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DOCTORAL THESIS

SOIL HEALTH IN URBAN AGRICULTURE WITH AN EMPHASIS ON ARBUSCULAR MYCORRHIZAL FUNGI

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Declaration of Authorship

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"There is only this now. It does not come from anywhere; it is not going anywhere. It is not permanent, but it is not impermanent. Though moving, it is always still. When we try to catch it, it seems to run away, and yet it is always here, and there is no escape from it. And when we turn around to find the self which knows this moment, we find that it has vanished like the past."

Alan Watts

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Abstract

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SOIL HEALTH IN URBAN AGRICULTURE WITH AN EMPHASIS ON ARBUSCULAR MYCORRHIZAL FUNGI

by Matthias SALOMON

Urban agriculture describes the production of food or raising of animals within city boundaries. It is a promising method to increase urban sustainability and to create resilient communities. However, our understanding of soil processes in these complex systems is limited and little addressed in previous scientific research. This thesis aimed to describe the soil health of urban agriculture systems based on field studies within the metropolitan area of Adelaide, South Australia. The natural occurrence of arbuscular mycorrhizal fungi (AMF) and their application via commercial products was emphasized as a tool to increase sustainability in urban agriculture.

Two independent surveys were undertaken: The first one captured twelve urban agriculture sites at one time point, whereas the second survey sampled three urban agriculture sites over the course of one year. Soil samples were collected and analysed according to physical, chemical, and biological soil properties. To analyse the potential use of commercial arbuscular mycorrhizal (AM) inoculants, these were evaluated in a global study towards their potential to colonize a host plant under controlled conditions. Some quality concerns were identified and addressed through a quality management framework. In a final step, typical urban agriculture substrates, such as potting mixes or composts, where evaluated and tested whether they support colonization of a self-propagated AMF culture. All results of this thesis were incorporated into two literature reviews, focusing on soil management principles of urban agriculture and the use of AMF as biofertilizers.

The results of this thesis showed generally fertile urban agriculture soils with heavy metal concentrations below national guideline limits. However, imbalanced plant nutrients were uncovered, such as consistently high concentrations of plant-available phosphorus and very low concentrations of mineral nitrogen during certain times of the year. Potential soil health constraints were identified and addressed through sustainable soil management principles and the use of urban waste products. The evaluation of commercial AM inoculants showed that, on a global scale, the majority of products failed to colonize a host plant under controlled conditions. Using a self-produced culture of AMF

with composts and potting mixes showed that the development of AMF is not inhibited as long as the substrate is not limiting plant growth.

This thesis provides novel insights into a broad range of urban agriculture soil health properties. It also highlights the use of AMF towards increased sustainability and productivity of urban food production. The evaluation of commercial AM inoculants revealed global quality concerns, which have been addressed through a proposed quality framework. Implementation of this framework into national guidelines would support the widespread adoption of AMF biofertilizers in food production systems.

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Chapter 1

Introduction

1.1 Introduction

The information within this thesis is presented primarily in the form of original research articles (Chapter 4 - 6 and 8) and review articles (Chapter 2 - 3 and 7), which have been, or are intended, for publication. The two review articles (Chapter 2 - 3), which immediately follow this short introduction, serve as the literature review for this thesis. Each research article also has its own introduction, the content of which will not be repeated in this first chapter, except where necessary to set the context for this study. This thesis introduction is intended to provide contextual information about the scope of this thesis and its structure. The Discussion will mainly focus on over-arching results and unanswered questions that arose throughout the thesis. This is then concluded by a description of emerging research topics within the areas of soil health, urban agriculture and arbuscular mycorrhizal fungi.

1.1.1 Significance of Urban Agriculture

According to the Food and Agriculture Organisation of the United Nations (FAO), urban agriculture is defined as "[...] the growing of plants and the raising of animals within and around cities" (Umesha et al., 2018). One of the earliest scientific publications mentioning urban agriculture is titled "Food and survival in Lusaka's self-help townships" (Ledogar, 1978). This article describes various efforts to improve food security in Zambia, after food imports from neighbouring countries were halted due to political unrest. If we go back even further in history, we find similar concepts, albeit under various popularised terms. "Victory gardens" were introduced during war times with the goal of increasing food security and reducing countries' dependency on food imports. In many instances, these measures were successful. For example, Victory gardens produced 40% of the fresh produce North Americans consumed in 1943 (Steinhauer, 2020). Today, urban agriculture is still an important component of food production in developing countries and essential for improving food security and the nutritional value of diets (Zezza & Tasciotti, 2010). Its significance can also be judged by flagship initiatives like the Urban Food Agenda (FAO, 2019). The Google trend for the search term 'urban agriculture' between 2004 and 2021 also highlights its importance in developing countries (see Figure 1). Most search requests were sent from Tanzania, a country where 20% of the population

are experiencing food insecurity (IPC, 2020). Among the top results are also developed countries like Canada. In such developed countries, the importance of urban agriculture for food security is almost negligible and participants are driven by reasons other than food security. Urban agriculture is increasingly seen as a multifunctional tool for sustainable city development with potential to improve urban ecosystems and social ties between communities (Orsini et al., 2020).

The major global challenges of our time are re-introducing the importance of sustainable cities, therefore bringing urban agriculture back into the global mindset. In the style of the war era's "victory gardens", a more contemporary term might now apply: "peace gardens". This term is not used elsewhere within this thesis, but convincing arguments are made that highlight urban agriculture's potential for sustainable food production and to improve urban ecosystems. These elements help in building resilient communities which in return builds peaceful societies. The recent COVID-19 pandemic highlighted the vulnerability of our food supply system and sparked interest in urban agriculture, home gardening and self-sufficiency (Lal, 2020).

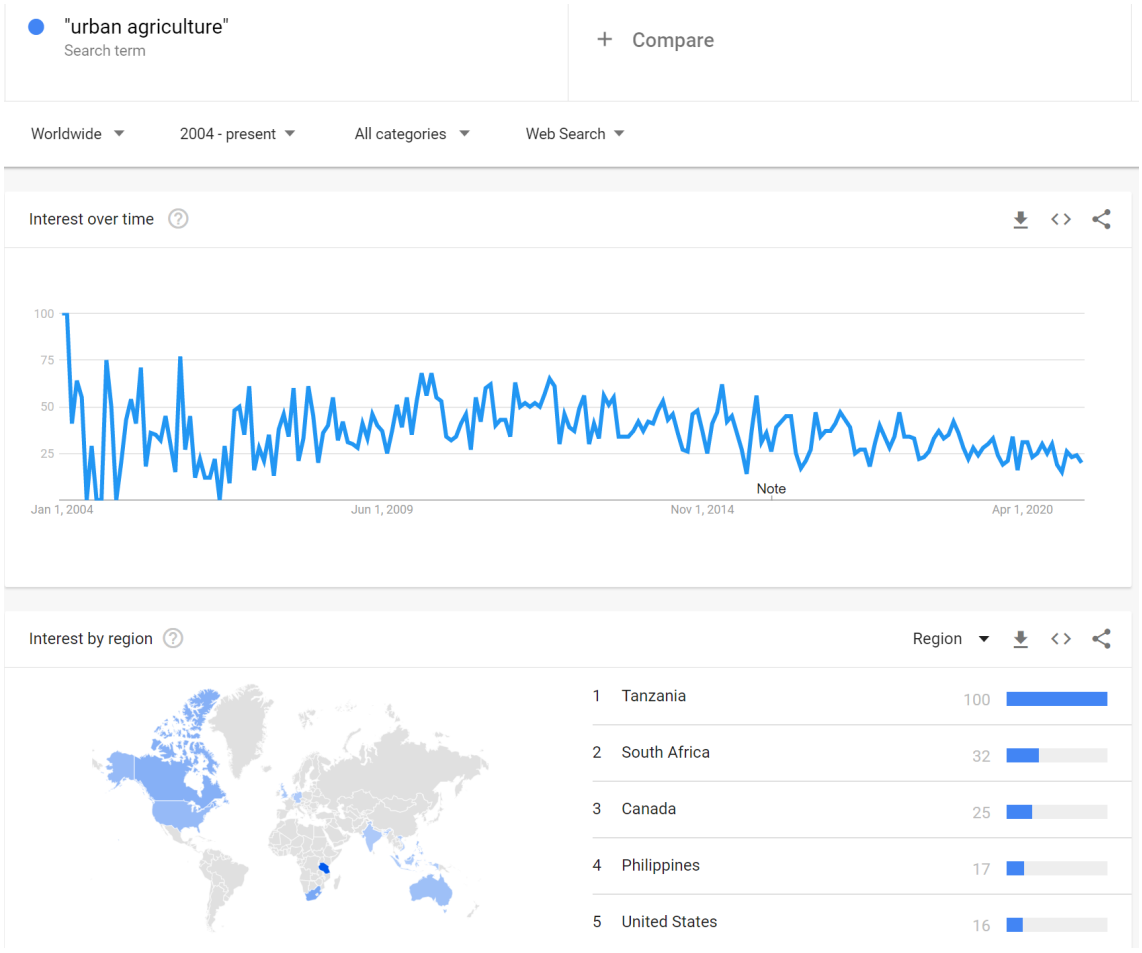

FIGURE 1.1: *Figure 1: Google Trend analytics for the search term "Urban agriculture". Accessed: 23/03/2021.*

1.1.2 Relevance of arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) form a symbiotic associations with most terrestrial plant species; these associates are called arbuscular mycorrhizas (AM). These "root fungi" inhabit the cortical cells of roots and facilitate the interface between the root and soil (Smith & Read, 2008). AMF were first described in 1845, but there was no knowledge about their symbiotic relationship with plants and their importance to ecosystems (Stürmer, 2012). Mosse (1953) was the first to describe how the addition of AMF spores to soil resulted in arbuscular mycorrhizal associations on strawberry plants. Subsequent research quickly unveiled their significance, which was increasingly recognised by scientists: "[...] in agricultural field conditions, plants do not, strictly speaking, have roots, they have mycorrhizas." (Begon et al., 1986). Yet, agricultural systems are rarely managed for the enhancement of arbuscular mycorrhizal performance. One reason is the difficulty to estimate their economic benefits (Gupta & Abbott, 2021). However, we see a different picture in academic research. With over 80% of all terrestrial plants forming an association with AMF, they have fast become one of the best-studied plant symbionts (Smith & Read, 2008). Consequently, there is an emphasis of the function of AMF in this thesis, particularly their role in urban agriculture systems. To use urban agriculture with the goal of increased sustainability in food production, it is necessary to understand how we can build "healthy" soils for increased crop productivity and how to maintain them. AMF are important indicators and determinants of soil health and should be managed accordingly (Gupta, 2020). Despite this, there is very little knowledge on the role of AM in urban agriculture systems; the work presented in this thesis seeks to address this knowledge gap.

1.1.3 Literature body and research gaps

Research on soil health in urban agriculture systems reveals a paucity of studies in the literature that deliver quantitative data on important soil properties or on nutrient flows. More studies have addressed related topics, such as the broader urban ecosystem or soil analysis for the purpose of urban development (see 2 for review). These topics have some commonalities when it comes to food production within city boundaries. One mutual research topic is the assessment of potential contamination in urban soils, and much of our current understanding can be applied to urban agriculture (Laidlaw et al., 2017). Many studies have also addressed urban soils and their role in the global carbon cycle (Brown et al., 2012). However, these results cannot be broadly applied due to the fundamental differences between urban agriculture soils and natural urban soils. The various facets of urban agriculture soils and its distinctive role within metropolitan areas warrant a dedicated research focus. To the contrary, an extensive amount of literature is available for AMF and their occurrence in various ecosystems. We also know about their importance for food production and soil health, but no data (to my knowledge) has been published on AMF in urban agriculture systems. The work from this thesis provided the first published data on AMF in urban agricultural systems (Salomon et al., 2020), and is further

supplemented by Chapters 2 and 5. Overall, we see an increased interest in urban agriculture from a scientific standpoint. The research output for soil health in urban agriculture has increased from 2.6 publications per year between 2000 and 2014, to 13.8 publications per year between 2015 and 2020 (Scopus search results for title/abstract/keywords: "urban agriculture" soil health). With increasing accessibility of advanced biotechnological methods, these studies will hopefully direct a stronger focus on the biological side of soil health within urban agriculture, as opposed to chemical and physical soil properties.

1.1.4 Research scope and structure of this thesis

The overarching aim of this thesis is to improve our current understanding of soil health in urban agriculture systems. Due to the importance of AMF and their role in soil ecology, AMF were emphasized in all research projects herein. The lack of scientific literature restricted the testing of specific hypotheses, thus the first project was designed as a city-wide soil health survey, using Adelaide, South Australia, as the case study (Chapter 4). These results contributed to our understanding of urban agriculture soils, and also form the base of this dissertation from which all other projects were developed. One inherent restriction of this survey-type study is that it can only capture a snapshot in time, whereas soils are a dynamic system. Repeated sampling over the course of 12 months attempted to capture the temporal dynamics and revealed some negative trends in soil health (Chapter 5). Based on the results of these two studies and an increasing number of scientific literature within this research topic, principles for soil management in urban agriculture systems have been devised (Chapter 2). This literature review is intended to highlight potential constraints on urban agriculture soils and how these can be addressed through appropriate management systems. It also provides a better understanding about the importance of soil health in urban agriculture systems and an extensive list of current literature.

