large proportions of reads are unassigned, we must bear this in mind.

4.3 General conclusions

Overall, when taking a holistic look at the evidence gathered in this thesis, an image of how Berlin's urban soils change in relation to the urban environment emerges. The most urban soils contain a high diversity of microorganisms, with fungal diversity increasing significantly. However, they often lack

stable aggregates, resulting in a poor soil structure. We also found evidence for the importation of large particles to these soils, presumably as a result of construction work. Interestingly, due to the high infiltration rates they display, Berlin's most urban soils appear capable of buffering the impacts of urban soil sealing. Plants living in these soils are likely to face stresses which make them vulnerable to parasitic attack, and these stresses are also likely to shape microbial community composition.

4.4 References

Cui, H., Ou, Y., Lv, D., Wang, L. X., Liang, A. Z., Yan, B. X., et al. (2020). Aggregate-related Microbial Communities and Nutrient Stoichiometry under Different Croplands. Ecol. Process. 9 (1), 9. doi:10.1186/s13717-020-00239-4

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs. Science 199, 1302–1310. doi: 10.1126/science.199.4335.1302

Delgado-Baquerizo, M., Eldridge, D. J., Liu, Y. R., Sokoya, B., Wang, J. T., Hu, H. W., et al. (2021). Global homogenization of the structure and function in the soil microbiome of urban greenspaces. Sci. Adv. 7:eabg5809. doi: 10.1126/sciadv.abg5809

Drew, G.C., Stevens, E.J. & King, K.C. Microbial evolution and transitions along the parasite–mutualist continuum. Nat Rev Microbiol 19, 623–638 (2021).

Lehmann, A., Zheng, W., and Rillig, M. C. (2017). Soil Biota Contributions to Soil Aggregation. Nat. Ecol. Evol. 1 (12), 1828–1835. doi:10.1038/s41559-017-0344-y

Lehmann, A., Zheng, W., Ryo, M., Soutschek, K., Roy, J., Rongstock, R., et al. (2020). Fungal Traits Important for Soil Aggregation. Front. Microbiol. 10, 2904. doi:10.3389/fmicb.2019.02904

Morris, E. K., Morris, D. J. P., Vogt, S., Gleber, S.-C., Bigalke, M., Wilcke, W., et al. (2019). Visualizing the Dynamics of Soil Aggregation as Affected by Arbuscular Mycorrhizal Fungi. ISME J. 13 (7), 1639–1646. doi:10.1038/s41396-019-0369-0

Pandey, P., V. Ramegowda and M. Senthil-Kumar (2015). "Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms." Frontiers in Plant Science 6: 14.

Schulz, B. and C. Boyle (2005). "The endophytic continuum." Mycological Research 109: 661-686.

United Nations, D. o. E. a. S. A., Population Division (2018) "World Urbanization Prospects: The 2018 Revision."

Wilpiszeski, R. L., J. A. Aufrecht, S. T. Retterer, M. B. Sullivan, D. E. Graham, E. M. Pierce, O. D. Zablocki, A. V. Palumbo and D. A. Elias (2019). "Soil Aggregate Microbial Communities: Towards Understanding Microbiome Interactions at Biologically Relevant Scales." Applied and Environmental Microbiology 85(14): 18.

Appendix

S.1 Chapter one supplementary materials

Supplementary table S.1.1. Loadings of all variables included in the PCA. Distances presented refer to radii surrounding sample plot, for which the variable value was calculated.

Response variable	PCA axis 1	PCA axis 2	PCA axis 3
Longitude	0.042	0.018	-0.240
Latitude	-0.004	0.038	0.176
Size of grassland patch	-0.045	-0.132	0.096
Share of dry grassland (100m)	-0.049	-0.017	-0.251
Share of dry grassland (500m)	-0.052	0.021	-0.282
Share of dry grassland (1000m)	-0.039	0.021	-0.234
Share of dry grassland (5000m)	-0.023	-0.093	-0.146
Air temperature	0.097	0.081	0.047
Urban climatic zone	0.202	-0.043	0.000
Sky view factor	0.034	-0.162	0.045
pH	0.115	-0.092	-0.244
Organic carbon	0.030	0.374	-0.020
Nitrogen	0.026	0.371	0.064
Sulphur	0.037	0.365	-0.026
Phosphorus	-0.005	0.017	0.181
Potassium	0.066	0.234	0.072
Copper	0.079	0.120	0.170
Zinc	-0.065	0.178	0.085
Cadmium	-0.074	0.174	0.235
Lead	-0.073	0.071	0.180
Nickel	-0.087	0.194	0.229
Water content	-0.045	0.329	0.119
Electrical conductivity	0.142	-0.027	-0.075
Cation exchange capacity	0.033	0.166	-0.209
C:N ratio	0.042	0.136	-0.227
C:S ratio	-0.098	-0.058	0.040
Slope	0.086	-0.034	0.042
Total plant cover	0.010	0.117	0.059
Herbaceous plant cover	0.069	0.029	0.141
Moss cover	0.019	0.148	-0.170
Plant litter cover	-0.089	0.002	-0.072
Herbaceous plant height	-0.020	-0.061	0.105
Soil sealing (100m)	0.217	-0.052	0.063
Soil sealing (500m)	0.243	0.000	0.000
Soil sealing (1000m)	0.241	0.035	-0.025
Population density (100m)	0.143	-0.049	0.174
Population density (500m)	0.202	-0.033	0.108
Population density (1000m)	0.214	-0.006	0.047
Floor area ratio (100m)	0.175	-0.065	0.093
Floor area ratio (500m)	0.217	0.014	0.046
Floor area ratio (1000m)	0.209	0.037	0.020
Urbanity index (100m)	0.181	-0.071	0.089

Urbanity index (500m)	0.219	-0.043	0.085
Urbanity index (1000m)	0.231	-0.006	0.065
Road density (100m)	0.146	-0.125	0.116
Road density (500m)	0.216	-0.101	0.056
Road density (1000m)	0.230	-0.025	-0.011
Road density (5000m)	0.213	0.104	-0.059
Road distance	-0.136	0.133	0.044
Railway density (100m)	0.072	0.087	-0.139
Railway density (500m)	0.122	0.148	-0.151
Railway density (1000m)	0.144	0.080	-0.106
Railway density (5000m)	0.149	0.000	-0.107
Railway distance	-0.120	-0.095	0.134
Distance from centre	-0.192	-0.122	0.031
Historical site connectivity	-0.080	-0.092	0.170

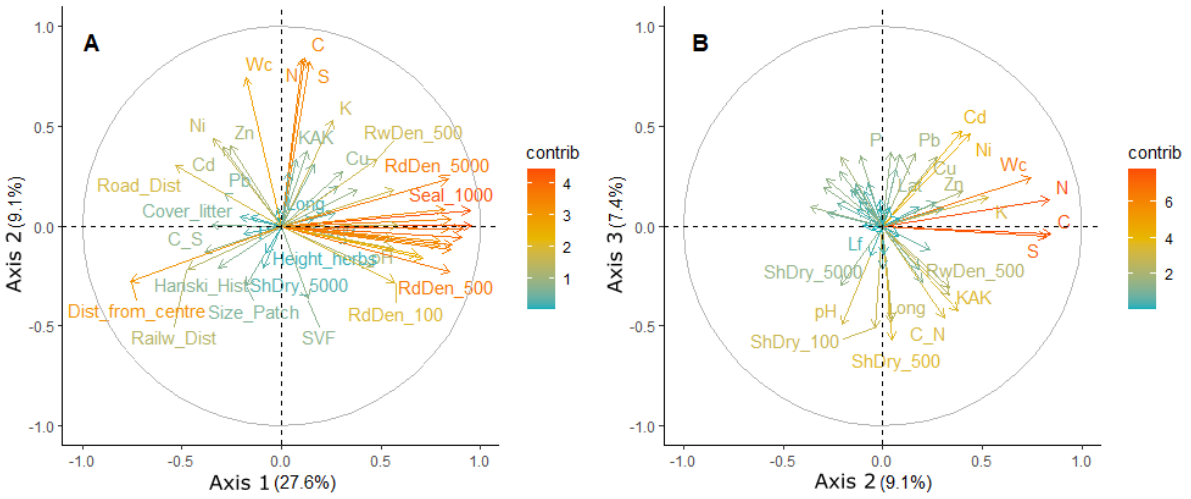
Supplementary table S.1.2. Summary of all statistical results of our study.

Kendall Correlations

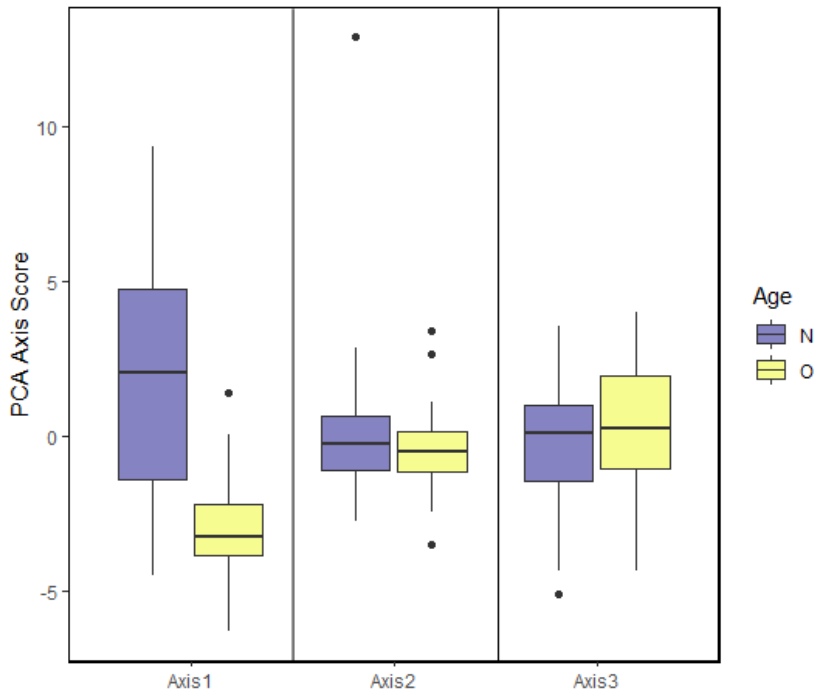
Variable 1	Variable 2	Z score	Kendall's Tau	P value
Average Infiltration Rate	Axis 1	2.62	0.25	0.0088
Average Infiltration Rate	Axis 2	-2.17	-0.20	0.030
Average Infiltration Rate	Axis 3	-0.83	-0.078	0.41
Standard Error Infilt. Rate	Axis 1	1.25	0.12	0.21
Standard Error Infilt. Rate	Axis 2	-2.17	-0.20	0.030
Standard Error Infilt. Rate	Axis 3	-0.59	-0.055	0.56
Average WSA%	Axis 1	-2.19	-0.20	0.029
Average WSA%	Axis 2	1.83	0.17	0.068
Average WSA%	Axis 3	1.53	0.14	0.13
Average MWD	Axis 1	2.44	0.23	0.015
Average MWD	Axis 2	1.23	0.12	0.22
Average MWD	Axis 3	-1.81	-0.17	0.070
Average Coeff. curvature	Axis 1	-0.63	-0.059	0.53
Average Coeff. curvature	Axis 2	0.25	0.023	0.81
Average Coeff. curvature	Axis 3	0.037	0.0035	0.97
Average Uniformity Coeff.	Axis 1	1.45	0.14	0.15
Average Uniformity Coeff.	Axis 2	0.40	0.037	0.69
Average Uniformity Coeff.	Axis 3	-0.77	-0.072	0.44
Average MED	Axis 1	0.47	0.045	0.64
Average MED	Axis 2	1.46	0.14	0.14
Average MED	Axis 3	1.32	0.13	0.19