The results contained in this thesis have been routinely communicated to urban agriculture participants in various seminars, and they shared great interest in this topic. These discussions contributed towards the development of another branch of research within my studies, which focused on assessing the quality of commercial AMF inoculants. What started as an Australian-based project, soon turned into a global study through cooperation with researchers from the USA and Switzerland (Chapter 6). This work clearly reflects the zeitgeist of incorporating AMF into our food production systems. To complement the findings of this study, an opinion paper was drafted to recommend a regulatory framework with the aim of quality improvement of commercial AMF inoculants (Chapter 7). Experts within the AMF community were invited to serve as co-authors and provide suggestions and improvements to the framework. To close the circle between AMF inoculants and urban agriculture, I then established whether commonly used urban agriculture substrates, such as potting mixes or composts, support the establishment of mycorrhiza (Chapter 8). The combination of soil-free plant substrates and AMF is not only a much underrepresented combination in the scientific literature, but

also enabled the provision of further insights into potential quality flaws of commercial substrates.

Overall, this thesis provides novel insights into the soil health of urban agriculture systems, commercial AMF inoculants and their behaviour in and suitability to urban agriculture soils. Literature reviews were provided at the start of this Thesis which summarize our current understanding.

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Chapter 2

Soil management principles in urban agriculture systems

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Contribution to the Paper	Conceptualization, data collection (literature review), writing – original draft		
Overall percentage (%)	95		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	03/05/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Abstract

The increasing urbanization of an ever-growing global population is coincident with major global challenges that are threatening our food security. Urban agriculture is as a multi-functional tool to improve urban living and to provide food security towards resilient communities. This review explores on the importance of urban agriculture and identifies a number of points that will help inform a shift towards actively managed urban agriculture soils. Firstly, common issues of soil health in urban agriculture systems are reviewed. These issues are then addressed by providing management principles for increased soil functioning of urban agriculture systems. These principles focus on improved soil nutrient and carbon pools and acknowledge the importance of the soil microbial community. Soil contamination with metal, organic and microbial contaminants, is then addressed through the discussion of options for mitigating potential risk factors. Together, this review provides an overview of our current understanding of soil health in urban agriculture systems. Where issues have been identified, these have been addressed by suggesting sustainable management principles.

2.1 Importance of urban agriculture

During the last decades, the world has seen an unprecedented increase in food production, which yielded a global average of 9747 kcal per person, per day, in 2013 (Berners-Lee et al., 2018). Although the rate of global food production exceeds its demand multiple times, food insecurity and malnutrition remains an actuality for over 2 billion people (Pérez-Escamilla, 2017). The strong increase in food production during the last decades was accompanied by a global population growth, which is estimated to have reached 7.7 billion in 2019. Projections show that the global population will increase to 9.7 billion by 2050 and 10.9 billion by 2100 (Desa, 2019). Most of this growth is happening in cities, leading to ever-expanding urban areas which will consume a further 2.4% of today's global croplands by 2030. The cropland that is being lost due to urbanization is 1.8 times more productive than the global average (Bren d'Amour et al., 2017). This development coincides with plateauing or decreasing crop yields in agricultural systems due to the effects of climate change Lobell et al., 2011. Other developments, such as soil degradation (Montanarella et al., 2016) or the emergence of new crop pathogens put further pressure on global food security (Fones et al., 2020). Projections show that at current rates, global food production will not meet its demand by 2050 (Ray et al., 2013). This global shift to the cities is seeing a greater disconnection between producers and consumers of food, both in terms of understanding, and distance (i.e. transport). To overcome these global challenges, urbanization should not only be seen as a causal factor for threatening our food systems, but also as a hotspot of sustainability for counteracting current and arising issues (Grimm et al., 2008). One promising method to implement these principles within metropolitan areas is urban agriculture (Deelstra & Girardet, 2000).

Urban agriculture describes the growing of food or raising of animals within city boundaries (Orsini et al., 2013). Since the industrial revolution, this kind of food production system lost its importance in richer economies (Mok et al., 2014). However, in lesser developed countries, urban agriculture is providing food for a substantial percentage of the population (Zezza & Tasciotti, 2010). In many African countries like Tanzania, urban agriculture is a common phenomenon with a dynamic development alongside growing cities (Drechsel & Dongus, 2009). Cuba is another prominent example for which urban agriculture was the key to feeding its population and to develop a sustainable agriculture program (Koont, 2011). In developed countries, urban agriculture is mainly practised by environmental activists and gardening enthusiasts, often for reasons other than food security. However, it has the potential to contribute towards local food production and, if managed correctly, can do so sustainably (Hume et al., 2021; McDougall et al., 2019). Recent shocks to the food system highlighted the susceptibility of current food production systems, which might also increase the value of urban agriculture in the developed world (Fanzo et al., 2018; Hobbs, 2020; Lal, 2020).

Urban agriculture is associated with a wide range of benefits. Starting with its social implications, various case studies highlighted how it can improve social inclusion and connectedness *via* gardening practices. It also offers alternative ways of educational development and training (Mok et al., 2014; Poulsen et al., 2017). Gardening has been associated with improved physical and mental wellbeing of participants (Soga et al., 2017). Research has also addressed the positive effects of healthy soils on the human health, potentially intensifying the importance of soil health in urban agriculture (G. Li et al., 2018). Economically, people who are taking part in urban agriculture were also rewarded with additional income. In less developed countries, this income significantly supplemented the regular income (Zezza & Tasciotti, 2010). By building social ties between gardeners and providing additional income and food security, urban agriculture can be considered a building stone for resilient communities (Ferreira et al., 2018).

On the ecological level, urban agriculture provides environmental services which are not only important for local communities but on the complete metropolitan scale. It plays an important role in increasing urban biodiversity and pollinator services (Hall et al., 2017). Urban agriculture, especially roof-top gardens, are useful methods for storm water management with improved quality and quantity of storm water runoff (Ackerman et al., 2014). Urban agriculture has the potential to re-use urban waste products as composts and to close the urban nutrient cycle (Wielemaker et al., 2018). Food production is happening in proximity to consumers, thereby reducing transportation costs and climate gas emissions (Lee et al., 2015). It allows flexible production methods with efficient space use and can range from low-cost and soil-based systems to more high-tech solutions (Mininni et al., 2018). One proposed option for urban food production involves the use of vertical farms with LED and hydroponic systems (Martin & Molin, 2019). Although these production systems have been reported to provide significant yield potential, their economic

viability in the near future has been questioned (Asseng et al., 2020). Also, such vertical farms would mainly address issues regarding food security, rather than environmental or sociological issues. Although we can expect technical advancements changing urban food production, soils will remain a key pillar of urban well-being (Celina et al., 2019; Kumar & Hundal, 2016).

2.2 Importance of soil management in urban agriculture

Urban soils need to be discussed from a new perspective once it involves urban agriculture and food production within city boundaries. Commonly, research on urban soils has focused on issues with direct relevance to urban and residential development, such as soil contamination and soil structure (Calzolari et al., 2020; Tiller, 1992). When this topic is opened up to urban agriculture, soils should be viewed under a broader 'soil health' framework. Soil health describes a holistic approach to assess and manage soils *via* measurable properties towards improved ecosystem functioning (Rinot et al., 2019). In an urban context, these services include: providing habitats for organisms; nutrient and pollutant retention and release; regulation and storage of water and CO_2; food provisioning through biomass production (Calzolari et al., 2020). The high spatial heterogeneity and rapid transformation of urban soils are another important argument towards judicious management strategies for urban soils and specifically for urban agriculture (De Kimpe & Morel, 2000).

Soil management is of special importance for sustainable food production. It has been shown that the management of soil health reduces the severity of soil-borne pathogens (Abawi & Widmer, 2000), improves physical soil properties and sequesters carbon (Williams et al., 2020). It allows for efficient nutrient cycling which is important when re-using urban waste products, such as municipal waste composts (Hernández et al., 2016; Nowak et al., 2015). The soil microbial community can be shaped towards increased resource use efficiency (Bowles et al., 2017) and issues of antimicrobial resistance genes can be mitigated (Gao et al., 2018). Recent studies found that low-density cities such as Adelaide or Sydney can be self-sustainable for vegetable production and could provide a significant amount of its overall food demand (Hume et al., 2021; McDougall et al., 2020). The actual amount of land that would need to be converted for urban agriculture is strongly depending on the projected yields. Soil management for improved soil health in urban agriculture systems can increase yields and therefore decrease the amount of land that needs to be converted to achieve self-sustainability.

2.3 Emerging issues in urban agriculture soils

2.3.1 Metal contamination

One of the first questions when evaluating urban soils focuses on potential contamination. Common soil contaminants include metals, polycyclic aromatic hydrocarbons (PAHs), phenols, pesticides and other organic compounds (NEPC, 2011). Due to the heterogeneous land use with fast turn-over rates among users, urban soils are especially prone to contamination. Long before the popularization of the term "urban agriculture", metal contaminated soils had been identified in urban gardening soils and residential backyards (Purves, 1966). Similar results have since been published with varying percentages of samples that tested above national health guidelines (Cheng et al., 2015; Laidlaw et al., 2018; Mitchell et al., 2014). Commonly found metal contaminants are lead (Pb), chromium (Cr), arsenic (As) or cadmium (Cd). In one study by (Clark et al., 2008), 81% of the tested gardens had Pb levels above the US EPA action limit of 400 mg kg^{-1}. Using raised beds with imported soil was initially successful to overcome this issue. However, Pb concentrations in raised beds were increasing over time, most likely due to re-contamination over wind-transported Pb particles from housing paint. Atmospheric decomposition is a common vector for many contaminants. Other common anthropogenic sources of metals contaminations stem from metal-surface runoffs, wastewater irrigation, burial of waste products or application of agrochemicals (Alloway, 2004). Where raised beds are used for mitigating issues over soil contamination, health issues can also arise from dust exposure from contaminated underlying and adjacent soil (Brown et al., 2016). When vegetables from contaminated sites are consumed, different plant species vary in their ability to accumulate metals. For example, Pb concentration in root tissue is up to three times higher as in leaf tissue (Clark et al., 2006). However, whereas green leafy vegetables might be less prone to metal uptake *via* roots, they could accumulate contaminations *via* atmospheric deposition. In this case, landscape variables such as buildings or vegetation buffers can be more significant variables than site-specific ones (Sung & Park, 2018). Besides the detrimental effects of soil metal contamination on human health, negative effects are also reported on the soil food web (K. Sharma et al., 2015) or beneficial insects (Gardiner & Harwood, 2017).

2.3.2 Organic contamination

Organic pollutants are another group of potential soil contamination which, just like metals, are of relevance to soil health and human health in urban agriculture systems. Previous research on urban soils has identified a range of potentially toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs) (Abdel-Shafy & Mansour, 2016), polychlorinated biphenyls (PCBs) (Wu et al., 2011), dioxins (Pussente et al., 2017) or DDT (dichlorodiphenyltrichloroethane) (Brodskiy et al., 2016). The European Chemicals Agency lists a total of around 143,000 chemicals for industrial use, which leaves many pathways for organic pollutants to enter the urban environment (Clarke & Smith, 2011). PAHs are a

group of carcinogenic compounds that are formed during fuel combustion and are highly persistent in the environment (Lal & Stewart, 2017). Similar to metal contaminants, they are also distributed *via* atmospheric deposition with high spatial variability within cities (Tang et al., 2005). The translocation of PAHs from soil to plants predominantly occurs *via* the root system for high-molecular-weight PAHs, whereas low molecular weight PAHs are rather taken up from the atmosphere by leaves (Fismes et al., 2002). Contamination with PAHs and other organic pollutants causes severe ecotoxicity with detrimental effects on the soil biota and plant growth (Eom et al., 2007). Two case studies on PAHs in rooftop urban agriculture systems identified only insignificant accumulation in vegetables (Gelman, 2014; Tusher et al., 2020). An assessment of soil pollution in an industrialised city in Spain revealed that of all tested PAHs, only Benzo(a)pyrene exceeded national guideline levels. Metal contaminations were of a higher concern (Boente et al., 2017). Research on peri-urban vegetable farms suggests that the occurrence of trace elements and organic contaminants is less site-specific but more dependent on crop species (Margenat et al., 2019). Another group of organic pollutants with detrimental effects for plant growth are herbicides. Herbicides with long half-life times (> 500 days) can be introduced during the composting process and result in observable effects when the finished compost is applied. Phytotoxic effects of composts have been reported in plant nurseries and gardens (Fauci et al., 2002).

2.3.3 Microbial contamination

Urban agriculture often takes place in raised beds with introduced soils, predominantly potting mixes or potting soils (Salomon et al., 2020). Where plants are grown in the natural soil, these are commonly amended with a range of organic fertilisers and soil conditioners, such as composts, manures or rock dusts (Salomon et al., 2020). Such organic products have been associated with a variety of soil-borne human or plant pathogens. For example, exposure to potting mixes and composts is a well-studied risk factor for Legionnaire's disease (Kenagy et al., 2017). Many other food contaminations stem from manures and composts, such as Salmonella sp., Escherichia coli or Listeria sp. (Z. Chen et al., 2018). These pathogens can transfer from contaminated soils and irrigation water to the edible parts of crops (Oliveira et al., 2011). These risk factors are also associated with the application of compost tea, an organic farming practice for soil improvement (M. Sharma & Reynnells, 2018). In one long-term field study on agricultural soil by Brochier et al. (2012), Enterococcus sp. and Clostridium perfringens were detected in compost amendments and in the soil. However, these pathogens were also detected in non-amended soils, highlighting their ubiquitous distribution. Ubiquitous pathogens such as Stenotrophomonas maltophilia have also been identified on leavy green vegetables from urban agriculture systems. In the isolated strains, antimicrobial resistances and different abilities to produce biofilms were found (D. Li et al., 2019). Another recent topic of research focuses on antimicrobial resistance genes (ARGs) in manures and their transmission in soils. This is of special relevance to urban agriculture, since over-fertilised soils are a common phenomenon and often stems from animal manures (Salomon et al., 2020;

Wielemaker et al., 2019). The development of ARGs is driven by the use of antimicrobial agents in animal farming for disease prevention and animal growth promotion. These ARGs could spread to human pathogens through horizontal gene transfer, rendering human antibiotic therapies ineffective. ARGs are commonly detected in animal manures and composting shows inconsistent effectiveness in their reduction (Qian et al., 2018; Wang et al., 2021). Plant endophytes can act as potential hosts for the transmission of ARGs from soils into plants (Wei et al., 2020). Various studies now identified wastewater and raw sewage sludge as a reservoir for AGRs in urban agriculture systems (Bougnom, McNally, et al., 2019; Bougnom et al., 2020; Bougnom, Zongo, et al., 2019).