Wilcoxon Rank Sum Tests

Variable 1	Variable 2	W score	P value
Average Infiltration Rate	Age	418	0.25
Standard Error Infilt. Rate	Age	344	0.90
Average WSA%	Age	234	0.039
Average MWD	Age	497	0.011
Average Coeff. curvature	Age	328	0.68
Average Uniformity Coeff.	Age	469	0.040
Average MED	Age	342	0.86
Axis 1	Age	623	1.9e-06
Axis 2	Age	384	0.58
Axis 3	Age	308	0.44

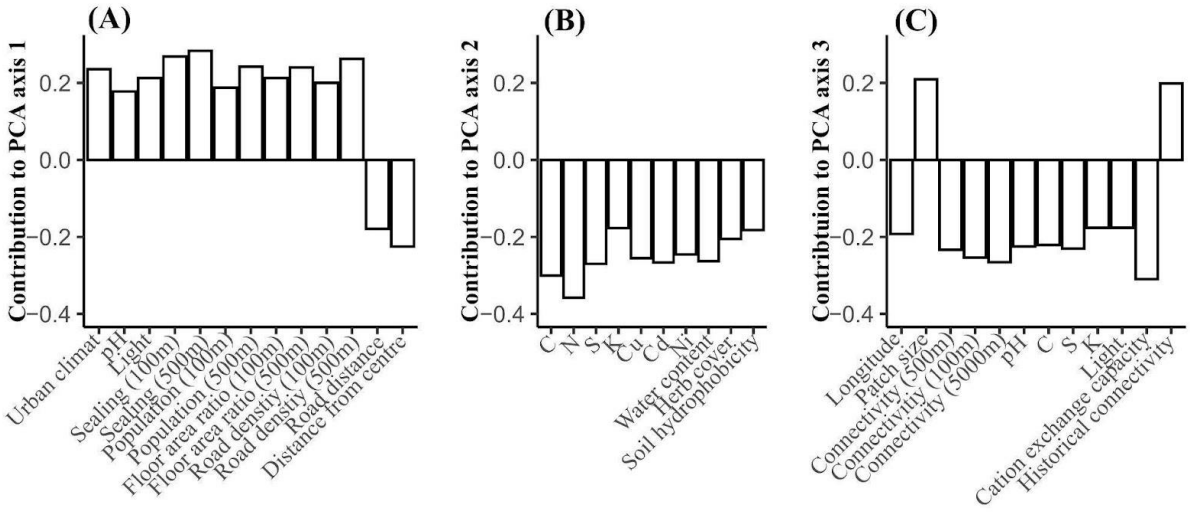


Supplementary Figure S.1.1. Biplots of data, demonstrating the collinearity of variables that form the three main axes of variation. The percentage of total variation in the dataset each axis represents is presented with the axis name.

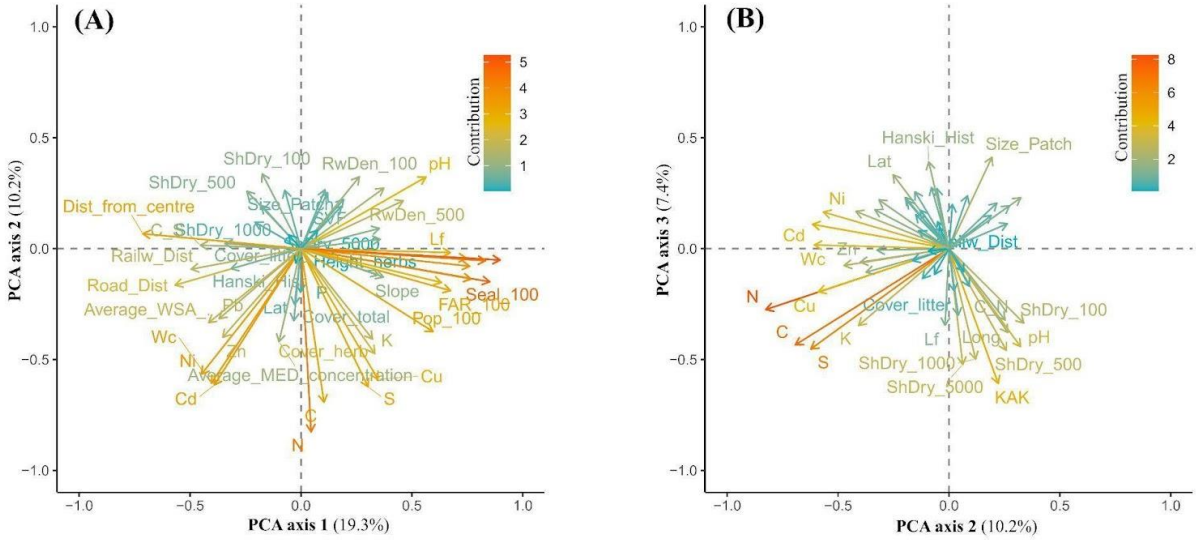


Supplementary figure S.1.2. Variation in PCA axes scores in sites divided by age group. N = New (post-1940), O = Old (pre-1940).

S.2 Chapter two supplementary materials



Supplementary Figure S.2.1. Environmental variables which are major contributors (contribution > 0.175 or <0.175) to each of the 3 PCA axes, which can be categorised as (A) urbanity, (B) soil chemistry and (C) site connectivity. These axes represent 19.3%, 10.2% and 7.4% of total dataset variation respectively.



Supplementary Figure S.2.2. Biplots of (A) axis 1 vs axis 2 and (B) axis 2 vs axis 3. Arrows are shaded according to contribution and proportion of total dataset variance represented by each axis is reported alongside axis titles. For descriptions of variables and individual loadings see Supplementary Table 1.

Supplementary Table S.2.1. Loadings of all of the environmental variables used in the PCA.

Environmental parameter name	Details	Axis 1	Axis 2	Axis 3
Long	Longitude	0.108263	0.265827	-0.37828
Lat	Latitude	-0.02874	-0.25189	0.33299
Size_Patch	Grassland size	-0.17753	0.193803	0.412
ShDry_100	Connectivity within 100m radius	-0.17502	0.337272	-0.33546
ShDry_500	Connectivity within 500m radius	-0.24392	0.259024	-0.4589
ShDry_1000	Connectivity within 1000m radius	-0.2072	0.120004	-0.49835
ShDry_5000	Connectivity within 5000m radius	-0.05594	0.062264	-0.52223
Air_temp_rank	Air temperature	0.350479	-0.11907	-0.13476
Urb_clim	Urban climate zone	0.746535	-0.04336	0.094744

SVF	Sky-view factor	0.182094	0.190275	0.149663
pH	pH	0.563406	0.323021	-0.44202
C	Organic carbon	0.10384	-0.69274	-0.4341
N	Nitrogen	0.045681	-0.82605	-0.27489
S	Sulphur	0.302377	-0.62303	-0.45257
P	Phosphor	-0.00162	-0.19728	0.230658
K	Potassium	0.322556	-0.40798	-0.34658
Cu	Copper	0.347324	-0.58931	-0.19488
Zn	Zinc	-0.35278	-0.40006	-0.0613
Cd	Cadmium	-0.39055	-0.61515	0.109552
Pb	Lead	-0.36927	-0.3159	0.219757
Ni	Nickel	-0.44884	-0.56622	0.164991
Wc	Water content	-0.40421	-0.60694	0.016428
Lf	Light flux	0.673614	-0.01785	-0.34591
KAK	Cation exchange capacity	0.192461	0.222184	-0.60808
C_N	C:N ratio	0.119408	0.252869	-0.32142
C_S	C:S ratio	-0.45383	0.015303	0.199173
Slope	Slope	0.37226	-0.12848	0.173078
Cover_total	Total plant cover	-0.03215	-0.32481	-0.00707
Cover_herb	Herb cover	0.332552	-0.47322	-0.07631
Cover_moss	Moss cover	-0.07299	0.263096	0.112061
Cover_litter	Litter cover	-0.3431	0.02341	-0.19005
Height_herbs	Height of herbs	-0.02344	-0.06551	-0.11655
Seal_100	Soil sealing within 100m radius	0.851339	-0.14968	0.152334
Seal_500	Soil sealing within 500m radius	0.898078	-0.05129	0.121642
Pop_100	Population within 100m radius	0.595258	-0.37385	-0.10646
Pop_500	Population within 500m radius	0.767086	-0.13676	0.080082
FAR_100	Floor:area ratio within 100m radius	0.674952	-0.18966	0.215393
FAR_500	Floor:area ratio within 500m radius	0.760939	-0.07905	0.275395
RdDen_100	Road density within 100m radius	0.633824	-0.15295	0.256134
RdDen_500	Road density within 500m radius	0.832211	-0.05447	0.270961
Road_Dist	Distance to nearest road	-0.56748	-0.16457	-0.05479
RwDen_100	Railway density within 100m radius	0.262971	0.323659	0.229126
RwDen_500	Railway density within 500m radius	0.461274	0.215742	0.20556
Railw_Dist	Distance to nearest railway	-0.49525	-0.09276	-0.01135
Dist_from_centre	Distance from city centre	-0.71294	0.067299	-0.10427
Hanski_Hist	Historical connectivity of site	-0.3155	-0.09116	0.390838
Infilt_Average	Mean infiltration rate	0.373491	0.272758	0.193074
Average_Coefficient_Uniformity	Soil particle uniformity	0.357114	0.041956	-0.30011
Average_Coefficient_Curvature	Soil particle grading	0.075224	0.083682	0.238632
Average_Mean_Weight_Diameter	Soil particle size	0.353295	0.091074	-0.1694
Average_WSA_.	Soil aggregate stability	-0.41644	-0.33395	0.17541
Average_MED_concentration	Soil hydrophobicity	-0.09916	-0.41973	0.167782

Supplementary Table S.2.2. List of models used to explore drivers of root colonisation and traits. These models were the input for Akaike model selection, the output of which can be seen in Supplementary Table X.

Model	**Model name**
PCA Axis 1	axis1
PCA Axis 2	axis2
PCA Axis 1 + PCA Axis 2 + PCA Axis 3	axes123mod
PCA Axis 1 + PCA Axis 2	axes12mod
PCA Axis 1 + PCA Axis 2 + PCA Axis 3 + Date of sampling	axes123daymod
PCA Axis 1 + PCA Axis 2 + PCA Axis 3 + Site age	axes123agemod
PCA Axis 1 + PCA Axis 2 + Age	axes12agemod
PCA Axis 1 * PCA Axis 2	axes12intmod

Age * PCA Axis 1 + Axis 2 + Axis 3	axes123Ageintmod
Age * Axis 1	axes1Ageintmod
Axis 1 + Axis 2 + Axis 3 + Date of sampling	axes123agedaymod
Date of sampling * Axis 1 + Axis 2	axes12dayintmod

Supplementary table S.2.3. Output of Akaike model selection. K = number of model parameters; AICc = Information score of the model, corrected for small sample sizes; Delta_AICc = Difference in information score between model and best model; AICcWt = AICc weight, proportion of variance explained by model; Cum.Wt = Cumulative AICc weights; LL = Log-likelihood of model. Model names are explained in Supplementary table 2.

AM fungi colonisation

Model names	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
axis1	3	364.89	0	0.26	0.26	-179.2
axis2	3	365.06	0.17	0.24	0.50	-179.29
axes12mod	4	366.55	1.66	0.11	0.62	-178.86
axes12agemod	5	367.12	2.23	0.09	0.70	-177.92
axes123mod	5	367.31	2.43	0.08	0.78	-178.02
axes1Ageintmod	5	367.78	2.89	0.06	0.84	-178.25
axes12intmod	5	368.48	3.59	0.04	0.89	-178.6
axes123agemod	6	368.6	3.71	0.04	0.93	-177.39
axes123daymod	6	369.72	4.83	0.02	0.95	-177.95
axes12dayintmod	6	369.83	4.94	0.02	0.97	-178
axes123Ageintmod	7	370.5	5.61	0.02	0.99	-177
axes123agedaymod	7	371.06	6.17	0.01	1	-177.29

Arbuscules

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
axis2	3	296.59	0	0.31	0.31	-145.05
axis1	3	296.8	0.21	0.28	0.59	-145.16
axes12mod	4	298.91	2.32	0.1	0.69	-145.04
axes123mod	5	299.34	2.75	0.08	0.77	-144.03
axes12agemod	5	300.28	3.7	0.05	0.82	-144.5
axes1Ageintmod	5	300.35	3.76	0.05	0.87	-144.54
axes12intmod	5	300.63	4.04	0.04	0.91	-144.67
axes123agemod	6	301.33	4.74	0.03	0.94	-143.75
axes123daymod	6	301.48	4.89	0.03	0.97	-143.83
axes123Ageintmod	7	302.87	6.28	0.01	0.98	-143.19
axes12dayintmod	6	303.28	6.69	0.01	0.99	-144.73
axes123agedaymod	7	303.51	6.92	0.01	1	-143.51

Non - AM fungi presence

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
axis1	3	426.49	0	0.33	0.33	-210
axes12mod	4	428	1.51	0.15	0.48	-209.58
axes12intmod	5	428.04	1.55	0.15	0.64	-208.38
axes123mod	5	428.42	1.93	0.13	0.76	-208.57
axes123daymod	6	430.19	3.71	0.05	0.81	-208.18
axes12agemod	5	430.44	3.95	0.05	0.86	-209.58

axes1Ageintmod	5	430.59	4.1	0.04	0.9	-209.66
axes123agemod	6	430.84	4.36	0.04	0.94	-208.51
axes12dayintmod	6	432.02	5.53	0.02	0.96	-209.1
axis2	3	432.35	5.86	0.02	0.98	-212.93
axes123agedaymod	7	432.76	6.28	0.01	0.99	-208.14
axes123Ageintmod	7	433.5	7.01	0.01	1	-208.5

Root hairs

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
axis1	3	388.74	0	0.33	0.33	-191.13
axes12mod	4	389.9	1.16	0.18	0.51	-190.53
axes123mod	5	390.91	2.17	0.11	0.62	-189.82
axes12agemod	5	391.83	3.08	0.07	0.70	-190.27
axes12intmod	5	392.05	3.3	0.06	0.76	-190.39
axes123agemod	6	392.4	3.66	0.05	0.81	-189.29
axes1Ageintmod	5	392.55	3.81	0.05	0.86	-190.64
axis2	3	392.89	4.15	0.04	0.9	-193.2
axes123daymod	6	393.07	4.32	0.04	0.94	-189.62
axes12dayintmod	6	393.49	4.75	0.03	0.97	-189.83
axes123agedaymod	7	394.74	6	0.02	0.99	-189.13
axes123Ageintmod	7	395.05	6.31	0.01	1	-189.28

Vesicles (log)

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
axis2	3	55.41	0	0.3	0.30	-24.46
axis1	3	55.78	0.37	0.25	0.56	-24.64
axes12mod	4	56.92	1.51	0.14	0.70	-24.04
axes12agemod	5	58.18	2.77	0.08	0.77	-23.45
axes12intmod	5	59.04	3.64	0.05	0.82	-23.88
axes123mod	5	59.11	3.7	0.05	0.87	-23.92
axes1Ageintmod	5	59.12	3.71	0.05	0.92	-23.92
axes12dayintmod	6	60.58	5.17	0.02	0.94	-23.38
axes123agemod	6	60.65	5.25	0.02	0.96	-23.41
axes123daymod	6	61.09	5.69	0.02	0.98	-23.63
axes123Ageintmod	7	61.91	6.51	0.01	0.99	-22.71
axes123agedaymod	7	62.64	7.23	0.01	1	-23.07

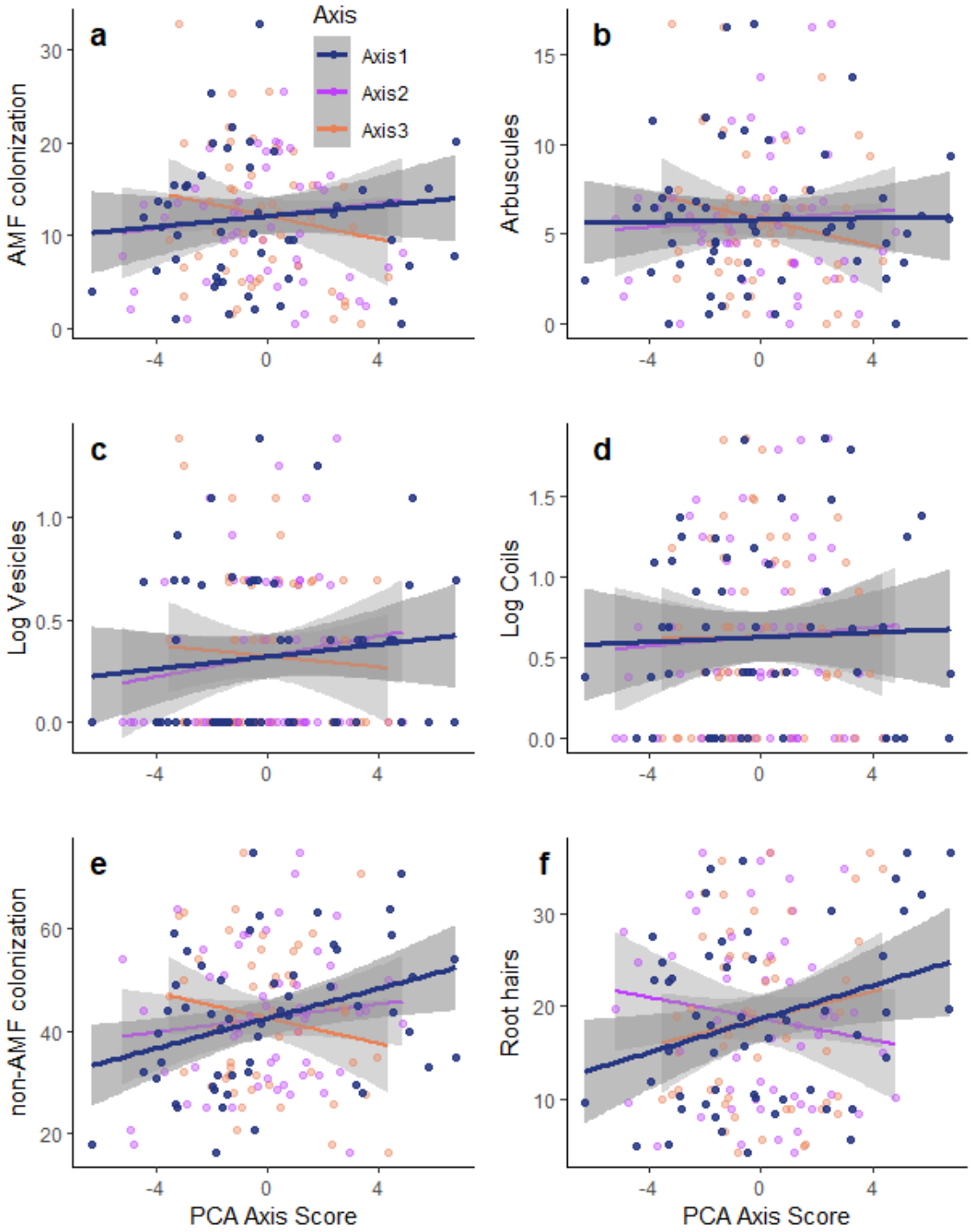
Coils (log)