2.3.4 Soil nutrients

Focusing on the major plant nutrients nitrogen (N) and phosphorus (P), most urban agriculture soils can be characterised as highly fertile. Especially levels of P and total N have been consistently high, whereas some N imbalances have been reported for the availability of mineral N in soils and leaf N content. Wielemaker et al. (2019) found in Dutch urban farms that mean nutrient inputs exceeded crop demands by roughly 450% for total N and 600% for P. Often, these P inputs were exceeding national application limits. Very high concentrations of plant-available P in soils were also been found by Salomon et al. (2020). Concentration of total N in these soils averaged at 0.7%, providing an adequate source of N for plant uptake after mineralisation (Peverill et al., 1999). High concentrations of P are not surprising, given its immobile nature in soils and the over application of manures or inorganic fertilisers by enthusiastic gardeners (Dewaelheyns et al., 2013). Most manures are effective P fertilisers with concentrations of up to 21 g P kg^{-1} total solids (TS) (Pagliari & Laboski, 2012). Many manures and composts have a small N:P ratio which results in the over application of P when N demands need to be covered (Shrestha et al., 2020). Following a more dynamic cycle, N uptake by plants is strongly affected by external influences such as temperature and precipitation, which are the leading drivers behind N mineralisation and losses through leaching or gaseous emissions (Peverill et al., 1999). With this in mind, concentrations of mineral N can become low, even when soils are adequately supplied with total N (Salomon et al., submitted). Analysing the shoot N content of urban agriculture plants revealed levels close to the lower limits for adequate supply (Arrobas et al., 2017). This issue is exacerbated because most urban agriculture sites are following principles of organic farming, which excludes the use of mineral fertiliser (Salomon et al., 2020). For organic farming systems, N availability is the most important yield-limiting factor. Although overall N inputs through organic fertilisers can be sufficient, the timing of N availability is often not meeting crop demand (Röös et al., 2018).

2.3.5 Soil carbon

Research on soil carbon (C) in urban agriculture revealed high concentrations in most gardening beds. In two different studies, these were between 3 and 10% total C, whereas conventional agriculture soils are more commonly between 0.5% and 2% (Dewaelheyns

et al., 2013; Kravchenko & Robertson, 2011; Salomon et al., 2020). Wielemaker et al. (2019) found input of organic material (OM) into urban agriculture systems between 700 and 138,100 kg OM ha^{-1} yr^{-1}. In this case, 84% of all farms applied OM above the estimated mean degradation rate of 2000 kg OM ha^{-1} yr^{-1} in the Netherlands. Because of its dynamic nature, C pools in soils are at risk of being lost if not managed accordingly. Repeated sampling of urban agriculture soil over one year revealed an almost linear decline in soil C. This was likely due to a reduction of OM input compared to previous years (Salomon et al., submitted). A similar decrease in soil organic content (SOC) was seen in West African urban agriculture systems after the addition of biochar, which lead to an initial sharp increase of SOC (Häring et al., 2017). These results also indicate an over proportional use of organic materials when establishing new garden beds for urban agriculture. Initially high C concentrations are then depleted through soil respiration of the labile C pool, if not replenished with more organic material. High soil respiration rates after the addition of compost have been reported in agricultural systems (Fabrizio et al., 2009). This rate is also depending on various compost quality parameters, such as compost stability, which describes the degree of organic matter decomposition. Compost stability for commercial products is specified in national guidelines, however, not applicable for self-made composts (Azim et al., 2018). Depending on the land use, urban soils can be naturally high in soil C, such as in urban grass lands and forests which were found to contain around 8% total C (Weissert et al., 2016). However, soil C is a dynamic system which can shift through anthropogenic changes, such as soil compaction, loss of biodiversity or changes to the soil structure (Trammell et al., 2017). Independent of the previous land use type of urban soils, judicious soil management is required to maintain soil C levels after the establishment of urban agriculture sites.

2.3.6 The soil microbiome

One emerging branch of soil research is focusing on the soil microbial community and its role in ecosystem functions. The importance of the "unseen majority" for terrestrial ecosystems has been sufficiently described (van der Heijden et al., 2008; van der Heijden & Wagg, 2013). Only recent advances in molecular technologies allowed this research to be conducted on a broader level, whereas research on urban agriculture systems is still limited. Focusing first on conventional agro-ecosystems, an increasing body of literature acknowledges the interactions between the plant-soil interface. This relationship is evidently affected by agricultural management practices, land use and soil disturbance (Chaparro et al., 2012). Keeping in mind common urban agriculture practices, such as excessive nutrient inputs, potential negative changes in the microbial community are possible (Leff et al., 2015). This has also been shown in one urban agriculture study where the microbial diversity decreased after an over-proportioned application of chicken manure (Salomon et al., submitted). However, research also suggests that common urban agriculture practices are favouring certain beneficial soil microbial communities, such as the arbuscular mycorrhizal fungi (AMF) (Salomon et al., 2020). Organic fertilization

in urban agriculture was also associated with relatively higher microbial enzyme activity than compared to inorganic fertilization (Igalavithana et al., 2017). Still, the limited body of research does not exclude the possibility of microbial pitfalls that are limiting soil health. Negative effect could include limited potential for disease suppression (Dignam et al., 2018). This scenario is speculative, but its likelihood increases whenever management practices are undertaken that differ too much from best practice soil management in conventional agricultural systems.

2.4 Principles of soil management in urban agriculture systems

2.4.1 Addressing soil contamination

Given the increased likelihood of potential contaminants within urban areas, urban agriculture should be undertaken on land which has been tested for contamination or where these are less likely to occur, such as in newly developed residential areas (Laidlaw et al., 2018). Introduction and accumulation of contaminants *via* atmospheric deposition can be reduced by planting or building barriers between crops and contamination sources, such as heavy traffic roads (Säumel et al., 2012). Plants with complex and hairy leaf morphology have proven particularly well for improving the surrounding air quality (Blanuša et al., 2020). Where soils are prone to contamination, or soil testing is not available, the use of raised beds with introduced soils can mitigate these risks. However, without wider-scale remediation efforts, raised beds could be re-contaminated within a few years (Clark et al., 2008). Raised beds are also associated with higher crop yields and less weeds (Miernicki et al., 2018). When using introduced soils such as composts or potting mixes, these need to be sourced from reliable producers to avoid the introduction of pollutants, pathogens or issues over phytotoxicity. Most countries have mandatory quality guidelines and many private associations provide quality labels following even higher standards (Cesaro et al., 2015). Compost has also been proposed as a tool for the remediation of soils polluted with organic or inorganic contaminants (Huang et al., 2016; Kästner & Miltner, 2016). One important mechanism is the adsorption and complexation of pollutants, which makes them unavailable for plant-uptake. Another similar approach to immobilize pollutants is the addition of biochar (Zhang et al., 2013). Further methods for soil remediation have been proposed, such as phyto-, myco-, or microbial remediation (Jin et al., 2018; Treu & Falandysz, 2017; Yadav et al., 2018). These measurements do not bypass national guidelines for soil contamination; however, they could be useful if contamination levels are close to the upper limit or if pre-emptive measurements are required. When using composts in urban agriculture systems, the use of green waste compost is likely to provide sanitary advantages over manure based composts (Avery et al., 2012). Hot composting is a widely used method to eliminate potential pathogens from compost. Common guidelines require 55 °C for 3-5 days to ensure adequate removal of pathogens (Azim et al., 2018). Various low-cost bioassays have been established to the evaluate the quality and phytotoxicity of composts, such as seed germination tests (Fauci

et al., 2002). Compared to mineral fertiliser, composts are less likely to favour herbivorous nematodes or could even reduce the abundance of root-knot nematodes, thereby providing further benefits towards soil health (Herren et al., 2020; Xiao et al., 2016).

2.4.2 Addressing soil nutrients and carbon

Soil carbon is an essential soil health parameter for soil structure and the retention of water and plant nutrients. For agro-ecosystems, soil organic carbon (SOC) between 1.5 and 2% are considered as the lower threshold (Trivedi et al., 2018). The carbon cycle is a dynamic system where soils can either act as a source or sink, depending on the amount of carbon inputs and losses. Research also highlighted the potential of urban soils for carbon sequestration with potential effects on mitigating climate change (Lorenz & Lal, 2015). One way to increase soil carbon is through maintaining an active vegetation with high plant biodiversity (S. Chen et al., 2018; Yang et al., 2019). Urban agriculture is commonly practiced with less automation, higher crop density and higher yields (Altieri et al., 1999). This allows the inclusion of companion planting systems, which has further positive effects on plant growth and subsequently soil health (Griffiths-Lee et al., 2020). Another method to increase soil carbon is through the application of organic materials, either as composts or as mulch (Pinamonti, 1998). Mulches can also prevent soil erosion and water evaporation (R. Li et al., 2021). It has also been shown to increase soil organic carbon (SOC) in urban forests (Sun et al., 2021) and soil microbial biomass in organic agriculture (Tu et al., 2006). The average annual garden waste is around 120 kg person^{-1} for England (Eades et al., 2020) or 150 kg person^{-1} for the Greater Brisbane region in Australia (Hla & Roberts, 2015). These numbers suggest a large resource of carbon that could be re-introduced into urban agriculture systems. Soil respiration rate in urban agriculture systems was measured as 1.3 g CO_2-C m^{-2} d^{-1}, which culminated towards 975 g CO_2-C loss through respiration per raised bed and year (Salomon et al., submitted). This would require the addition of almost 5 kg of compost to counteract C-losses through soil respiration (assumptions: raised bed area = 2 m^2, total C compost = 20%). When using raised beds with introduced soil, C-loss through soil respiration can also be observed as substrate shrinking and is often found in immature composts (Gruda, 2019). Although more compost could be applied to re-fill garden beds, this might lead to the accumulation of P. Ideally, once desired soil P concentrations have been reached, organic amendments should be focused towards providing adequate levels of C and N. Common green waste products like grass clippings or wood chips can raise the C:N ratio of soils and composts to desired values, without adding excessive amounts of P (Vandecasteele et al., 2017). Another common urban waste product with a broad N:P ratio and good composting qualities is spent coffee grounds (N:P = 30:1) (Liu & Price, 2011). Urban agriculture's intensive production system allows gardeners to apply an adaptive fertilization regime which is adjusted to the current growth stage and nutrient demand of plants. Rather than applying a sizeable amount of fertiliser for the whole growing season, fertiliser should be applied in smaller quantities but at higher frequencies. Agricultural studies showed

that fertiliser efficiency increased when the application is timed to meet the nutrient demands of plants and applied more frequently (Abbasi et al., 2013; da Silva et al., 2018; Ma & Herath, 2016). Issues over high salinity and EC can be avoided by using this practice, which is especially important for manure-based composts. Such composts often show an extremely high EC due to the presence of soluble salts. Over-application could cause depressed plant growth or "plant burn" (Gondek et al., 2020; Reddy & Crohn, 2012). When applied at smaller quantities, these salts can be washed out or taken up by plants, before accumulating to phytotoxic levels.

2.4.3 Soil management practices for increased soil biota function

Soils contain a rich biodiversity that exceeds that of aboveground biodiversity by several orders of magnitudes. The soil biota is governing nutrient cycling, turnover of soil organic material (SOM) and pathogen suppression. All these factors are critical for soil health and should be kept at self-sustaining levels to avoid losses of important ecosystem functions (Thiele-Bruhn et al., 2012; Wagg et al., 2014). Common practices for increased soil biodiversity include the use of minimum tillage, high plant biodiversity, and organic farming practices (Bowles et al., 2017; Mijangos et al., 2006; van Capelle et al., 2012). Adversely, the use of agrochemicals has been linked with negative effects towards the soil biota and soil fertility (Cycoń & Piotrowska-Seget, 2009; Prashar & Shah, 2016). Studies on urban agriculture soil health found higher microbial richness and enzyme activities in soils that have been fertilised organically compared to inorganic (Igalavithana et al., 2017). It was also suggested that principles of organic farming and high plant biodiversity resulted in an abundance of arbuscular mycorrhizal fungi (AMF) within urban agriculture systems (Salomon et al., 2020). These symbiotic organisms have been shown to provide a broad range of important ecosystem functioning, such as nutrient uptake of plants and suppression of soil-borne diseases (Baum et al., 2015; Rillig, 2004; Schouteden et al., 2015). The importance of microbial communities has been repeatedly proven, such as with improved P uptake of plants in no-till soil (Köhl et al., 2014). Research in agricultural systems repeatedly highlighted the advantages of minimal soil disturbance practices. Intensive tillage and ploughing is linked to disturbed soil aggregate stability and soil microbial communities with far-reaching effects on soil nutrient retention and overall soil health (Nunes et al., 2018). Translated to urban agriculture system, this method of reduced soil disturbance is commonly referred to as "no-dig gardening" (Guitart et al., 2015; Wesselow & Mashele, 2019). Although this practice has not been the subject to much scientific research, one could assume that low soil disturbances in urban agriculture systems harbours similar advantages as in conventional agricultural systems. Especially in raised beds with introduced potting mixes and composts, issues of soil compaction are reduced, and most plants can be grown without previous soil preparation (Miernicki et al., 2018). Another well studied advantage linked with the application of compost is the suppression of soil-borne pathogens (De Corato, 2020). Compost can also be applied in a liquid solution, commonly referred to as "compost tea", which provides

similar disease suppressive effects. Care must be taken to produce sanitary compost tea to avoid the introduction of pathogens (Martin St., 2015; On et al., 2015).