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
axis2	3	94.89	0	0.34	0.34	-44.2
axis1	3	95	0.1	0.32	0.66	-44.25
axes12mod	4	97.15	2.25	0.11	0.78	-44.16
axes12dayintmod	6	98.89	4	0.05	0.82	-42.53
axes12intmod	5	98.93	4.03	0.05	0.87	-43.83
axes1Ageintmod	5	99.1	4.2	0.04	0.91	-43.91
axes12agemod	5	99.56	4.67	0.03	0.94	-44.14
axes123mod	5	99.58	4.69	0.03	0.97	-44.15

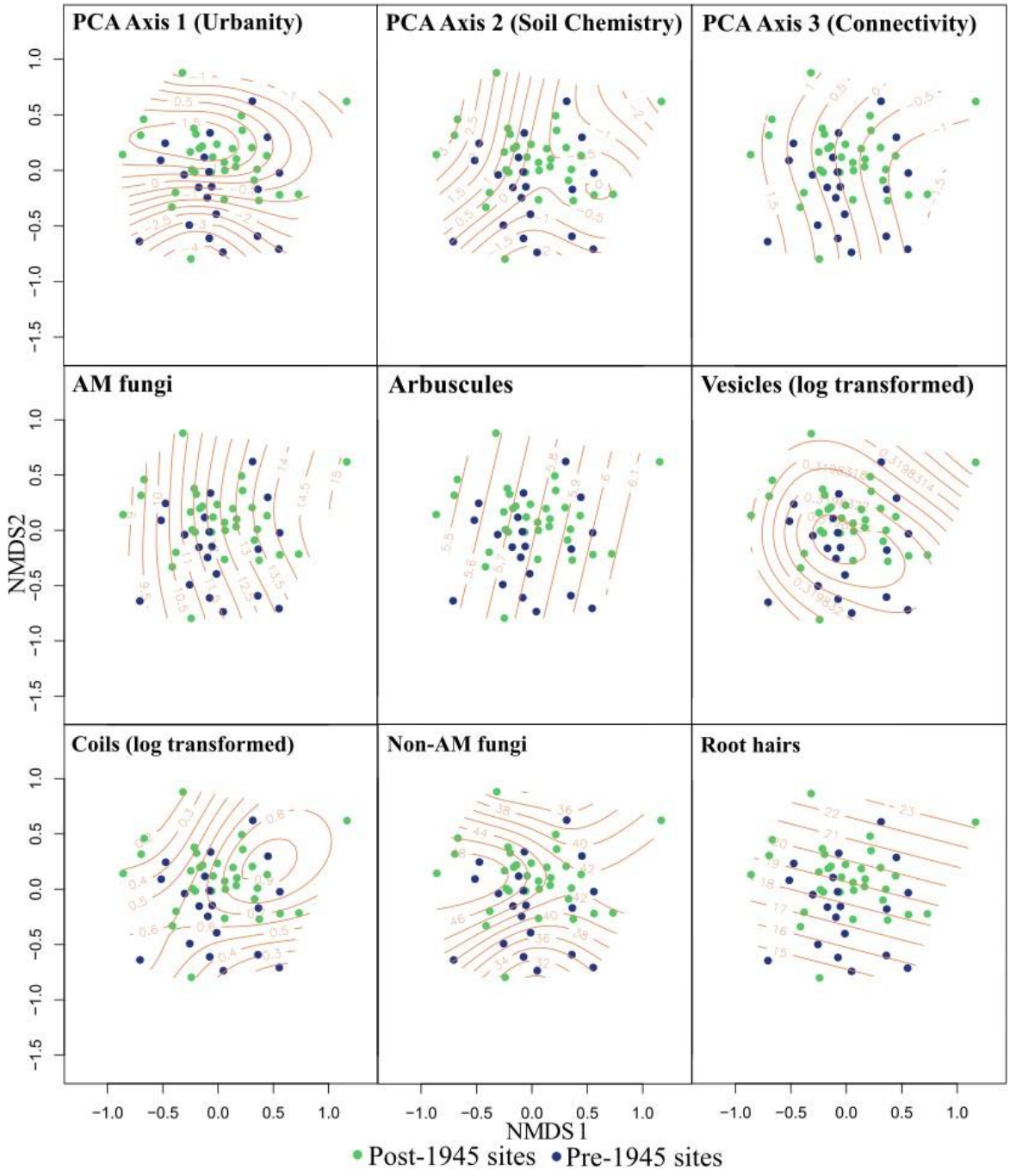
axes123daymod	6	101.91	7.02	0.01	0.98	-44.04
axes123agemod	6	102.11	7.21	0.01	0.99	-44.14
axes123Ageintmod	7	104.23	9.34	0	1	-43.87
axes123agedaymod	7	104.54	9.65	0	1	-44.03

Supplementary table S.2.4. Output of the linear regressions used to explore the drivers of root colonisation and traits. Models were selected by Akaike model selection (Supplementary Table 3.)

Response	**Model**	**R2**	**F**	**p**
AM fungi	Axis 1 + Axis 2	0.028	0.732	0.486
Arbuscules	Axis 1 + Axis 2	0.005	0.125	0.883
Non-AM fungi	Axis 1	0.117	6.763	0.012
Root hairs	Axis 1	0.094	5.283	0.026
Vesicles (log)	Axis 1 + Axis 2	0.037	0.967	0.387
Coils (log)	Axis 1 + Axis 2	0.005	0.135	0.874



Supplementary Figure S.2.3. Responses of all root traits to all three PCA axes. Lines represent linear model fits, shaded areas represent 95% confidence intervals. For statistical summaries, please see Supplementary Table 4. Please note that due to the plotting three variables in each plot, the number of points appears inflated.



Supplementary Figure S.2.4. NMDS plots of plant communities across study sites (2D stress = 0.246). The age of the grassland sites is represented by colour. In each plot a different parameter is portrayed using contour lines. These parameters include the environmental syndromes represented by PCA axes 1, 2 and 3, in addition to the root traits measured in this investigation.

Supplementary Table S.2.5. Output of Akaike model selection of models including a parameter representing plant community. This was only carried out for response variables where an NMDS plot demonstrated a potential pattern (Supplementary Figure 4). The NMDS axis included in each model as a proxy for plant community was selected according to the direction of the correlation seen in the NMDS plot. K = number of model parameters; AICc = Information score of the model, corrected for small sample sizes; Delta_AICc = Difference in information score between model and best model; AICcWt = AICc weight, proportion of variance explained by model; Cum.Wt = Cumulative AICc weights; LL = Log-likelihood of model.

AM fungi

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
Axis1 + Axis2 * NMDS1	6	358.34	0	0.39	0.39	-172.25
Axis1 + Axis2 + NMDS1	5	359.17	0.84	0.26	0.65	-173.95
Axis1 * NMDS1 + Axis2	6	359.48	1.14	0.22	0.87	-172.83
NMDS1	3	360.59	2.26	0.13	0.99	-177.05
Axis1 + Axis2	4	366.55	8.21	0.01	1	-178.86

Arbuscules

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
NMDS1	3	294.4	0	0.67	0.67	-143.95
Axis1 + Axis2 + NMDS1	5	297.44	3.05	0.15	0.81	-143.08
Axis1 + Axis2 * NMDS1	6	298.78	4.38	0.07	0.89	-142.48
Axis1 + Axis2	4	298.91	4.51	0.07	0.96	-145.04
Axis1 * NMDS1 + Axis2	6	299.95	5.55	0.04	1	-143.06

Root hairs

Model	K	AICc	Delta_AICc	AICcWt	Cum.Wt	LL
Axis1	3	388.74	0	0.28	0.28	-191.13
NMDS2	3	388.85	0.1	0.27	0.55	-191.18
Axis1 + NMDS2	4	388.85	0.11	0.27	0.81	-190.01
Axis1 * NMDS2	5	390.92	2.18	0.09	0.91	-189.82
Axis1 * NMDS2 + Axis2	6	391	2.25	0.09	1	-188.58

Supplementary Table S.2.6. Output of multiple linear regression models, in this case for response variables which appeared to be influenced by plant community. The table includes a breakdown of coefficients for each component of the model. Models were selected using Akaike model selection, the results of which are presented in Supplementary Table 5.

AM fungi

Model	R2	F	p	
Axis 1 * NMDS1 + Axis 2	0.162	3.509	0.014	
Coefficients:				
Component	**Estimate**	**St. Error**	**t**	**p**
Axis 1	0.544	0.300	1.816	0.076
NMDS1	8.946	2.638	3.392	0.001
Axis 2	0.924	0.430	2.148	0.037
Axis1:NMDS1	1.169	0.811	1.442	0.156

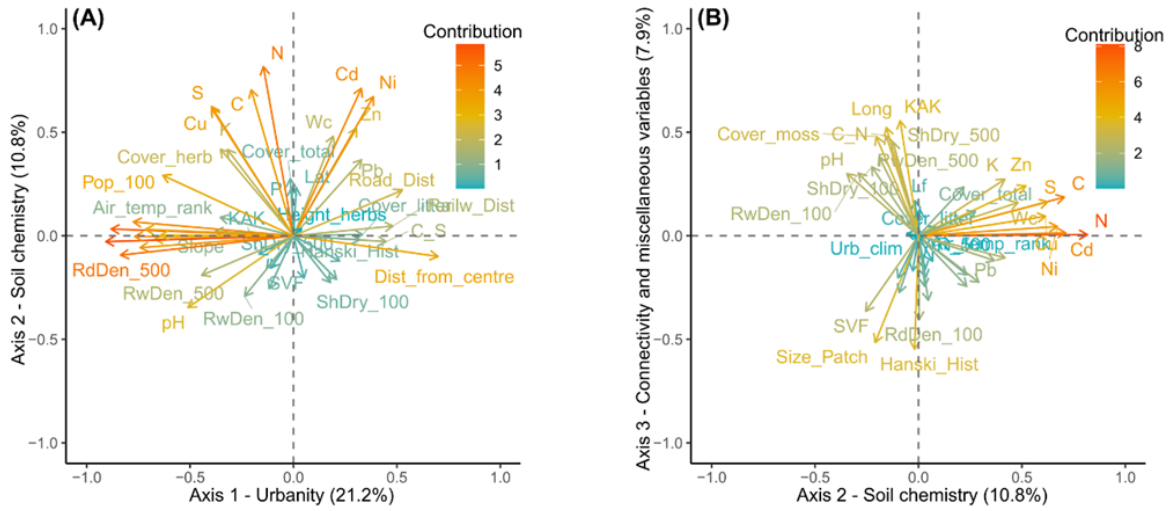
Arbuscules

Model	R2	F	p	
Axis 1 + Axis 2 + NMDS1	0.019	1.338	0.273	
Coefficients:				
Component	**Estimate**	**St. Error**	**t**	**p**
Axis 1	0.0818	0.165	0.496	0.622
Axis 2	0.293	0.242	1.210	0.232
NMDS1	2.841	1.468	1.936	0.059

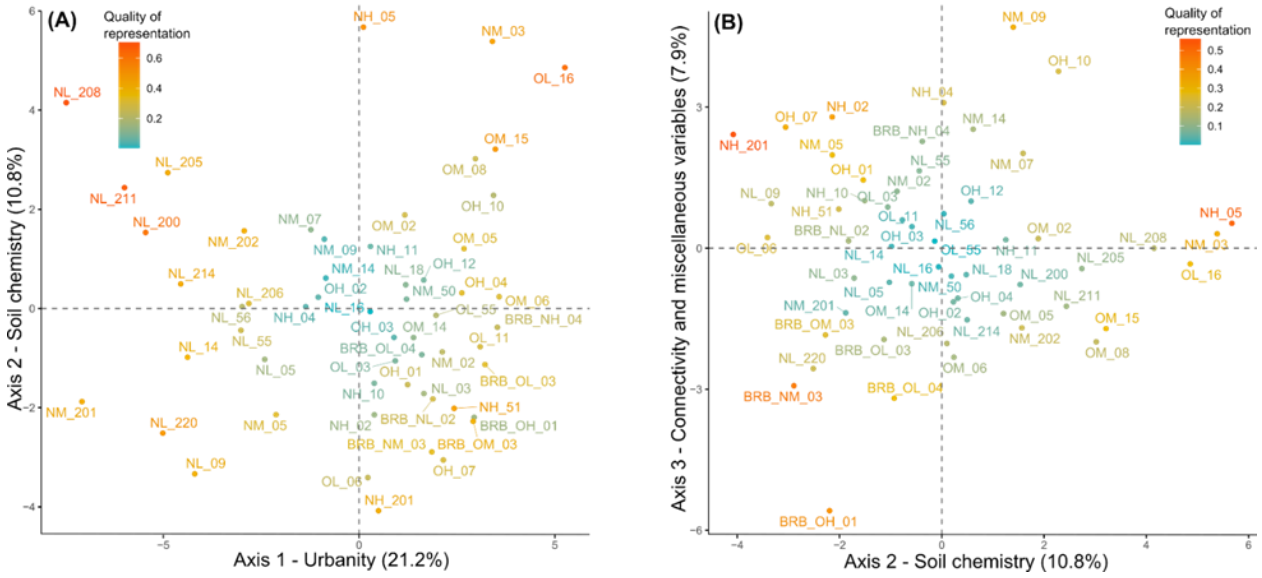
Root hairs

Model	R2	F	p	
Axis 1 + NMDS2	0.097	3.777	0.030	
Coefficients:				
Component	**Estimate**	**St. Error**	**t**	**p**
Axis 1	0.643	0.428	1.502	0.139
NMDS2	5.551	3.783	1.467	0.149

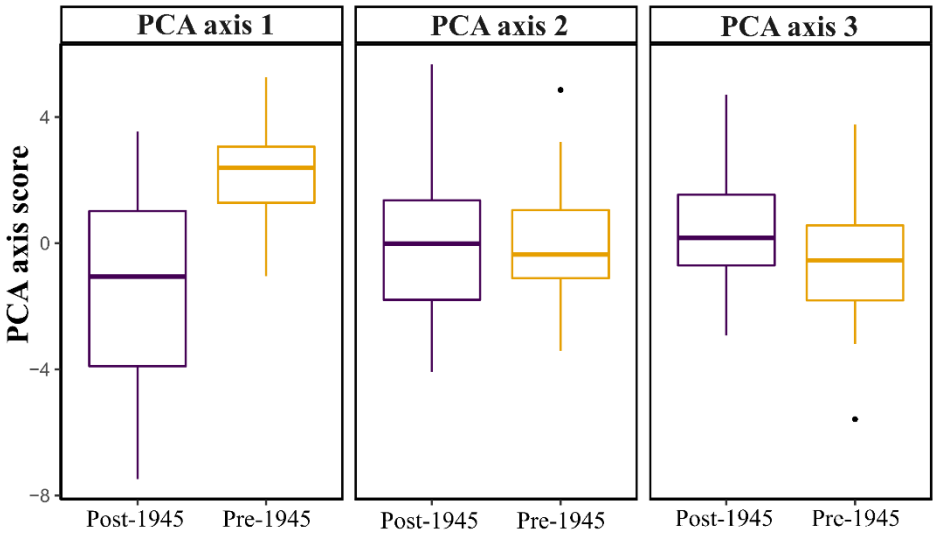
S.3 Chapter three supplementary materials



Supplementary Figure S.3.1. Biplots of the PCA of environmental variables. (A) PCA axes 1 and 2. (B) PCA axes 2 and 3. Percentages of total variance are presented by axis labels. Abbreviations for variables are presented in Supplementary table 1.



Supplementary Figure S.3.2. Plots of study sites according to their PCA scores. (A) Axes 1 and 2. (B) Axes 2 and 3.



Supplementary Figure S.3.3. Boxplot of PCA scores, separated by different age-classes of site. Please note, higher PCA axis 1 score means sites are less urban than those with lower scores.

Supplementary Table S.3.1. PCA variable loadings for environmental data. These variables are plotted in biplots in Supplementary Figure 1.

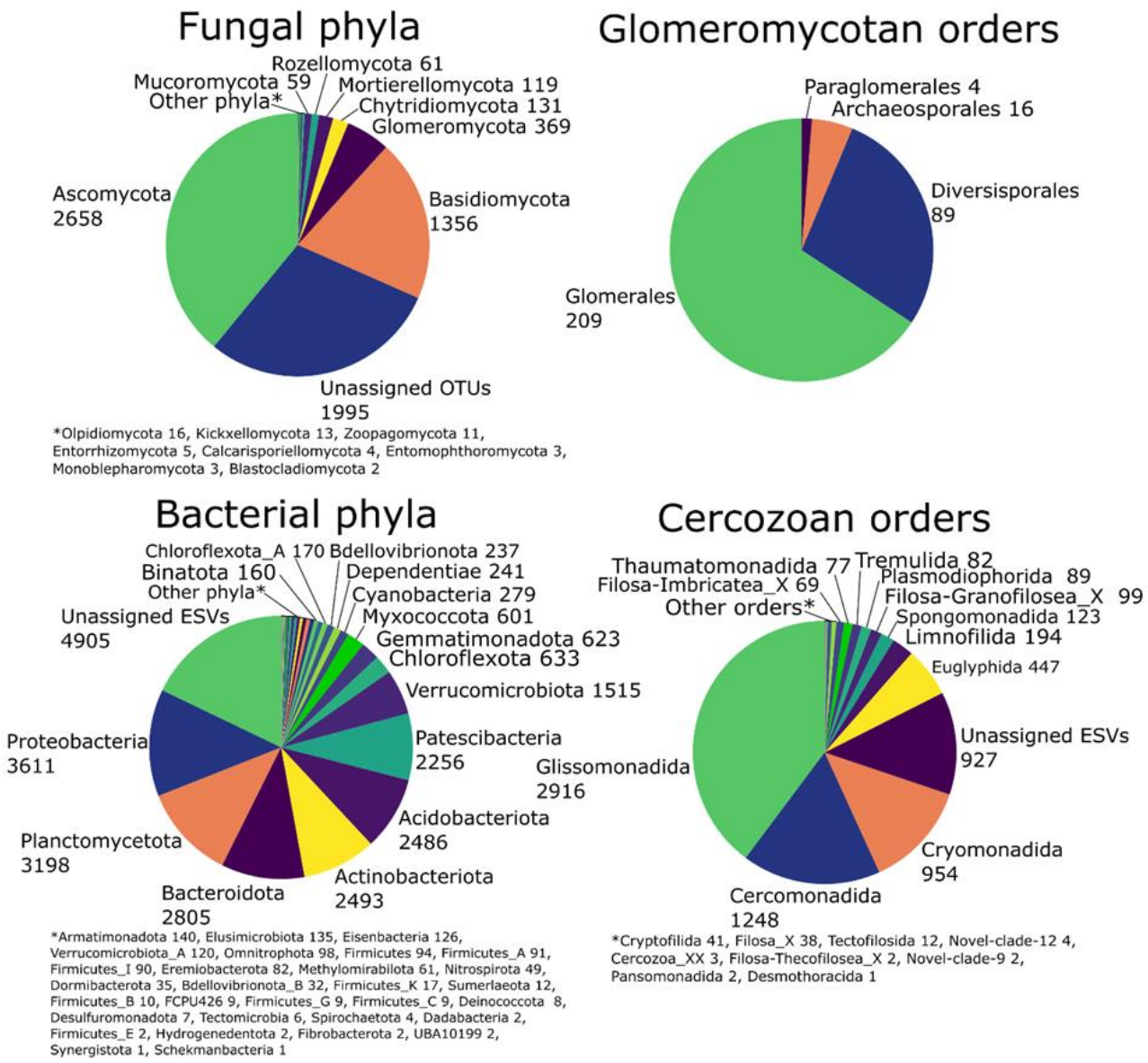
Variable abbreviation	Environmental variable	Axis 1	Axis 2	Axis 3
Long	Longitude	-0.04227	-0.07136	0.280696
Lat	Latitude	0.004518	0.10704	-0.13103
Size_Patch	Patch size	0.068194	-0.09608	-0.27659
ShDry_100	Patch connectivity (100m)	0.058653	-0.10354	0.178869
ShDry_500	Patch connectivity (500m)	0.073249	-0.05616	0.242745
Air_temp_rank	Air temperature	-0.11615	0.040923	0.023029
Urb_clim	Urban climate zone	-0.24311	-0.02614	0.006044
SVF	Sky view factor	-0.03849	-0.1171	-0.19566
pH	pH	-0.16724	-0.15885	0.161177
C	Organic C	-0.06703	0.323445	0.100279
N	Nitrogen	-0.0477	0.374607	0.003028
S	Sulphur	-0.13002	0.28663	0.088025
P	Phosphorous	-0.00251	0.107148	-0.10118
K	Potassium	-0.10492	0.191407	0.146732
Cu	Copper	-0.1295	0.281841	0.050615
Zn	Zinc	0.099866	0.238147	0.129596
Cd	Cadmium	0.107571	0.326857	0.007538
Pb	Lead	0.107995	0.168502	-0.06402
Ni	Nickel	0.126567	0.308139	0.024159
Wc	Water content	0.062939	0.220806	0.086501
Lf	Electron flux	-0.21989	-0.00736	0.091332
KAK	Cation exchange capacity	-0.0546	-0.04074	0.298374
C_N	C:N ratio	-0.03626	-0.07164	0.259529
C_S	C:S ratio	0.146928	-0.01278	-0.09077
Slope	Slope	-0.12443	0.009522	-0.06163