2.5 Conclusion and future outlook

Increasing urbanization remains a catalyst for many global challenges. At the same time, urban areas are also providing important resources that can be used to mitigate many of the occurring issues. With greener cities as a planning ideal, urban agriculture has been proposed as a multi-functional method that allows efficient use of urban spaces with positive impacts on the social, ecological and economic scale. This ideal has been reinforced during the COVID-19 pandemic which highlighted the susceptibility of urban areas and the modern food system. With this in mind, urban agriculture is likely to play an important role in increasing urban sustainability and the resilience of local communities.

One crucial element towards reaching this goal is the proper management of soil health in urban agriculture systems. Soil health has far reaching effects on most aspects involving crop productivity. It is governing plant-nutrient cycles which is of special importance when relying on urban waste products as fertilizer. Through increased soil microbial functioning, it could reduce the dependency on agrochemicals. Overall, soil health is directly related to crop yield and the amount of land that would need to be allocated towards urban agriculture in order to achieve certain levels of urban self-sufficiency. Potential health concerns of growing food in an urban environment can be mitigated through a variety of soil management practices.

The concept of greener cities has been recognized by urban planners as a method to improve human health, social interactions and the local microclimate (Nieuwenhuijsen et al., 2017). Urban agriculture as one implementation for greener cities takes advantage of urban resources and helps in creating resilient communities. The term “smart cities” has been used for interweaving the principles of urban agriculture with advanced technologies, such as aquaponics (dos Santos, 2016). This evolution of urban agriculture does not come as a surprise. Food production has been advancing rapidly in recent years, involving further development of existing technologies (Kodali et al., 2016) or even the introduction of new paradigms, like edible insects (Premalatha et al., 2011). With this in mind, the future of urban food production might just look as heterogeneous as the urban landscape itself. However, it is not foreseeable that these developments will lead to a redundancy of healthy urban soils. As outlined in this review, urban soils provide many ecosystem services and allow for the most simplistic and cost-effective way of food production. Healthy soils should be considered as the backbone of greener cities and resilient local communities.

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Chapter 3

Biofertilizers: assessing the effects of arbuscular mycorrhizal fungi on soil health

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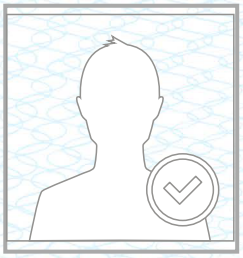

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3.1 Introduction

Soils provide a variety of important ecosystem services and are the foundation of global biogeochemical cycles such as carbon, water and plant nutrients. They host an abundance of microorganisms, ranging from the microscopic to the macroscopic level (Adhikari & Hartemink, 2016). Healthy soils have the capacity to provide those ecosystem functions that are appropriate to its surrounding and to do so in a sustainable way. As such, healthy soils are the foundation of most food production systems, ecosystems and urban settlements (Keesstra et al., 2016; Salomon et al., 2020). Many developments of the past and present have led to land loss and land degradation. Anthropogenic activities that heavily impact soils on a global scale include intensive agriculture, deforestation and land disturbances, such as for urban settlements or mining. Those impacts can be complex and, as many systems are interwoven with one another, can result in a cascade of events with broader implications on its surroundings. Estimates suggest that up to 45% of global land areas are degraded, which may undermine the well-being of 1.5 billion people (Gibbs & Salmon, 2015). Major drivers of land degradation are soil erosion, acidification, land clearance, salination or pollution through heavy metals and petrochemicals (Olsson et al., 2019).

The previous decades saw a variety of concepts and names that were used for soil assessment. Among those are soil fertility, soil quality, soil capability and, recently, soil health. Similarly, the objectives and methods of each concept progressed throughout the years. The earliest forms of soil assessment were emphasizing the suitability for crop growth, whereas newer concepts consider the multifunctionality of whole ecosystem services. Soil assessment can be done on soil biology (e.g. microbial communities), chemistry (e.g. pH and nutrients) and physics (e.g. bulk density and texture). Current developments in soil analysis allowed a shift towards the inclusion of soil biology as commonly used indicators, which were previously more focused on soil chemistry and physics. Following, we adhere to the term 'soil health' to describe sustainable and resilient ecosystem services (Bünemann et al., 2018).

The sustainability of many current agricultural practices has been questioned. For example, one consequence of suboptimal soil management is the release of soil carbon into the atmosphere which is about the same magnitude as carbon emissions caused by current deforestation events (Sanderman et al., 2017). The concentration of carbon in the soil is tightly linked with important soil characteristics, including soil aggregation and soil microbial biomass (Wilson et al., 2009). As a consequence, the loss of soil carbon leads to deteriorated soil health and contributes to elevated atmospheric carbon dioxide (CO_2) concentrations (Rumpel et al., 2020). Intense farming systems put further pressure on soil fertility and can result in nutrient depletion. Such soils lack the capacity of replenishing essential plant nutrients, and crop yields are only sustained through the application of

fertilizers (Tan et al., 2005). As a further consequence, soils might see a loss of biodiversity which deprives them of their essential ecosystem functions (van der Heijden et al., 2008).

Soils are home to a wide range of biota that have beneficial effects on plant growth and soil functioning. One of those groups is the mycorrhizal fungi. Mycorrhizal fungi follow a cosmopolitan distribution and are one of the main drivers of soil microbial interactions. Their hyphae can build up to 40 m g^{-1} of soil (Smith et al., 2004) and reach a biomass of 700–900 kg ha^{-1} (Wallander et al., 2001). Mycorrhizal fungi can be broadly categorized into ectomycorrhizas, ericoid mycorrhizas, orchid mycorrhizas and arbuscular mycorrhizas (Smith & Read, 2008). These mycorrhizas differ in their physiology, symbiotic strategies and taxonomic classification. However, they all form mycorrhizal associations with host plants that can be described as a mutualistic symbiosis. Only in particular cases or circumstances are these associations parasitic (Smith & Read, 2008).

Arbuscular mycorrhizas are plant–fungal associations in which the fungus enters the plant root and forms specialized structures, the arbuscules, which are used for nutrient exchange with the host plant (see Figure 3.1). Arbuscular mycorrhizal fungi (AMF) are obligate symbionts for 80% of terrestrial plant species and most crop plants. This mutualistic relationship is improving plant nutrition by aiding in the uptake of phosphorus and zinc. In return, the plant delivers photosynthates and lipids to the fungal symbiont (Smith & Read, 2008). Besides improved plant nutrition, AMF play a crucial role in soil health and are key indicators for describing soil quality. AMF have been found to improve many aspects of soil health and counteract the negative impacts caused by inappropriate soil management (Jeffries et al., 2003). At the same time, common agricultural practices led to a diminished abundance of AMF in the soil. Such practices include simplified crop rotations, application of mineral fertilizer or soil disturbances, such as cultivation (Verbruggen & Toby Kiers, 2010).

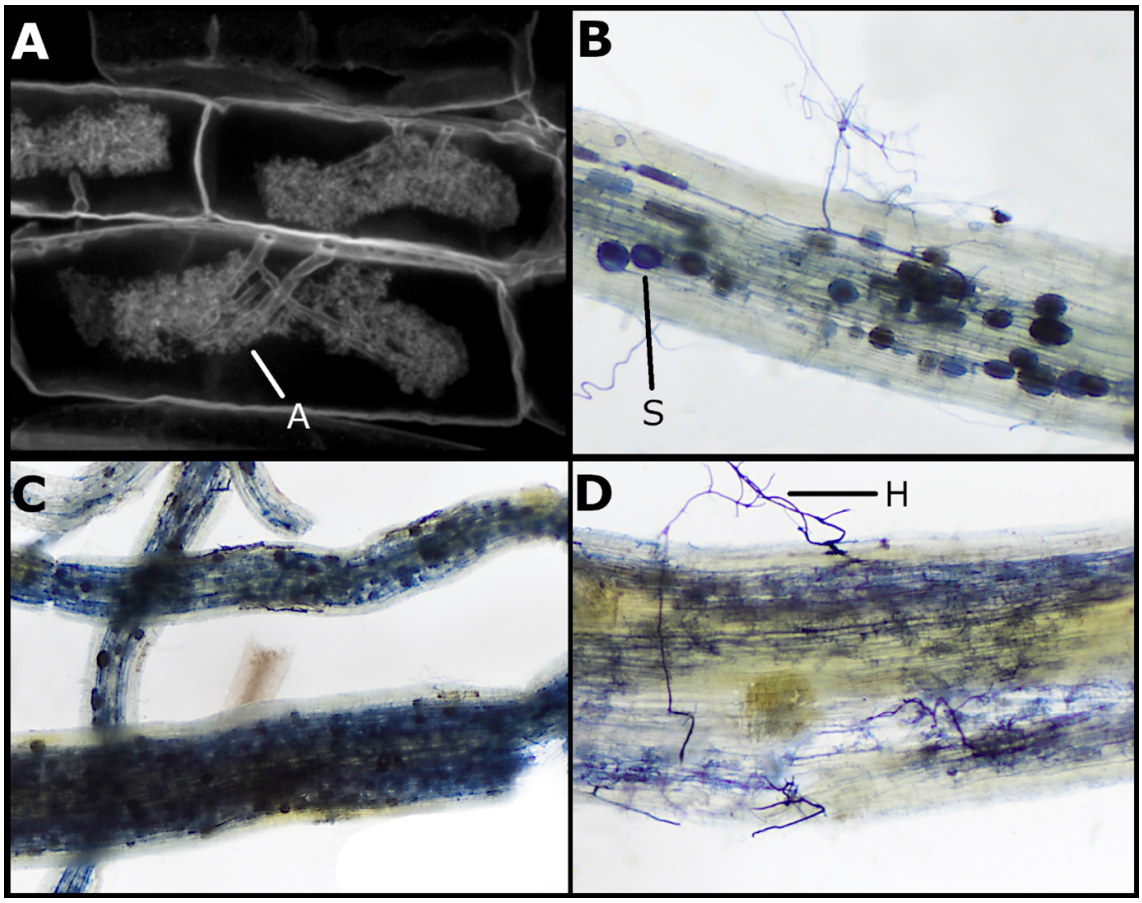

FIGURE 3.1: *(a) Mycorrhizal arbuscules inside Pisum sativum root cells; (b–d) colonized maize roots with many intraradical spores and extraradical hyphae. A = arbuscule, S = spore, H = hyphae. (a): Photo courtesy of Ryan Geil, published with kind permission from Peterson and Massicotte (2004) and NRC Press, © Canadian Science Publishing or its licensors.*

One proposed way to increase soil health in a sustainable way is the application of AMF as a biofertilizer. AMF biofertilizers are designed to bolster natural mycorrhizal communities when those have been impaired or to introduce new mycorrhizal isolates with improved functional traits. The AMF inoculum is embedded in a carrier material which might include further additives, such as organic additives (e.g. humic acids) or other plant growth-promoting microorganisms such as *Trichoderma spp.*, *Bacillus spp.* or other microorganisms. Such biofertilizers have been proposed as an alternative to mineral fertilizers and pesticides and have been known to increase yield resilience by supporting plants against abiotic stress (Berruti et al., 2016). While AMF biofertilizers hold enormous potential to improve sustainability, their integration into broad-scale applications has been challenging. The mass production of AMF propagules is defined by its symbiotic life cycle in which mycorrhizal fungi require a host plant to propagate. This production method is linked to phytosanitary issues and requires much care to exclude plant pathogens. Although AMF can be cultured axenically, it is currently not economical to do so on a broad scale. Furthermore, most root culture systems require genetically modified hairy roots, which are produced using tumor-inducing (Ti) plasmids (Adholeya et al., 2005; Berruti et al., 2016). Recent studies demonstrated that AMF cannot produce

fatty acids themselves and rely on their host plants for providing them. Thus, it is possible that specific growth media might be designed in the future, in which it is possible to grow AMF without host plants (Kameoka et al., 2019). Further issues arise when applying AMF inoculum to field soil, which already contains an established microbial community. Under those circumstances, survival and establishment of the introduced AMF needs to be evaluated (Bender et al., 2019; Rodriguez & Sanders, 2015). Furthermore, AMF show a certain host selectivity and might not perform consistently in a crop rotation (Hoeksema et al., 2010).

Although the production and application of AMF biofertilizer come with certain challenges, an increasing number of companies are attracted by its potential economic value (Vosátka et al., 2012). The global market for microbial inoculants is expected to reach US$3.622 billion by 2024, of which the mycorrhizal fungi are a major sector (LLP, 2019). In most countries, the term 'biofertilizer' is not legally defined and is therefore lacking regulations and minimum standard requirements. This situation led to an unregulated market in which a high percentage of commercial AMF biofertilizers fail to induce mycorrhizal colonization. To this date, a number of scientific studies are available where the majority of tested inoculants showed unsatisfying results (Corkidi et al., 2004; Tarbell & Koske, 2007) (Salomon et al., under review). This situation is undermining the potential of AMF biofertilizer with bigger implications for sustainability in food production and consumer protection. Scientific research might be sabotaged when researchers rely on commercial mycorrhizal inoculants.

AMF biofertilizers hold the potential to significantly reduce our carbon footprint on this earth. The management of AMF can be a sustainable option to reduce agrochemicals and to increase soil health at the same time. As outlined above, the production and application of AMF biofertilizers can be challenging and needs further research before they can be adapted on a broader scale. However, those challenges should not be a reason to undermine the potential of AMF, nor should the interest in AMF applications be belittled as a 'recurring evolution' (Hart et al., 2018). Instead, the management and understanding of the soil microbiome should be treated as a valuable tool for humanity to stay within its planetary boundaries (Rockström et al., 2009). Following, we will address the key issues of how AMF can help to improve soil health and outline promising developments towards the use of AMF biofertilizers (see Table 3.1).