Cover_total	Total plant cover	-0.00499	0.126734	0.063009
Cover_herb	Herbaceous plant cover	-0.11573	0.191926	-0.05669
Cover_moss	Moss cover	0.016616	-0.09397	0.257433
Cover_litter	Plant litter cover	0.109648	0.003004	0.069054
Height_herbs	Height of herbaceous plants	0.012718	0.01591	-0.10854
Seal_100	Soil sealing (100m)	-0.28898	0.015073	-0.08716
Seal_500	Soil sealing (500m)	-0.29745	-0.01293	-0.00033
Pop_100	Population (100m)	-0.20718	0.134006	-0.12049
Pop_500	Population (500m)	-0.25398	0.03096	-0.06599
FAR_100	Floor area ratio (100m)	-0.23459	0.014356	-0.1314
FAR_500	Floor area ratio (500m)	-0.25175	-0.00145	-0.01996
RdDen_100	Road density (100m)	-0.21052	0.001619	-0.21729
RdDen_500	Road density (500m)	-0.27406	-0.04277	-0.14694
Road_Dist	Distance to nearest road	0.172214	0.100789	0.126739
RwDen_100	Railway density (100m)	-0.07737	-0.13363	0.162583
RwDen_500	Railway density (500m)	-0.14554	-0.08738	0.205132
Railw_Dist	Distance to nearest railway	0.157683	0.021756	-0.1674
Dist_from_centre	Distance from city centre	0.229113	-0.04579	-0.10906
Hanski_Hist	Historical site connectivity	0.104988	-0.00865	-0.294

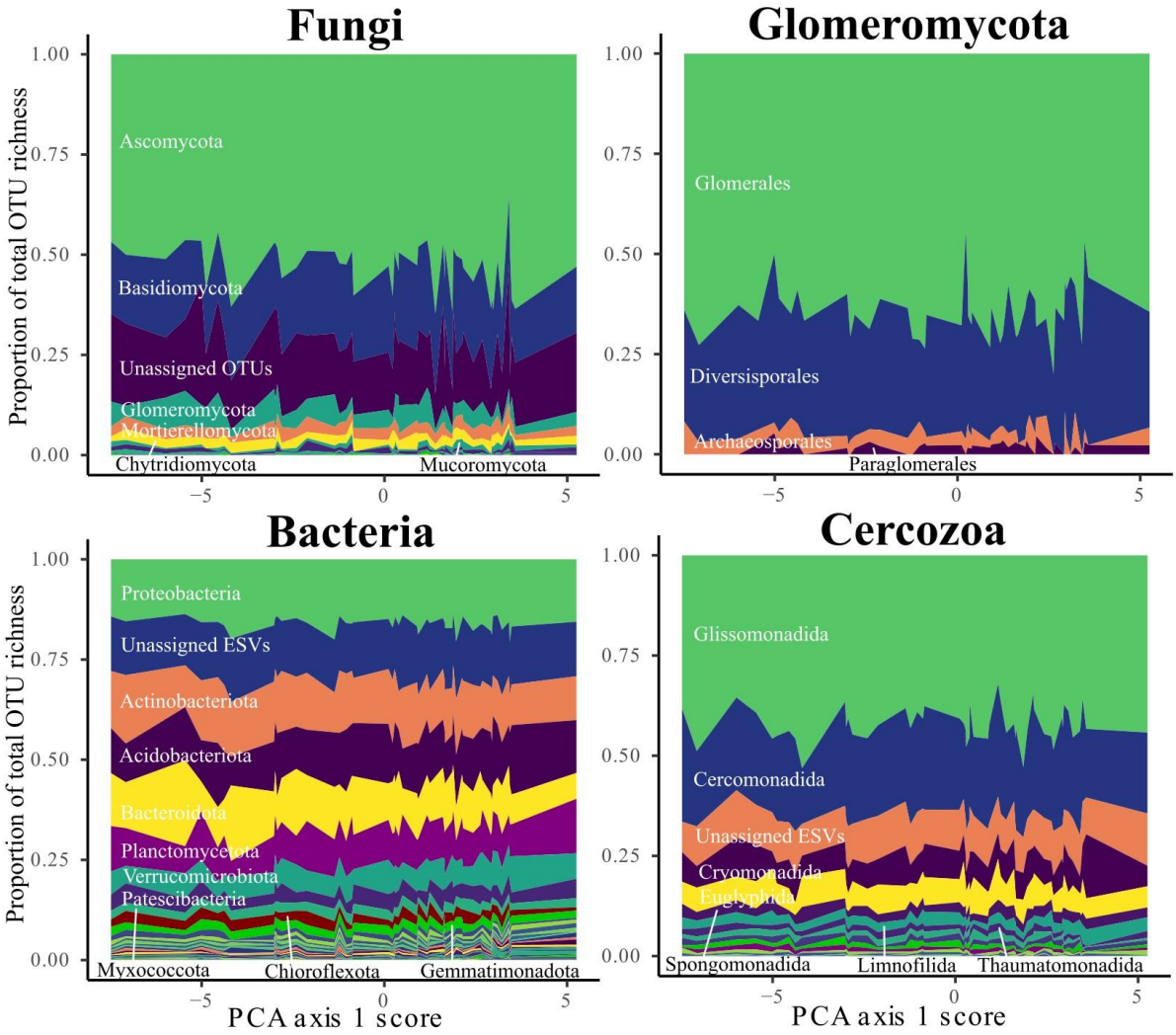
Supplementary Table S.3.2. A breakdown of OTU richness and read numbers which are present in each organism dataset used in this study.

Dataset (total OTUs	Taxonomic group	OTUs (percentage of total)	Reads (percentage of total)
Fungi 6805 OTUs 1530510 reads	Ascomycota	2658 (39%)	993032 (65%)
	Basidiomycota	1356 (20%)	354757 (23%)
	Glomeromycota	369 (5%)	16079 (1%)
	Chytridiomoycota	131 (2%)	14561 (1%)
	Mortierellomycota,	119 (2%)	90116 (6%)
	Unassigned at phylum level	1995 (29%)	44912 (3%)
	10 other phyla: see Supp. Fig. 4	177 (3%)	17053 (1%)
Glomeromycota 318 OTUs 882648 reads	Glomerales	209 (66%)	478423 (54%)
	Diversisporales	89 (28%)	377819 (43%)
	Archaesporales	16 (5%)	26073 (3%)
	Paraglomerales	4 (1%)	333 (<1%)
Bacteria 27469 OTUs 965520 reads	Proteobacteria	3611 (13%)	172934 (18%)
	Planctomycetota	3198 (12%)	52110 (5%)
	Bacteroidota	2805 (10%)	81169 (8%)
	Actinobacteriota,	2493 (9%)	139341 (14%)

	Acidobacteriota	2486 (9%)	215623 (14%)
	Patescibacteria	2256 (8%)	14362 (1%)
	Verrucomicrobiota	1515 (6%)	87091 (9%)
	Unassigned at phylum level	4905 (18%)	82836 (9%)
	38 other phyla: see Supp. Fig. 4	4200 (15%)	120054 (12%)
Cercozoa 7330 OTUs 569088 reads	Glissomonadida	2916 (40%)	217554 (38%)
	Cercomonadida	1248 (17%)	120220 (21%)
	Cryomonadida	954 (13%)	84382 (15%)
	Euglyphida	447 (6%)	56482 (10%)
	Limnofilida	194 (3%)	7253 (1%)
	Spongomonadida	123 (2%)	11652 (2%)
	Unassigned at the order level	927 (13%)	40612 (7%)
	14 other orders: see Supp. Fig. 4	521 (7%)	30960 (5%)



Supplementary Figure S.3.4. Pie charts of the relative numbers of OTUs present in each of the four datasets used in this study. The taxonomic levels at which this is presented are the following: Fungi, phylum level; Glomeromycota, order level; Bacteria, phylum level; Cercozoa, order level.



Supplementary Figure S.3.5. Area plots of relative proportions of reads attributed to different taxonomic groups within each dataset across the urbanity gradient. For the fungi, the low-read non-labelled phyla are: Rozellomycota, Olpidiomycota, Zoopagomycota, Kickxellomycota, Calcarisporiellomycota, Entorrhizomycota, Monoblepharomycota, Entomophthoromycota, Blastocladiomycota.

Low-read bacterial phyla present but not labelled in the figure are: Chloroflexota_A, Binatota, Cyanobacteria, Firmicutes, Methylomirabilota, Nitrospirota, Bdellovibrionota, Firmicutes_I, Dependentiae, Armatimonadota, Eisenbacteria, Elusimicrobiota, Firmicutes_A, Eremiobacterota, Omnitrophota, Verrucomicrobiota_A, Dormibacterota, Bdellovibrionota_B, Firmicutes_K, Sumerlaeota, Deinococcota, Desulfuromonadota, FCPU426, Firmicutes_B, Firmicutes_G, Firmicutes_C, Dadabacteria, Spirochaetota, Tectomicrobia, Firmicutes_E, Hydrogenedentota, Fibrobacterota, UBA10199, Synergistota and Schekmanbacteria.