3.2 Arbuscular mycorrhizal fungi and soil health: addressing the key issues

3.2.1 Improved soil structure and stability

Soil structure is defined as the spatial arrangement of soil particles to form a three-dimensional matrix consisting of mineral and organic particles (aggregates) and porous

spaces in between. Soil stability describes the disintegration forces necessary to disrupt this matrix. Soil structure is a key indicator of soil health as it influences a variety of important soil characteristics. Well-structured soils facilitate root growth, aeration and water infiltration into deeper soil layers, while providing water retention at the same time. High soil stability prevents soil from wind and water erosion. Soil structure and stability are generally influenced by soil physical, chemical and microbial influences. Within the microbial influences, AMF are one of the main contributors towards soil structure and stability.

The effects of AMF on soil structure are of direct and indirect nature. The direct effects include processes in which fungal mycelium enmeshes soil particles into bigger units, including soil microaggregates (<0.25 mm) and macroaggregates (>0.25 mm) (Miller & Jastrow, 1990). The indirect effects are describing how AMF can influence plants and microbial communities which then influence the soil structure (Tisdall & Oades, 1982). At the macroaggregate level, soil structure through AMF is mainly improved due to the physical force provided by the hyphal entanglement of soil particles (Miller & Jastrow, 2000). As mentioned before, AMF hyphae can build up to 40 m g^{-1} of soil (Smith et al., 2004). At the same time, the hyphal diameter is about ten times smaller than that of fine roots, allowing hyphae to penetrate even micropores (<30 μm) (Smith & Read, 2008). The well-known biochemical compound Glomalin and the group of Glomalin-related soil proteins are a biochemical pathway for soil aggregation. This group of proteins is thought to act like a glue for soil particles (Driver et al., 2005). However, there are open questions regarding their quantification and release into the soil and if they are specific to AMF (Rosier et al., 2006). Fungal growth is further linked to a variety of other extracellular organic compounds that have been shown to improve the soil structure, for example, through changes in the surface polarity (Gebbink et al., 2005) or carbon deposition. Mycorrhizal effects on the formation of microaggregates are less researched but hypothesized to work through physical forces on primary soil particles. The turgor pressure of hyphae during their growth could eliminate spatial constraints that would otherwise prevent the formation of microaggregates. Similarly, this physical force could align particles and bind them with organic matter (Rillig & Mummey, 2006).

Indirect effects of AMF on soil structure describe its effect on plants and soil communities, which, in return, can influence soil structure and stability. The influence of plant communities on the soil structure has been repeatedly shown, for example, in the context of agricultural (Munkholm et al., 2013) or natural ecosystems (Pérès et al., 2013). The host-selectivity of AMF thereby promotes certain plant species over others, which ultimately leads to changes in soil structure (Van Der Heijden et al., 2006). The effects of AMF on single plants are mainly evolving around an increase in the ratio of root to shoot biomass as a result of the mycorrhizal symbiosis. Following is a cascade of events with potential positive outcomes on the soil structure, such as increased rhizodeposition, soil entanglement by fine roots, increased root decomposition and changes to the soil

TABLE 3.1: *Impact of AMF on soil health: overview of soil ecosystem functions provided by AMF and their underlying mechanism.*

Soil ecosystem function	Mechanism	Reference
Uptake of plant nutrients and stimulation of plant growth	Improved uptake of phosphorus, nitrogen and micronutrients	(Watts-Williams & Cavagnaro, 2012)
	Increased plant biomass	(van der Heijden et al., 1998)
	Improved drought resistance	(Sanchez-Diaz & Honrubia, 1994)
	Protective effects against root diseases	(Hol & Cook, 2005)
	Induction of systemic pathogen resistance	(Pozo & Azcón-Aguilar, 2007)
Improved soil structure and stability	Hyphal entanglement of soil particles	(Miller & Jastrow, 1990)
	Carbon deposition	(Rillig & Mummey, 2006)
	Changes in surface polarity of soil particles	(Rillig, 2005)
	Alignment of soil particles	(Tisdall, 1991)
	Eliminating spatial constraints	(Six et al., 2004)
	Indirect effects through changes in plant and microbial communities	(Rillig & Mummey, 2006)
Alleviation of soil contamination	Immobilization of metals	(French, 2017)
	Up-regulation of plant detoxification genes	(Jiang et al., 2016)
	Removal of contaminants from plants through fungal structures	(Göhre & Paszkowski, 2006)
	Increased plant vigour through improved plant nutrition	(Vogel-Mikuš & Regvar, 2006)
	Degradation of organic pollutants	(F. Wang et al., 2020)
Carbon sequestration	Significant carbon sink into the soil	(Douds et al., 2000)
	Increased plant community productivity	(Zhang et al., 2012)
	Improved soil structure	(Wilson et al., 2009)
Nutrient retention	Improved nutrient uptake of plants	(Smith & Read, 2008)
	Immobilization in fungal structures	(Watts-Williams & Cavagnaro, 2012)
	Improved soil structure	(Cavagnaro et al., 2015)

water regime (Piotrowski et al., 2004). AMF can have further effects on the composition and quantity of soil microorganisms, which in return lead to changes to the soil structure (Rillig & Mummey, 2006; van der Heijden et al., 2008).

3.2.2 Soil contamination

Soils are considered contaminated once they contain substances (e.g. organic pollutants), elements (e.g. toxic metals) or microorganisms (e.g. pathogens) above normal concentrations or above national guideline levels (FAO, 2015). Soil contamination is a diverse topic which makes it difficult to quantify it on a global scale. The extent of soil contamination is nevertheless alarming and considered one of the major threats to soil functioning. Although soil contamination can be of natural origin, anthropogenic activities are considered as the main causes (FAO, 2015).

One of the main issues of soil contamination is the accumulation of toxic metals, for example, through the application of agrochemicals, biosolids, wastewater or mining (Wuana & Okieimen, 2011). The earliest studies about the protective effects of mycorrhizal fungi against toxic metals involved ericoid mycorrhizal fungi (Bradley et al., 1981). Since then it has been repeatedly demonstrated that also AMF can alleviate stress caused by increased metal concentrations in soils (Hildebrandt et al., 2007). It is almost a confusing phenomenon, since some metals are necessary micronutrients for plant life and their uptake is promoted by AMF (Watts-Williams & Cavagnaro, 2012). This uptake of micronutrients and the protective effect against toxic levels demonstrate the complex interaction between soils, the soil microbiome and plants. Studies at contaminated sites revealed the presence of AMF communities (Vogel-Mikuš et al., 2005), albeit their diversity is often reduced compared to non-contaminated sites. However, those remaining AMF species might be better adapted to high concentrations of toxic metals (Del Val et al., 1999; Galli et al., 1994). The underlying mechanisms are either changing the fate of the metals in the soil or change the plant's response towards them. One established mechanism is the immobilization of metals in intra- and extraradical fungal structures through metallothioneins or other chelating agents (French, 2017; Lanfranco, 2007). Interactions between metals and Glomalin-related soil proteins have been reported as well, leading to similar forms of immobilization (Yang et al., 2017). Other proposed mechanisms are related to changes in the plant physiology which are caused by the AM symbiosis. These changes involve the upregulation of plant genes involved in detoxification (Jiang et al., 2016) or the removal of contaminants from plant roots through fungal structures such as arbuscules (Göhre & Paszkowski, 2006). More recent studies also investigated the effects of AMF on organic pollutants in soil. The mechanisms involved are similar to those of potentially toxic metals but also include the degradation of pollutants through enzymes. Again, the overall protective effect is composed of multiple mechanisms and interactions between soil microorganisms and plants (F. Wang et al., 2020). The effects of many other common pollutants such as pesticides and micro-plastics are still poorly investigated.

Especially the effects of mixed pesticides in combination with other soil stress factors on soil health are still poorly understood (Rillig, Ryo, et al., 2019).

3.2.3 Carbon sequestration

Soils are the largest terrestrial carbon storage. The amount of carbon that is stored in soils exceeds that of the atmosphere and the global plant biomass combined (Scharlemann et al., 2014). The carbon cycle is one of the most important processes on Earth to which almost every organism is linked in one way or another. Plants are one of the main drivers of this process and as such also their symbionts at the root–soil interface. Plants forming associations with AMF allocate up to 20% of carbon to AMF and plants forming ectomycorrhizas can allocate up to 50% of their assimilates, demonstrating that mycorrhizal associations are substantially involved in the carbon cycle (Soudzilovskaia et al., 2015). The prehistoric events that saw a decline in atmospheric carbon are linked to the emergence of deeply rooted trees around 450 million years ago and angiosperms around 130 million years ago. Mycorrhizal associations have been confirmed in root fossils dating back more than 400 million years ago (Remy et al., 1994) and are, therefore, considered crucial for the evolution of terrestrial plants, indicating that their role in carbon cycling might be of equal importance (Taylor et al., 2009). Soil carbon concentration is an important factor for soil health and is the foundation of almost all other soil components. Having high concentrations of soil carbon is therefore considered valuable for 'healthy soils'. However, due to unsustainable management, soils are increasingly releasing carbon into the atmosphere in the form of CO_2. This development is aggravating climate change when soils could actually be used as carbon storage and bind atmospheric CO_2 into the ground. One initiative predicts that an annual growth rate of 0.4% soil carbon could have significant effects on soil health and contribute to limit global warming at the same time (Kon Kam King et al., 2018).

Studying the impact of AMF on the soil carbon cycle is a difficult undertaking on an ecosystem level. Pot studies under controlled conditions allow to separate the carbon inputs between roots and AMF. However, on an ecosystem level, this becomes increasingly complicated, as an almost unmanageable amount of processes interact with AMF and vice versa. On the level of a single plant, mycorrhizal associations are translocating photosynthates from the plant directly into the soil. Hexose is the preferred metabolite for AMF which is sourced from plants and then used for growth and reproduction. Estimates suggest that between 5% and 20% of plant-derived carbon is translocated to AMF associations (Douds et al., 2000). The overall carbon sink through AMF mycelium thereby accumulates to 50–900 kg carbon ha^{-1} (Zhu & Miller, 2003). Although this carbon drain might suggest negative impacts on plant growth, most mycorrhizal plants react to mycorrhizal colonization by producing more biomass. AMF provide many advantages such as improved nutrient uptake which then leads to higher photosynthetic rates. Another mechanism on how AMF improve carbon sequestration is their positive effect on soil stability and soil aggregation. Mycorrhizas are critical components of the terrestrial carbon

cycle and shape plants and soils alike. Conversely, depriving soils of their mycorrhizal potential leads to implications that go beyond the effects on a single plant (Wilson et al., 2009).

3.2.4 Nutrient retention

High concentrations of plant nutrients can be challenging when those are not fully integrated into the ecosystem. Nutrient availability that is exceeding its demand leads to nutrient loss, either through leaching, gaseous emissions or erosion. This situation is mainly observed after the application of fertilizers, for example, in the agricultural context. Nutrients that are mobile within the soil matrix can leach out and make them inaccessible to the root system. Various forms of nitrogen are at risk of being lost through gaseous emissions, such as in the form of nitrous oxide (N_2O). Immobile nutrients such as some forms of phosphorus are more susceptible to soil erosion than leaching. Although phosphorus can also bind to mobile particles and then leach through the soil. Such excess nutrients can have negative impacts on their surroundings. Leached or wind-dispersed nutrients contaminate water ways and groundwater, thereby damaging the aquatic biodiversity. N_2O is a potent greenhouse gas with significant global warming potential. The overall fertilizer efficiency is sobering, especially when considering the global scale of fertilizer application. It is estimated that 30% of nitrogen and 15–30% of phosphorus are lost in the same year of application (Quan et al., 2020; Syers, 2006).

One method by which AMF can improve nutrient retention is obviously due to its role in plant nutrition, especially in the case of phosphorus. Without mycorrhizal associations, plant roots are inside a depletion zone once all immobile nutrients (like phosphorus) around the roots are taken up, making them dependent on nutrients moving to roots via mass flow, usually a slow process. Plants with mycorrhizal colonization can escape this depletion zone through the hyphae which can penetrate a bigger soil volume than roots by itself. This way, phosphorus gets bound into fungal and plant biomass which immobilizes it and protects it from erosion and leaching. Furthermore, mycorrhiza show enhanced mineralization of complex-bound or organic forms of phosphorus which would otherwise be unavailable to plant roots and susceptible of leaching. Another essential plant nutrient that is impacted by AMF is nitrogen. To this date, the exact interactions between AMF and the various forms of nitrogen are still to be investigated (Hodge & Fitter, 2010). However, it is known that AMF can take up nitrogen in the form of ammonium (NH_4^+) and amino acids. Evidence suggests that even other organic forms of nitrogen might be involved, especially since AMF are equipped with nitrogen reductase genes. The net balance of AMF and its role in nitrogen uptake on plants differ between studies. Whereas some studies show almost no contribution, other studies come to the opposite result. Although the effect of AMF on the nitrogen uptake of the plant is variable, its impact on reducing nitrogen losses has been proven repeatedly (Cavagnaro

et al., 2015).