Low-read cercozoan orders present but not labelled are: Filosa-Imbricatea_X, Filosa-Granofilosea_X, Tremulida, Plasmodiophorida, Filosa_X, Cryptofilida, Tectofilosida, Cercozoa_XX, Filosa-Thecofilosea_X, Novel-clade_12, Desmothoracida.

Supplementary Table S.3.3. PERMANOVA output, using both sequential and partial models, 9999 permutations

			Fungi			Glomeromycota			Bacteria			Cercozoa		
			R2	F	p	R2	F	p	R2	F	p	R2	F	p
Sequential model	Axes1, 2 and 3	Axis 1	0.048	2.570	<0.001	0.028	1.483	0.033	0.065	3.366	<0.001	0.051	2.808	<0.001
		Axis 2	0.039	2.061	<0.001	0.032	1.710	0.006	0.057	2.982	<0.001	0.046	2.496	<0.001
		Axis 3	0.026	1.382	0.0148	0.030	1.607	0.011	0.032	1.650	0.030	0.025	1.343	0.053
	Axes 1, 2, 3 and age	Axis 1	0.048	2.575	<0.001	0.028	1.485	0.028	0.065	3.358	<0.001	0.051	2.810	<0.001
		Axis 2	0.679	2.065	0.012	0.032	1.713	0.006	0.057	2.975	<0.001	0.046	2.498	<0.001
		Axis 3	0.026	1.386	0.012	0.030	1.609	0.013	0.032	1.646	0.031	0.025	1.344	0.051
		Age	0.021	1.090	0.210	0.020	1.070	0.337	0.017	0.898	0.585	0.019	1.031	0.347
Marginal model	Axes 1, 2 and 3	Axis 1	0.048	2.556	<0.001	0.027	1.451	0.039	0.057	2.957	<0.001	0.050	2.749	<0.001
		Axis 2	0.039	2.055	<0.001	0.033	1.719	0.004	0.058	3.009	<0.001	0.046	2.500	<0.001
		Axis 3	0.026	1.383	0.011	0.030	1.607	0.014	0.032	1.650	0.029	0.025	1.343	0.051
	Axes 1, 2, 3 and age	Axis 1	0.033	1.732	<0.001	0.031	1.633	0.010	0.032	1.660	0.030	0.027	1.500	0.020
		Axis 2	0.039	2.069	<0.001	0.032	1.699	0.007	0.058	3.012	<0.001	0.046	2.513	<0.001
		Axis 3	0.021	1.344	0.019	0.032	1.666	0.008	0.029	1.521	0.044	0.023	1.238	0.096
		Age	0.021	1.090	0.216	0.020	1.070	0.345	0.017	0.898	0.577	0.019	1.031	0.341

Supplementary Table S.3.4. Results of simple and partial mantel tests, using Pearson correlations and 9999 permutations.

		Fungi		Glomeromycota		Bacteria		Cercozoa	
	Model	R2	p	R2	p	R2	p	R2	p
Simple Mantel tests	PCA axis 1	0.155	0.024	0.022	0.358	0.148	0.025	0.159	0.021
	PCA axis 2	0.224	0.007	0.151	0.018	0.341	<0.001	0.293	<0.001
	PCA axis 3	0.137	0.071	0.025	0.354	0.077	0.168	0.057	0.247
	Geographic distance	0.162	0.010	0.122	0.016	0.089	0.076	0.082	0.099
Partial Mantel tests	Axis 1 + geographic distance	0.164	0.023	0.028	0.315	0.154	0.023	0.162	0.021
	Axis 2 + geographic distance	0.225	0.006	0.152	0.017	0.342	<0.001	0.294	<0.001
	Axis 3 + geographic distance	0.110	0.114	0.003	0.477	0.063	0.201	0.040	0.309
	Axis 1 + Axis 2	0.133	0.042	0.004	0.469	0.124	0.046	0.136	0.040
	Axis 1 + Axis 3	0.163	0.022	0.023	0.349	0.153	0.021	0.163	0.019
	Axis 2 + Axis 3	0.234	0.004	0.153	0.015	0.346	<0.001	0.296	<0.001

Supplementary Table S.3.5. Results of hierarchical partitioning of taxonomic group richness. Presented are the most important PCA axis alongside the percentage of variance this represents within each taxonomic group, and the Kendall correlation coefficient between this axis and the OTU richness of the taxonomic group.

Dataset	Taxonomic group	Most significant axis	Explained variance	Correlation coefficient
Fungi	Ascomycota	Axis 1	86.3%	-0.30
	Basidiomycota	Axis 3	43.6%	0.22
	Chytridiomycota	Axis 1	68.4%	-0.26
	Glomeromycota	Axis 1	85.8%	-0.33
	Mortierellomycota	Axis 2	75.5%	0.31
	Olpidiomycota	Axis 1	100.0%	-0.45
	Rozellomycota	Axis 2	84.4%	0.32
	Zoopagomycota	Axis 2	67.8%	0.24
	OTUs unassigned at phyla level	Axis 1	80.4%	-0.32
Glomeromycota	Archaeosporales	Axis 1	72.2%	-0.23
	Diversisporales	Axis 2	85.7%	0.22
	Glomerales	Axis 2	70.9%	0.28
Bacteria	Actinobacteriota	Axis 1	81.2%	-0.32
	Armatimonadota	Axis 2	87.6%	-0.20
	Bacteroidota	Axis 1	69.9%	-0.30
	Bdellovibrionota_B	Axis 1	87.8%	-0.27
	Chloroflexota	Axis 2	70.4%	-0.43
	Chloroflexota_A	Axis 1	87.8%	-0.38
	Deinococcota	Axis 2	82.8%	-0.39
	Dormibacterota	Axis 2	50.2%	0.33
	Eremiobacterota	Axis 1	48.1%	0.29
	FCPU426	Axis 1	43.6%	0.27
	Firmicutes_B	Axis 1	95.4%	-0.31
	Gemmatimonadota	Axis 1	65.3%	-0.24
	Methylomirabilota	Axis 1	49.1%	-0.28
	Myxococcota	Axis 1	51.6%	-0.22
	Nitrospirota	Axis 1	69.9%	-0.26
	Proteobacteria	Axis 1	53.4%	-0.21

	Sumerlaeota	Axis 1	70.0%	-0.25
	UBA10199	Axis 2	50.0%	-0.24
	OTUs unassigned at phyla level	Axis 2	56.6%	-0.20
Cercozoa	Cercomonadida	Axis 2	98.4%	0.23
	Cercozoa_XX	Axis 1	68.2%	-0.37
	Cryptofilida	Axis 2	51.8%	0.24
	Filosa_X	Axis 2	96.5%	0.34
	Filosa-Imbricatea_X	Axis 1	64.4%	-0.25
	Filosa-Thicofilosea_X	Axis 2	89.0%	0.21
	Limnofilida	Axis 2	85.5%	0.40
	Plasmodiophorida	Axis 1	97.8%	-0.32
	Tremulida	Axis 2	73.1%	0.24

Supplementary Table S.3.6. Species which are likely to be indicators of highly urban sites. Presented are the results of indicator species analysis for the top 18 most urban sites according to PCA axis 1 score.

Dataset	Species	Point biserial correlation coefficient	p value
Fungi	*Septoglomus viscosum*	0.446	0.0026
	Purpureocillium lilacinum	0.437	0.0032
	Ganoderma adspersum	0.403	0.0091
	Keissleriella culmifida	0.378	0.0318
	Claroideoglomus claroideum	0.378	0.0051
	Clonostachys rosea	0.375	0.0186
	Thanatephorus cucumeris	0.375	0.004
	Mortierella alpina	0.371	0.0165
	Olpidium brassicae	0.365	0.0079
	Septoglomus viscosum	0.363	0.0137
	Thanatephorus cucumeris	0.362	0.019
	Robillarda sessilis	0.358	0.034
	Holtermanniella takashimae	0.343	0.0171
	Glomus aggregatum	0.341	0.0191
	Nectria ramulariae	0.339	0.0269
	Buckleyzyma aurantiaca	0.339	0.0205

	Schizothecium conicum	0.333	0.0096
	Mortierella antarctica	0.329	0.0098
	Preussia flanaganii	0.317	0.0053
	Tolypocladium album	0.309	0.0499
	Myrmecridium phragmitis	0.305	0.0054
	Lectera longa	0.304	0.032
	Mucor hiemalis	0.301	0.0302
	Filobasidium stepposum	0.296	0.0094
	Gibberella tricincta	0.282	0.0466
	Naganishia adeliensis	0.273	0.0165
	Mortierella alpina	0.236	0.029
Glomeromycota	*Scutellospora calospora*	0.284	0.0319
Bacteria	*Nitrospira C japonica*(RS_GCF_900169565.1)	0.556	4.00E-04
	SCN-69-37_sp001724025(GB_GCA_001724025.1)	0.538	1.00E-04
	QHWT01_sp003222675(GB_GCA_003222675.1)	0.528	4.00E-04
	UBA6082_sp002428665(GB_GCA_002428665.1)	0.52	0.0024
	Solirubrobacter soli(RS_GCF_000423665.1)	0.519	9.00E-04
	Gp1-AA17_sp003223515(GB_GCA_003223515.1)	0.512	9.00E-04
	AV55_sp003219415(GB_GCA_003219415.1)	0.51	3.00E-04
	*Lysobacter*_sp001427225(RS_GCF_001427225.1)	0.506	7.00E-04
	SCN-70-22_sp001724275(GB_GCA_001724275.1)	0.498	0.0012
	QHWT01_sp003222675(GB_GCA_003222675.1)	0.497	6.00E-04
	OLB17_sp001567505(GB_GCA_001567505.1)	0.479	0.0014
	Microlunatus phosphovorus(RS_GCF_000270245.1)	0.469	0.0012
	*Cellulomonas*_sp000426185(RS_GCF_000426185.1)	0.465	0.0025
	Luteitalea pratensis(RS_GCF_001618865.1)	0.457	0.0028
	Pedobacter panaciterrae(RS_GCF_001636695.1)	0.455	0.0033
	UBA11741_sp002427845(GB_GCA_002427845.1)	0.453	0.0034
	*Williamsia*_sp002095395(RS_GCF_002095395.1)	0.453	0.0021
	Oligoflexus tunisiensis(GB_GCA_001748245.1)	0.449	0.0043
	Fimbriiglobus ruber(RS_GCF_002197845.1)	0.446	0.0039
	Solirubrobacter soli(RS_GCF_000423665.1)	0.443	0.0047
	Nonomuraea solani(RS_GCF_900108335.1)	0.442	0.0068