Another important mechanism on how AMF help in nutrient retention is through effects on soil and plant water relations. There is some evidence that AMF affect plant water transport and can support plant water uptake (Augé et al., 2015; Bowles et al., 2016). Enhanced plant water uptake will consequently reduce the amount of leachate and the amount of nutrients being transported down the soil profile, which can also reduce N_2O emissions (Lazcano et al., 2014). In addition, the above-mentioned effects of AMF on enhanced soil aggregation can lead to significant effects on water retention. Both mechanisms together directly impact the amount of leaching in soils (Cavagnaro et al., 2015). Altogether, the impact of nutrient retention due to AMF is compelling. Nutrient retention of up to 80% has been reported for nitrogen and up to 60% for phosphorus (Corkidi et al., 2011; van der Heijden, 2010). Gaseous emissions of N_2O were reduced between about 30% and 50% after the application of nitrate fertilizer (Bender et al., 2015; Bender et al., 2014).

3.2.5 Arbuscular mycorrhizal fungi biofertilizer production

As outlined in the previous sections, mycorrhizal fungi have been linked to increased soil health and increased plant vigour. They are key players in the soil microbiome and at the soil–root interface. These characteristics make them promising alternatives to agrochemicals which can be used in sustainable agriculture and ecosystem restoration. The idea of using AMF as biofertilizers is almost as old as the systematic research of AMF itself. However, most work to transfer the scientific research into efficient applications has been rather futile up to this date. AMF biofertilizers are still considered a niche product and are mostly limited to the hobby market or some horticulture systems (Rouphael et al., 2015). The main reasons for the narrow application range being high costs (Berruti et al., 2016), unreliable product quality (Salomon et al., under review) and questions regarding the establishment under field conditions (Rodriguez & Sanders, 2015). Regardless of those issues, their huge environmental and economic potential is fuelling continuous research and major investments from agrochemical companies (Vosátka et al., 2008).

The earliest systems for the propagation of AMF used host plants in pot cultures and sterilized soil, which were then inoculated with the desired species. Preferred host plants are maize or sorghum, as their fast-growing root system allows substantial sporulation (Ijdo et al., 2011). To this date, it is still the most common method for mass production of mycorrhizal fungi, as it is relatively cheap and can be easily upscaled (Ijdo et al., 2011). This *in vivo* system underwent multiple modifications, such as the closed bag system which would reduce the costs for maintenance and helped to exclude potential phytopathogens (Walker & Vestberg, 1994). Multiple publications describe the use of soilless substrate, such as sand (Jentschke et al., 1999), which could further help with phytosanitary issues and eliminate the need for soil sterilization. It is even possible to produce on-site inoculum on agricultural waste products, yielding 3600 spores in 100 mL^{-1} of soil

(Chaiyasen et al., 2017). The simplicity of *in vivo* systems allows easy propagation and maintenance of mycorrhizal cultures. Major bottlenecks for the use in commercial biofertilizer production are phytosanitary issues and the difficulty to extract spores from the substrate, for example, to be further concentrated or to be used with different carrier materials (see Figure 3.2).

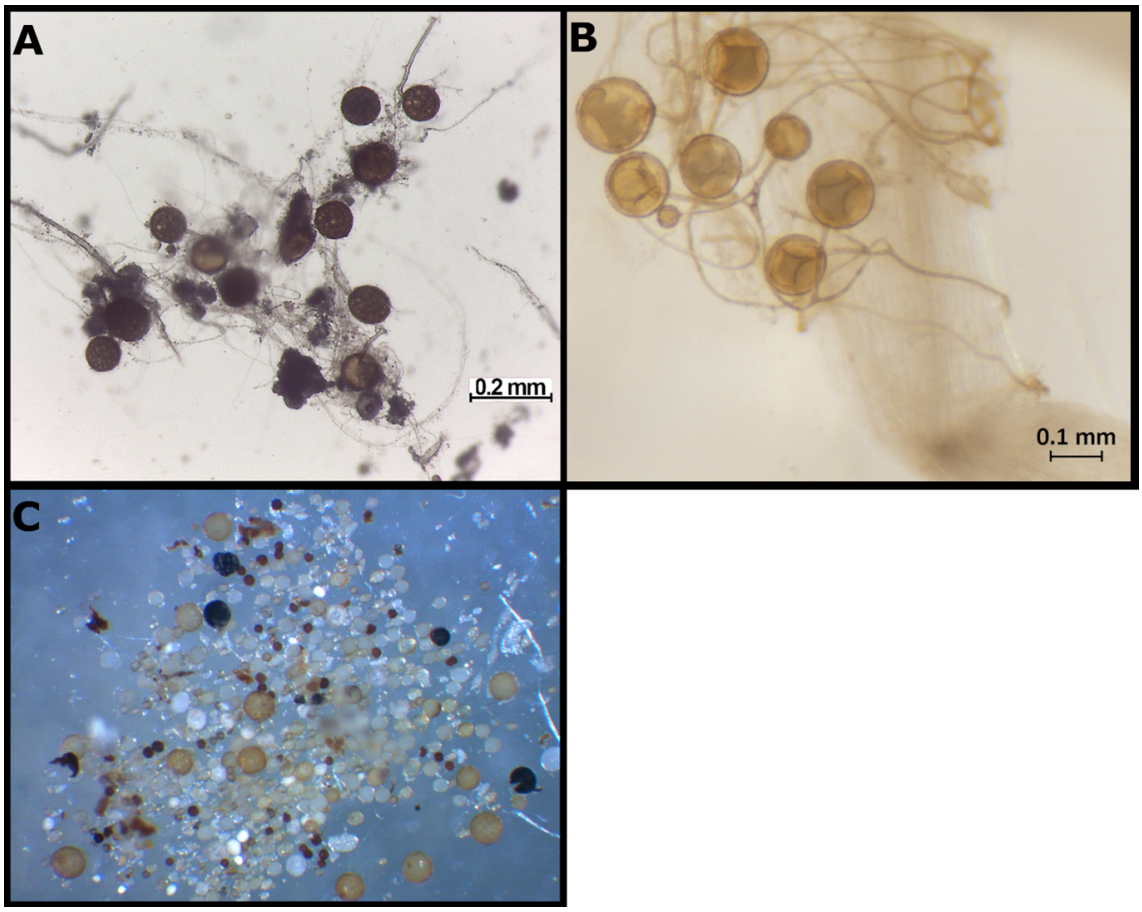

FIGURE 3.2: *A: Extracted AMF spores from a commercial product. B: Spores of an in vitro culture of Rhizophagus clarum growing on tomato roots. C: Spores of various sizes and colours as can be found in natural soils. C: Photo courtesy of Luise Köhl.*

The need for axenic AMF systems led to the development of *in vitro* cultures on Ti-transformed hairy roots (Declerck et al., 1996). The motivation behind this development was not only its potential use for the production of biofertilizers but also providing a research tool for the study of AMF. The original method made it possible to produce up to 10,000 spores of *Glomus versiforme* within 4 months. The spore production could be further increased by using a split-plate design (65,000 spores) (Douds et al., 2000) or whole plants instead of hairy roots (Voets et al., 2005). The latest research even demonstrated the asymbiotic sporulation of *Rhizophagus irregularis* using culture medium with fatty acids (Kameoka et al., 2019). AMF propagules in axenic systems can be easily extracted and are free of plant pathogens by default. They can be added to a variety of carrier materials which makes them applicable for most production systems. However, axenic cultures require trained personal and high material costs which make it too expensive for most

biofertilizer applications. Also, not all AMF species can be cultured *in vitro* and continuous subculturing has been shown to lead to changes in functional traits (Kokkoris & Hart, 2019). Some producers question the ability of axenic cultures to establish in the field (e.g. because the AMF did not adapt and grow under real soil conditions), and this is an issue that needs further testing.

Other approaches for the production of AMF inoculum include hydroponic or aeroponic systems. To this date, there have been a variety of adaptations, each with customized designs and slightly different nutrient solutions. One of the earliest works used the nutrient flow technique (NFT) to produce *Glomus mosseae* propagules on maize, yielding up to 50% mycorrhizal root infection (Elmes & Mosse, 1984). This system provided good growth conditions with relatively little root disturbance to protect the fungal structures. But also deep water culture systems with reduced aeration times have been successfully used (Hawkins & George, 1997). Aeroponic systems have certain advantages over hydroponics, such as increased root aeration which supports plant growth and reduces the likelihood of certain diseases. Nutrients and moisture are applied as aerosols around the roots. Common systems convert the nutrient solution into micro-droplets (spray nozzles), mist (atomizers) or ultrasonic fog (piezo elements) (Jarstfer & Sylvia, 1995). With regard to the production of mycorrhizal inoculum, aeroponics put less physical stress onto the root systems than hydroponics. Production rates of up to 50 spores cm^{-1} of colonized roots have been reported (Hung & Sylvia, 1988). Aeroponics and hydroponics allow easy spore extraction through sieving the roots and moist roots can be further processed to sheared-root inoculum using a common food blender. Sheared-root inoculum can only be made from wet roots and then stored for up to 1 month, whereas unprocessed and dried roots retain their inoculum potential for up to 2 years, but are not suitable for the production of sheared root inoculum (Sylvia & Jarstfer, 1992). Those examples provide proof of the flexibility of AMF production systems and explain how they can be advanced to fulfill specific requirements. Most companies do not disclose their production methods due to issues about intellectual property. However, we can assume that successful companies developed efficient production systems which might be based on the mentioned hydroponic or aeroponic systems. Systems that have been made public involve an airlift bioreactor (Jolicoeur et al., 1999) or a semi-hydroponic set-up (Declerck, 2009).

3.3 Managing arbuscular mycorrhizal fungi for soil health

3.3.1 Agriculture

The management of AMF in agriculture has been subject to recent discussions which were mainly focused on insufficient correlations between the yield of agricultural crops and their mycorrhizal colonization (Ryan & Graham, 2018). It is obvious that many plants

can grow without AMF assuming that sufficient amounts of fertilizers are added. However, it is important to consider the whole spectrum of ecosystem services which are provided by AMF, especially those related to soil health (Chen et al., 2018). Those additional services are often not as visible as crop yields but nevertheless of high economic and ecological importance (Rillig, Aguilar-Trigueros, et al., 2019). Especially in times of extreme weather events, good soil health and functioning mycorrhizal symbiosis can be considered as a 'health insurance' for crops to achieve yield resilience (Rivero et al., 2018). However, such things are often difficult to measure, scientifically and monetarily. Moreover, AMF can help to reduce the reliance on fertilizers and make agriculture more sustainable. Future research should focus on the role that AMF can play for soil ecological engineering, producing the same amount of food with less inputs.

Mycorrhizal colonization of crop plants through indigenous AMF can be increased through changes in agricultural practices. Key variables which have been identified are the use of cover crops and the type of tillage system (Bowles et al., 2017). Those results indicate that mycorrhizal colonization of summer crops increases by 30% when working with minimal soil disturbance and cover crops instead of barrow fallows. Furthermore, the AMF species richness increased by 11%, highlighting the sensitivity of certain species to soil disturbance. Further research focused on the comparison between organic and conventional agriculture, whereas organic systems are usually associated with higher AMF abundance and richness. Again, tillage and cover crops have been considered as major influences, as well as the impact of fertilization (Borriello et al., 2012). Mineral fertilizers are generally associated with reduced AMF abundance and species richness, caused by their high availability and effects on soil chemistry (Oehl et al., 2004). Most organic farms also incorporate more diverse crop rotations and take advantage of the many positive effects of legumes and mixed crops on soil health (Verbruggen et al., 2010).

Research shows that soils can be actively managed to support AMF and increase soil health. Such measures include organic farming, permanent crop cover, crop rotation and the inclusion of temporary pastures in rotations. However, these methods come with limitations. Organic farming is relying on ploughing and tilling as means to control weeds and phytopathogens. Arid farmlands in many parts of the world do not support multiple crops within one season. It is especially those harsh environments that could benefit from the many advantages of AMF. Canola is an important cash crop in organic and conventional farming systems alike. However, being a non-mycorrhizal plant, it has negative impacts on following crops in terms of mycorrhizal colonization (Valetti et al., 2016). Where options to manage indigenous AMF are limited or weakened by non-mycorrhizal crops, soils can be actively inoculated with AMF biofertilizer. This inoculum could be adapted to the local conditions and provide optimized plant and ecosystem functions (Sanders, 2010).

Although AMF provide multiple ecosystem services, they need to provide clear economic benefits to find their way into most broad-scale applications. Assuming that most

farmers do not get direct fiscal support to protect their soils, the costs of biofertilizer need to be weighed against the cost of potential fertilizer-savings or yield and quality gains. Major factors for this cost-benefit analysis are the amount of necessary inoculum and the specific mycorrhizal growth response of the crop. Due to its relatively low seed density, potato is a crop with strong prospects for profitable AMF biofertilization. Hijri, 2016 demonstrated in field trials over 4 years an average yield increase of about 4 t ha^{-1}. The profitability threshold was reached in almost 80% of all trials while an average of 71 spores was applied per seed potato at the time of planting. Tawaraya et al., 2012 achieved significant savings in phosphorus fertilization for the Welsh onion (*Allium fistulosum*). Typical for this crop, the seeds were preplanted under greenhouse conditions where seedlings were either treated with *Glomus etunicatum* 'R10' or left uninoculated. Plants were then transplanted into the field under various fertilization treatments. The yield of the inoculated plants at 300 mg P_2O_5 kg^{-1} soil was similar to those in 1000 mg P_2O_5 kg^{-1} soil and not inoculated, whereas the costs of the difference in superphosphate application were double the costs of the inoculum. Not part of this equation is the savings in environmental costs due to potential fertilizer run-off and/or leaching. For future research, it is important that on-farm experiments are being performed under real-world agricultural conditions and that inoculation tools that are practically feasible for farmers are provided.

Other common agricultural crops are more difficult to inoculate at reasonable costs, mostly due to higher seeding rates or smaller yield gains after inoculation. Most studies in the field of AMF do not include an economic analysis and use laboratory cultures of AMF rather than commercial inoculants. Economic analysis on self-propagated cultures would be unreasonable as they are not produced on a commercial scale and therefore produced at much higher costs. Ignoring the economic factors of biofertilization, myriads of studies show that field inoculation with AMF is producing promising outcomes for plant growth (Al-Karaki et al., 2004; Cely et al., 2016; Pellegrino & Bedini, 2014; Thompson et al., 2013) and soil health (see Table 3.2). In terms of efficient application of AMF for large seed quantities, seed coating is a promising solution to deliver not only AMF but also other beneficial microorganisms directly to the plant (Rocha, Ma, Souza-Alonso, et al., 2019). Successful implementation of this technique, in combination with AMF and agricultural crops, has been reported (Oliveira et al., 2016; Rocha, Ma, Carvalho, et al., 2019), even in combination with fungicidal seed coating (Cameron et al., 2017).

3.3.2 Ecological restoration

The science of ecological restoration is focused on trajectories of change, that is, on the succession, assembly and state transition of natural communities. The specific aims range from re-introduction of single species to population and community restorations. Broadly, ecological restoration can be defined as the assisted recovery of ecosystems that have been degraded or destroyed. Due to the various positive effects of AMF on the ecosystem and plants, they are considered a promising tool to drive this restoration process. In that way, they can increase the survivability of plant species in contaminated soils

TABLE 3.2: *Various meta-analysis on AMF involving their effect on plant growth and nutrient uptake. No additional analysis is presented here.*

Analysed interaction	Outcome	Reference
Photosynthesis under salt stress	Alleviation of salinity stress and increased photosynthetic rate	(Y. Wang et al., 2019)
Wetland plant performance	Significant benefits, even under flood conditions	(Ramírez-Viga et al., 2018)
Nutritional and non-nutritional factors in crops	Positive effects on nutrient uptake, soil aggregation, water flow, disease resistance	(Delavaux et al., 2017)
Potato yield	Increased yields due to AMF inoculation and with economic benefits	(Hijri, 2016)
Response of wheat to AMF	AMF field inoculation increased nutrient uptake and dry weight	(Pellegrino et al., 2015)
Copper, manganese and iron concentration in crops	Significant role for copper and iron, limited role for manganese	(Lehmann & Rillig, 2015)
Zinc nutrition in crop plants	Positive impacts on Zn concentration in shoot, root and fruit	(Lehmann et al., 2014)
Nutrient uptake of tomato	Particularly beneficial for phosphorus and zinc	(Watts-Williams et al., 2014)
Plant growth under water stress	Improved drought resistance of plants	(Jayne & Quigley, 2014)
Mycorrhizal responsiveness in crop plants and wild relatives	No evidence of decreased mycorrhizal responsiveness	(Lehmann et al., 2012)
Context-dependant plant response to AMF	Plant response most positive when plants are phosphorus limited	(Hoeksema et al., 2010)

or favor certain plants over others, such as unwanted neophytes. Ultimately, restoration efforts in combination with AMF lead to the enhanced establishment of plant communities with positive effects on soil health (Asmelash et al., 2016).

In most cases, natural ecosystems do not allow active soil management such as in the case of agriculture. Most restoration efforts are focusing on transplanting and seeding of plants. Various studies demonstrated that AMF inoculation can be successfully merged with those methods. Zhang et al., 2012 applied a mix of three lab-cultured AMF species to grassland by drilling holes and refilling them with the inoculum. Over 3 years, the number of established seedlings and their community productivity was significantly higher than in the uninoculated control. White et al., 2008 drilled seeds with inoculum into the soil, as well as broadcasting both onto the surface, achieving similar colonization results. However, in this case, there were no positive mycorrhizal effects due to the inoculation, which might be caused by high levels of phosphorus in the soil and a high abundance of

native AMF communities. The efficiency between native and introduced AMF is still to be debated. Williams et al., 2012 pre-inoculated tree cuttings, whereas inoculum made of native AMFs and a natural forest performed better than a commercial one. The positive effects of seedling inoculation with natural AMF communities had also been demonstrated in other studies (Emam, 2016). It becomes evident that field inoculation of natural ecosystems with AMF is a feasible option for ecological restorations, and, as shown in some studies, can have long-lasting effects over multiple years. To this date, the species effectiveness between native and introduced AMF is not fully understood. However, we do have a good understanding of the importance that soil microorganisms have on above-ground ecological complexes. Managing below-ground mycorrhizas seems to be one important step in order to harbor the full potential of restoration efforts. Khan, 2006 defined the term 'Mycorrhizoremediation' as an enhanced form of phytoremediation.

3.4 Conclusion

In this chapter we highlighted how AMF can enhance soil health and how they could be used as biofertilizers, either in agriculture or ecological restoration. The main mechanisms by which AMF improve soil health include improved soil structure and the immobilization of potentially toxic metals. The latest research even showed that AMF can enhance the degradation of organic pollutants. Due to the nature of their symbiosis, they act as a significant carbon sink for the soil, thereby increasing soil carbon. Carbon sequestration for soils is not only improving soil health but can also be used to trap atmospheric CO_2 back into the soil, thereby contributing to mitigate climate change. In the context of climate change, we also addressed the potential of AMF to reduce gaseous nutrient losses from soils, such as in the form of N_2O. This gas is mineralized from other forms of nitrogen in the soil and is a potent climate gas. AMF further reduce the leaching of nitrogen into the ground water and phosphorus-loss through soil erosion. Nutrient loss from soils is not only impacting soil fertility but is also critical with regard to the surrounding ecosystem, especially the aquatic biodiversity.

The highlighted effects of AMF on soil health can be used to increase sustainability in food production and to restore degraded soils. It is obvious that AMF could be applied as biofertilizers in various scenarios. Their economic and ecological potential was also commercialized by a variety of companies all around the world. The production and application of AMF is challenging and requires expertise. For people working in the field of mycorrhizal research, it is not surprising that many of those products fail to induce mycorrhizal colonization. This was also confirmed by some studies where only a fraction of the tested products were considered viable. During the past centuries, a variety of AMF production systems have been proposed which uses soil, hydroponics, aeroponics, or axenic cultures. Each system has its advantages and disadvantages, and to this date, the mass production of high-quality AMF propagules is an expensive endeavor. Nevertheless, investments in mycorrhizal products are fueled by an endless stream of mycorrhizal research that highlights again and again the positive effects of AMF and how it could be

used to reduce our footprint on this earth. The value of AMF field inoculation has been proven for a variety of scenarios, such as agriculture systems or to enhance phytoremediation efforts. In some cases where the targeted host plant is exceptionally responsive to mycorrhiza and where only relatively small amounts of inoculum are necessary, the use of AMF biofertilizer can be economically viable already today.

3.5 Future trends in research

In recent years, particularly with the advent of high throughput sequencing, our knowledge of soil microbial communities has increased greatly. It has long been known that soil microorganisms, including AMF, provide essential ecosystem services which we are now able to better quantify. The next great step in soil ecology involves the assignment of soil functions to microbial communities. In doing so, we will be able to quantify, and then predict, the impacts that management and other causes of environmental change (e.g. climate change) have on soils and the ecosystem services they provide. Armed with this knowledge we will then be able to better understand how to best manage systems to support the benefits of the soil microbiome. Such knowledge will also assist in the development of reliable biofertilizers. Those biofertilizers are not limited to AMF but the whole spectrum of beneficial soil microorganisms. One utopian vision of the future would involve a replacement of most agrochemicals with biofertilizers.

We have observed and also predict a continued interest in more sustainable farming systems in the future. Such systems seek to produce more food, on less land and with fewer inputs. At the same time, they are equipped against adverse conditions, such as the increasing occurrence of extreme weather events. This is a tall order but one we cannot afford to not meet in the context of an increasing global population and increased food requirements in the coming decades. Moreover, we have observed a growing interest in agricultural paradigms that is shifting towards biologically regulated nutrient supply, rather than the importation of externally sourced synthetic inputs. This development would help to 'close the loop' and minimize the movement of resources on and off farm.

This is an exciting time to be studying soil ecology. There are many challenges in the here and now, and in the future to come. We contend that the soil microbiome, including mycorrhizal fungi, may hold many of the answers to meet those challenges and to do so in a sustainable way.

3.6 Where to look for further information

The following articles and books provide a good overview of the subject:

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- Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. (1995): Working with Mycorrhizas in Forestry and Agriculture, Australian Centre for International Agricultural Research, Canberra.
- Declerck, S., Strullu, D-G. and Fortin, A. (2005): In Vitro Culture of Mycorrhizas, Springer, Berlin Heidelberg.
- Fisseha, A., Bekele, T. and Birhane, E. (2016): The Potential Role of Arbuscular Mycorrhizal Fungi in the Restoration of Degraded Lands, Front Microbiol. 7: 1095.

Research associations

- International Mycorrhiza Society (http://mycorrhizas.org)

AMF collections

- Banque Européenne des Glomeromycota (BEG) (https://www.i-beg.eu)
- Glomeromycota *In Vitro* Collection (GINCO) (https://www.mycorrhiza.be/ginco-bel/)
- International Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) (https://invam.wvu.edu).

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Chapter 4

Urban soil health: A city-wide survey of chemical and biological properties of urban agriculture soils

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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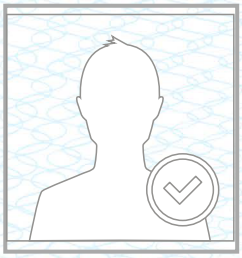

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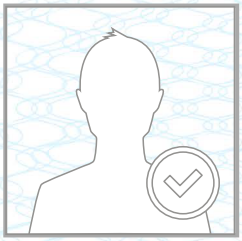

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Urban soil health: A city-wide survey of chemical and biological properties of urban agriculture soils

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ABSTRACT

The integration of urban green spaces into modern city planning is seen as a promising tool to offset the drawbacks of ever-expanding cities. Urban agriculture is a common method to implement such strategies and to increase urban sustainability with a special focus on food security. Due to their location, urban farms are highly influenced by past and present anthropogenic activities which can threaten both soil health and food safety. This study includes 12 urban agriculture sites in the metropolitan area of Adelaide, Australia. It is the first of its kind to focus on soil health in urban agriculture systems with a further emphasis on mycorrhizal fungi. Descriptive information about each site, the biodiversity of the selected plots and soil samples from different depths and locations were collected and analysed for chemical and biological parameters. Seven metals, total and plant-available (Colwell) phosphorus and available nitrogen were measured in soils. A glasshouse bioassay was also conducted to determine the abundance of beneficial arbuscular mycorrhizal fungi in the soils and the change of root colonization after inoculation with the mycorrhizal fungus *Rhizophagus irregularis*. Results showed a generally high biodiversity of plants that correlated with site activity (commercial or community garden) and which could potentially be used for urban biodiversity conservation. Metal concentrations in soils were below national guidelines levels for all samples, although sites with previous industrial history showed elevated levels when compared to sites without industrial history. The use of raised beds with introduced soils eliminated differences in previous land-use history, thereby providing a good option to support cleaner production. Gardening soils were considered highly fertile, with plant-available (Colwell) P concentrations exceeding recommended levels for most horticultural crops, while soils were adequately supplied with nitrogen. Most plant nutrients were derived from freely available urban waste streams and integrated via composting. Various urban waste streams could be used to counter-act imbalanced soil nutrients. Arbuscular mycorrhizal fungi were present in all sites, indicating that the practiced soil management is sustainable from a microbial perspective. Given their important role in supporting plant nutrition, and potential to reduce the need for external nutrient inputs, they provide an important focal point for achieving clean and sustainable urban food production. The results were incorporated into a framework for the management of urban soil health.

1. Introduction

The global population is expected to reach more than 9 billion by 2050, with most of this increase to occur within urban areas (United Nations, 2019). In terms of land use, urban areas are projected to grow up to 80% by the year 2030, with most of this increase happening in developing countries (Mahendra and Seto, 2019). As a consequence, around 2% of the world's current arable land will be lost due to urbanisation (Bren d'Amour et al., 2017). These developments lead to various social, economic and environmental challenges that need to be addressed accordingly in the context of urban planning. The integration of urban green spaces is seen as a promising strategy to offset many drawbacks of ever-expanding cities and to increase urban sustainability. Urban green spaces can also contribute to food security, which is of special importance for developing countries. This implementation is called

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urban agriculture (Skar et al., 2019).

Urban agriculture refers to food production systems inside city boundaries or densely populated areas. As such, it makes significant contribution to social, economic and ecological quality (Miccoli et al., 2016). It is a global phenomenon which is of special importance for food security in developing countries. Estimates suggest that the scale of urban agriculture grows linearly with the urban growth of countries in equatorial Africa (Lee-Smith, 2010). Developing countries in Asia show a similarly high participation of urban dwellers in agriculture, which is considered an important source of livelihood (Zezza and Tasciotti, 2010). Urban agriculture in more developed countries has a stronger focus on social components rather than food production and is often associated as a leisure activity or as a form of ecological activism. To date, the driving forces behind urban agriculture appear to be less concerned with food security, and more so with social, cultural and ecological factors (Mok et al., 2014). However, in the context of climate change, and other shocks to the food system (e.g. the recent Covid-19 pandemic), there is renewed interest in urban agriculture as a means to secure a supply of clean food in all regions of the world. Especially when regional transport of foods may be affected by pandemic-induced controls on movement. In the pursuit of urban sustainability and sustainable food production, there is further need to re-evaluate urban agriculture on a global scale (Skar et al., 2019).