*Haloferula*_sp000739615(RS_GCF_000739615.1)	0.441	0.0025
HRBIN40_sp002898275(GB_GCA_002898275.1)	0.441	0.0055
*Chitinophaga*_sp900110995(RS_GCF_900110995.1)	0.439	0.0058
Actinoplanes atraurantiacus(RS_GCF_900215205.1)	0.435	0.0036
UBA11740_sp003168335(GB_GCA_003168335.1)	0.433	0.0039
Luteitalea pratensis(RS_GCF_001618865.1)	0.429	0.0134
Litorilinea aerophila(GB_GCA_002148365.1)	0.428	0.0018
Luteitalea pratensis(RS_GCF_001618865.1)	0.425	0.0074
GWC2-73-18_sp001794945(GB_GCA_001794945.1)	0.424	0.005
Ohtaekwangia koreensis(RS_GCF_900167975.1)	0.423	0.0072
Fimbriiglobus ruber(RS_GCF_002197845.1)	0.423	0.0091
*Agromyces*_sp001429165(RS_GCF_001429165.1)	0.418	0.0038
UNC496MF_sp900116125(RS_GCF_900116125.1)	0.418	0.0263
UBA11740_sp003168335(GB_GCA_003168335.1)	0.417	0.0275
*Aquamicrobium*_sp001427385(RS_GCF_001427385.1)	0.415	0.0274
RBG-16-40-8_sp001769925(GB_GCA_001769925.1)	0.411	0.0148
Gp7-AA10_sp003223695(GB_GCA_003223695.1)	0.41	0.006
UBA2421_sp002343075(GB_GCA_002343075.1)	0.408	0.0162
*Nocardioides*_sp001425025(RS_GCF_001425025.1)	0.408	0.006
Chthoniobacter flavus(RS_GCF_000173075.1)	0.408	0.0104
*Arthrobacter*_A_sp003268655(GB_GCA_003268655.1)	0.408	0.0022
Gp6-AA56_sp003222395(GB_GCA_003222395.1)	0.407	0.0261
OLB13_sp001567485(GB_GCA_001567485.1)	0.405	0.01
GWC2-73-18_sp001794945(GB_GCA_001794945.1)	0.405	0.0137
Luteitalea pratensis(RS_GCF_001618865.1)	0.405	0.0075
Paraclostridium benzoelyticum(RS_GCF_001006285.1)	0.403	0.0074
Methylobacter luteus(RS_GCF_000427625.1)	0.403	0.0274
Pirellula staleyi(RS_GCF_000025185.1)	0.402	0.0155
RBG-16-71-46_sp001780165(GB_GCA_001780165.1)	0.401	0.0272
*W-Chloroflexi-9*_sp002840675(GB_GCA_002840675.1)	0.4	0.0304
Kouleothrix aurantiaca(GB_GCA_001399705.1)	0.396	0.0308
UBA2475_sp002319075(RS_GCF_002319075.1)	0.396	0.0143
*Nocardioides*_sp000620645(RS_GCF_000620645.1)	0.395	0.0089
Chthoniobacter flavus(RS_GCF_000173075.1)	0.393	0.0092

*Palsa-739*_sp003139545(GB_GCA_003139545.1)	0.391	0.0191
*Brevundimonas*_sp001427825(RS_GCF_001427825.1)	0.39	0.0188
Gp6-AA56_sp003222395(GB_GCA_003222395.1)	0.389	0.0298
Kouleothrix aurantiaca(GB_GCA_001399705.1)	0.389	0.0153
PALSA-1355_sp003153375(GB_GCA_003153375.1)	0.388	0.0181
QHXM01_sp003222945(GB_GCA_003222945.1)	0.387	0.0114
Bin18_sp002238415(GB_GCA_002238415.1)	0.386	0.0203
Micromonospora lupini(RS_GCF_000297395.2)	0.386	0.0299
Gp6-AA56_sp003222395(GB_GCA_003222395.1)	0.385	0.0173
Gp18-AA60_sp003225335(GB_GCA_003225335.1)	0.384	0.0189
Gp6-AA56_sp003222395(GB_GCA_003222395.1)	0.384	0.0174
Phyllobacterium brassicacearum(RS_GCF_003010955.1)	0.383	0.0158
UBA2421_sp002343075(GB_GCA_002343075.1)	0.382	0.0238
2011-GWC2-44-17_sp001029695(GB_GCA_001029695.1)	0.382	0.0257
XYD1-FULL-53-11_sp001770165(GB_GCA_001770165.1)	0.382	0.023
Gp6-AA56_sp003222395(GB_GCA_003222395.1)	0.382	0.022
*Opitutus*_sp003054705(RS_GCF_003054705.1)	0.382	0.0209
67-14_sp001897355(GB_GCA_001897355.1)	0.38	0.0166
Pedobacter nyackensis(RS_GCF_900176505.1)	0.379	0.03
Paenibacillus T cellulosilyticus(RS_GCF_003182255.1)	0.378	0.0051
Mesorhizobium metallidurans(RS_GCF_000350085.1)	0.375	0.0252
Chryseolinea serpens(RS_GCF_900129725.1)	0.374	0.0189
OLB17_sp001464455(GB_GCA_001464455.1)	0.373	0.0285
Niastella vici(RS_GCF_002077945.1)	0.37	0.0194
Gp6-AA56_sp003222395(GB_GCA_003222395.1)	0.37	0.0243
Chryseolinea serpens(RS_GCF_900129725.1)	0.369	0.0235
Chthoniobacter flavus(RS_GCF_000173075.1)	0.366	0.0107
AV2_sp003218935(GB_GCA_003218935.1)	0.365	0.0309
Kouleothrix aurantiaca(GB_GCA_001399705.1)	0.365	0.0272
Pirellula staleyi(RS_GCF_000025185.1)	0.365	0.0293
AV55_sp003219415(GB_GCA_003219415.1)	0.364	0.0316
Turicibacter sanguinis(RS_GCF_000178255.1)	0.364	0.0485
Microvirga ossetica(RS_GCF_002741015.1)	0.363	0.0299
HRBIN33_sp002923375(GB_GCA_002923375.1)	0.362	0.0323

UBA11741_sp002427845(GB_GCA_002427845.1)	0.36	7.00E-04
QHBO01_sp003243965(GB_GCA_003243965.1)	0.359	0.0312
Steroidobacter denitrificans(RS_GCF_001579945.1)	0.358	0.0328
Arenimonas composti(RS_GCF_000426365.1)	0.357	0.0189
Dyadobacter beijingensis(RS_GCF_000382205.1)	0.357	0.0459
*Singulisphaera*_sp900129635(RS_GCF_900129635.1)	0.355	0.0287
Ga0077555_sp001464855(GB_GCA_001464855.1)	0.355	0.0328
Luteitalea pratensis(RS_GCF_001618865.1)	0.355	0.035
UBA854_sp002295885(GB_GCA_002295885.1)	0.355	0.0492
*Blastococcus*_sp900188025(RS_GCF_900188025.1)	0.354	0.0267
Fen-1137_sp003142855(GB_GCA_003142855.1)	0.354	0.0405
Gp7-AA10_sp003223695(GB_GCA_003223695.1)	0.354	0.0347
Devosia epidermidihirudinis(RS_GCF_000971295.1)	0.353	0.0288
PMNU01_sp002952755(RS_GCF_002952755.1)	0.352	0.0478
QHVH01_sp003222245(GB_GCA_003222245.1)	0.351	0.0326
Gp6-AA56_sp003222395(GB_GCA_003222395.1)	0.35	0.0283
UBA2421_sp002343075(GB_GCA_002343075.1)	0.348	0.0465
*Conexibacter*_A_sp000688095(RS_GCF_000688095.1)	0.347	0.0417
Palsa-1315_sp002737345(GB_GCA_002737345.1)	0.346	0.0408
*Pseudoxanthomonas*_A_sp900104085(RS_GCF_900104085.1)	0.344	0.041
UBA2421_sp002343075(GB_GCA_002343075.1)	0.344	0.0301
AR5_sp003220265(GB_GCA_003220265.1)	0.344	0.0406
QHWT01_sp003222675(GB_GCA_003222675.1)	0.342	0.0306
Chthoniobacter flavus(RS_GCF_000173075.1)	0.341	0.0096
JOSHI-001_sp002198735(RS_GCF_002198735.1)	0.341	0.0445
2-12-FULL-60-19_sp001798595(GB_GCA_001798595.1)	0.34	0.0462
*Sphingopyxis*_sp900108295(RS_GCF_900108295.1)	0.339	0.0297
*Phenylobacterium*_sp001557235(RS_GCF_001557235.1)	0.338	0.0335
QHWT01_sp003222675(GB_GCA_003222675.1)	0.336	0.0498
Microlunatus phosphovorus(RS_GCF_000270245.1)	0.335	0.0108
QHWT01_sp003222675(GB_GCA_003222675.1)	0.333	0.045
Enhygromyxa salina_C(GB_GCA_000737335.3)	0.33	0.0258
Mucilaginibacter pineti(RS_GCF_900101875.1)	0.329	0.0285
*Rhizobacter*_sp000799305(RS_GCF_000799305.1)	0.329	0.0472

	Ohtaekwangia koreensis(RS_GCF_900167975.1)	0.328	0.03
	Kouleothrix aurantiaca(GB_GCA_001399705.1)	0.322	0.0474
	QHVH01_sp003222245(GB_GCA_003222245.1)	0.318	0.0132
	UBA4722_sp002404295(GB_GCA_002404295.1)	0.302	0.0306
Cercozoa	*Nudifila producta*	0.388	0.0115
	Euglypha rotunda	0.386	0.0112
	Spongomonas minima	0.363	0.0081
	Paracercomonas compacta	0.352	0.0216
	Nucleocercomonas praelonga	0.343	0.0309
	Sorosphaera veronicae	0.338	0.0224
	Neocercomonas jutlandica	0.33	0.0473
	Trachelocorythion pulchellum	0.328	0.0376
	Sandona aporians	0.325	0.0304
	Paracercomonas virgaria	0.306	0.0172
	Tremula longifila	0.28	0.0259