There are many mechanisms involved through which urban agriculture is able to contribute to sustainable food production and urban sustainability. One of the biggest advantages is its ability to produce food locally and to reduce transportation routes (Lee et al., 2015). The greatest reductions in greenhouse gas emissions can be achieved by growing high-yielding seasonal food that would otherwise be imported (Kulak et al., 2013). The integration of urban waste streams for nutrients and organic matter enables urban agriculture systems to reach a high self-sufficiency for most plant nutrients and even up to 100% for phosphorus (Wielemaker et al., 2018). Many case studies have shown that urban agriculture is highly adaptable and often tailor-made for the specific needs of the local residents and their surrounding environment. This multifunctionality allows for efficient land-use in densely populated areas (Lovell, 2010) and even in areas with poor or unknown soil conditions (Armar-Klemesu, 2000). It can also be shaped to specifically provide urban ecosystem services such as pollination, pest control or climate resilience (Lin et al., 2015).

Urban agriculture comes in various forms and shapes, and especially developed countries see an increase in more advanced systems such as hydroponics in combination with vertical gardening and LED light systems. However, the most common form of urban agriculture is using either the natural soil or a soil-based medium in raised beds or containers (Mok et al., 2014). The importance of urban agriculture for sustainable urban planning and food security warrants the need for detailed investigations of urban soil health in the context of food production. To this day, most of this research focused on soil contamination due to anthropogenic activities. Such results are often individual to each sampling location, and show high variability according to their particular surroundings (Säumel et al., 2012). Information on soil fertility in terms of available plant-nutrients and soil microbial activity is scarce. The available studies agree that urban agriculture sites have an ample supply of plant nutrients which are derived from various forms of urban waste streams (Wielemaker et al., 2019). Research also indicates that using organic fertilizers rather than inorganic forms is associated with higher microbial activity due to carbon inputs to soil (Igalavithana et al., 2017). In terms of yield-efficiency, high outputs have been reported, however, often at benefits-to-cost ratios similar to conventional farms. In a hypothetical scenario, most inputs could have been substituted with local renewables, thereby increasing the sustainability of those systems (McDougall et al., 2019).

Maintaining and enhancing soil health is commonly cited as a high priority in urban farming communities. While measuring soil health is difficult, one approach that can be used is to assess impacts on key soil biota. To this end, arbuscular mycorrhizal fungi (AMF) are a near-ubiquitous group of soil fungi that colonise the roots of the majority of terrestrial plant species (Siddiqui and Pichtel, 2008). These resulting associations, arbuscular mycorrhizas (AM), can provide many important ecosystem services, including improved nutrient uptake and decreased nutrient losses caused by leaching or soil erosion (Rillig et al., 2019) and are oftien cited as an important indicator of good 'soil health'. Colonization of roots by AMF can also alleviate effects of metal toxicity in plants (Watts-Williams and Cavagnaro, 2012), increase plant pathogen resistance and improve the soil structure. All of the aforementioned benefits of AMF are relevant in the contexts of both urban and conventional agriculture practices (Siddiqui and Pichtel, 2008). Although AM have an important role to play in sustainable production systems, the status of AM in urban agricultural systems has not, to our knowledge, been studied previously.

This study includes the results of a survey of physiochemical and biological properties of soil from urban agricultural sites across a major metropolitan city (see Supp. Fig. 1). The research involved 12 urban sites, which were described according to their design and plant biodiversity. Soil samples were collected and tested for a number of different soil parameters. This analysis answers questions regarding soil nutrient potential and contamination with potentially toxic metals. Soil collected for the sites was also used in a greenhouse bioassay to gain information on the soil's biological properties, namely its mycorrhizal potential. Following, the term "mycorrhizal potential" is used to describe the soil's potential to promote colonization of roots by AMF.

2. Material and methods

The selected sites were dominated by community gardens ($n = 10$), but also included two commercial production sites in an urban setting. The sites were surveyed in September–October (Austral Spring), 2017 and soil physicochemical properties were measured. The same soils were used in a glasshouse bioassay experiment with the aim to assess their mycorrhizal potential.

2.1. Site selection

All sites were within a 15 km radius of the City of Adelaide (see Supp. Fig. 2A). The City of Adelaide (Longitude S-34.93°, Latitude E138.60°) has a population of approximately 1.3 million people with a varied history (post-European settlement in 1836) of urban, agricultural, and industrial land use (see below). Using publicly available data, a total of 17 urban agriculture sites were identified as potential survey sites. Selection criteria were a minimum size of 200 m^2 and evidence of active food production. Of the 17 sites identified, representatives of 12 sites agreed to being included in this study. For confidentially, the precise locations and names of some sites are not identified here.

2.2. Survey: site characterization and sampling

Prior to visiting sites, further information was gathered using publicly available web sites as well as current and historical satellite imagery. This contextual information includes local land use context, garden size and number of garden beds. Information on historical land use was supplemented and/or confirmed during site

visits. Upon arrival at each site, the number of beds was recorded and if production took place in raised beds or not. At each site, gardening beds with evidence of active farming were identified and four or five representative beds randomly selected for more detailed investigation and sampling (see Supp. Fig. 2B).

The dimensions of the beds sampled at each site were measured, and the identity and abundance of plants species being grown at the time was recorded. The source of the soil (i.e., indigenous or imported potting soil) in the production areas was recorded, and where possible, information on the nature of amendments (e.g., manure, compost, etc.) was recorded. Although no sites were formally certified as organic, all sites followed basic principles and ethos of organic farming. These principles mainly included the use of organic pesticides over synthetic ones and abstinence of any mineral fertilizers.

Soil was collected from each bed by taking five soil cores from the 0–10 cm soil layer using a 10 cm diameter auger. Those five cores were then combined at the bed level to produce one composite sample per bed. At two of the sites, cropping was in rows rather than beds, thus soil samples were taken from an area of 1.5 × 2.5 m, which was equivalent to the typical bed size at the other sites.

In an effort to characterise underlying soil conditions at each site, soil samples were also taken from across the site in the non-cultivated area (e.g. in the space between the beds), later referred to as the 'underlying soil'. These samples were taken from four separate locations randomly distributed across the site (i.e. n = 4). Samples were taken from the underlying soil for the 0–10 cm and 10–30 cm soil layers using a 5 cm diameter auger; at some sites it was not possible to sample to a depth of 30 cm due to high soil strength. All soils collected were stored in air-tight plastic bags and placed in a travel refrigerator at 4 °C until their return to the laboratory, where they were processed immediately.

2.3. Survey: soil physicochemical analysis

Upon return to the laboratory, soil samples were carefully mixed and any coarse woody (or other) debris removed using a 2 cm sieve. The sieved soil was then divided into subsamples for analysis as follows. The first sub-sample was used for determination of soil gravimetric moisture content after drying at 105 °C for 48 h. The second sub-sample was used for colorimetric determination of mineral N (ammonium and nitrate) on 2 M KCl soil extracts as described in Cavagnaro et al. (2006). The third subsample was air-dried at 40 °C for at least 48 h and used for further physicochemical analysis: soil pH and EC (1:5 water extract) was measured using a TPS WP-81 pH, TDS, Temperature & Conductivity Meter (EnviroEquip Biolab, Australia). Plant-available (Colwell) P was determined colorimetrically in soil samples collected from the garden beds, using Murphey & Riley colour reagent after extraction in 0.5 M sodium bicarbonate solution for 16 h (Cavagnaro et al., 2006). Total Dumas carbon (C) and N analysis was performed by Australian Precision Ag Laboratory (see http://www.apal.com.au/, last accessed May 2019). The concentration of metals in the soil was determined on soil digests in aqua regia and perchloric acid, followed by analysis for the individual elements: Arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), nickel (Ni), phosphor (P), lead (Pb) and zinc (Zn), by inductively coupled plasma optical emission spectroscopy (ICP-OES, PerkinElmer Avio 200). The reference soil ACU-4 was used as certified reference material with recovery rates between 89% and 106%. The instrument detection limits (on a soil basis) were 0.028 mg kg^{-1} for As, 0.012 mg kg^{-1} for Cd, 0.1 mg kg^{-1} for Cu and Mn, 0.028 mg kg^{-1} for Ni and 0.1 mg kg^{-1} for Pb and Zn.

2.4. Bioassay

Mycorrhizal fungi are often cited as a key indicator of soil health and as having a role to play in clean and sustainable production systems. In order to investigate the potential for indigenous and introduced (*Rhizophagus irregularis*, see below) AMF to colonise the roots of plant grown in the soils collected from the sites, a glasshouse bioassay experiment was undertaken. Due to the limited amount of soil from some sites following physicochemical analysis, it was not possible to conduct the supplemented inoculation (i.e. *R. irregularis*) treatment on every collected sample; however, 80% of the soils could be inoculated, with n = 50 in the test of indigenous AMF inoculum potential, and n = 40 in the test of impacts on soil after supplemental inoculation with *R. irregularis*.

The culture of *R. irregularis* (WFVAM10) has been used in previous studies and was found to result in good mycorrhizal root colonization (Watts-Williams and Cavagnaro, 2012). The culture is regularly propagated in a closed pot culture system with *Tagetes patula nana* as a host plant. On average, 7 spores g^{-1} inoculum were present, as well as a variable number of infected root pieces. This source of mycorrhiza inoculum has previously been found to provide high levels of AM colonization under a range of conditions.

The glasshouse bioassay was performed as follows: tomato (*Lycopersicon esculentum* cv. 76R) seeds were surface-sterilized and pre-germinated on double autoclaved sand mixture, before being transplanted into the final substrate after the development of the first true leaf. The final substrate consisted of 150 g of the collected garden bed soils mixed with 150 g of double autoclaved fine sand. *R. irregularis* inoculum was added (10% w/w) for the supplemented treatment while keeping the same final weight. Plants were grown in an environmentally controlled greenhouse from November to December 2017 (Austral Spring-Summer) and randomized weekly. Plants were watered daily using reverse osmosis (RO) water, and no other nutrients were added.

Plants were destructively harvested 36 days after transplanting, and roots and shoots were separated before being dried at 65 °C. At harvest, a subsample of the fresh roots was taken and stored in 50% ethanol for 24 h. Mycorrhizal colonization was quantified using the gridline intersect method after staining with ink and vinegar (Vierheilig et al., 1998). Shoots were ground to a fine powder before being analysed for the elements calcium (Ca), copper (Cu), iron (Fe), potassium (K), sulphur (S), magnesium (Mg), manganese (Mn), phosphor (P) and zinc (Zn) by ICP-AES (as described above). To obtain information about the presence of indigenous mycorrhizal spores in the collected soil samples, a subsample of the collected soils (n = 27) was processed according to (Merryweather and Moyersoen, 1997) as follows: depending on the available soil, between 10 and 30 g dry soil was weighed as biological triplicates and wet-sieved on 27 μm and 450 μm sieves for spore extraction. The extract was then centrifuged in a 50% sugar solution for further cleaning. The supernatant was separated and washed three times with RO water. Spores were then placed onto a 45 mm glass dish with four circular walls in between (nematode counting dish) and counted using a dissecting microscope (Olympus SZ-PT) between 80–100× magnification.

2.5. Statistical analysis

Survey: the data was not normally distributed and was therefore analysed using the non-parametric Kruskal–Wallis one-way analysis of variance with Bonferroni correction. In order to identify differences between the variables 'location' (garden beds, underlying soil 0–10 and 10–30 cm) or 'previous industrial history' (yes/no) (see below), site means (e.g. averaged across beds) were used as replicates. However, when comparing sites, individual samples

were used as replicates. Where significant differences were identified, post hoc tests were performed using Fisher's Least Significant Difference. In order to explore the relationship between different variables (e.g. total P and plant-available (Colwell) P), simple linear regression modelling was undertaken.

Bioassay: data was not normally distributed and Kruskal–Wallis one-way analysis of variance with Bonferroni correction was used in order to reveal differences between groups. Where significant differences were identified, post hoc tests were performed using Fisher's Least Significant Difference. Individual samples were used as replicates and analysed with the grouping factor Inoculation (none/*R. irregularis*). Again, simple linear regression modelling was used to explore relationship between different variables (e.g., shoot P concentration and soil P concentration).

All data was analysed with the software R in the version 3.5.0, using the package 'agricolae' 1.2 (CRAN, 2018) for non-parametric Kruskal-Wallis analysis with Fisher's Least Significant Difference as post hoc test. Principal component analysis was performed using the function 'prcomp' and 'lm' was used for the coefficient of determination R^2.

3. Results

3.1. Site characterization

The sites included in this study (Table 1), were on average approximately 0.1 ha in size, but ranged from 210 to 15,000 m^2. Whereas at nine of the sites production was predominantly conducted in raised beds using introduced soil or potting mix, at two of the sites it was in beds formed from the natural soil and supplemented with self-made or externally sourced compost. The remaining site grew crops in the natural soil without any organic amendments. Across all sites, an average of 35% of the available area was dedicated to production (as garden beds, chickens, beekeeping and fruit trees), and the remainder was used for pathways, storage facilities (e.g. sheds), and other non-production oriented activities. There was an average of 29 beds at each of the 10 community garden sites, which was similar to the average number of gardeners (23) at each of these sites. At the two commercial sites, production was set up in rows rather than beds. While the community gardens provided a mix of activities ranging from food production to social inclusion and educational activities, the two commercial enterprises focused solely on food production. Compost was produced and used at all but two of the sites (one commercial and one community garden). Further nutrients were imported, typically in the form of commercially available municipal green-waste compost and/or animal (predominantly horse) manure. Most sites were located between residential allotments and often in close proximity to park lands or other nature reserves (see Table 1).

All sites together had a total plant species richness of 73 species in the production areas surveyed, and at the individual site level, ranging from one to 21 species (Table 1). On the bed level, species richness ranged from one to twelve species. The most abundant crops were varieties of onions, lettuce, cabbage, broad beans and carrots, all of which are typical winter crops grown in South Australia. Plant richness and biodiversity (Shannon-Index) varied greatly between the sites and in some cases beds only contained one plant species. The Shannon-Index was used as a biodiversity index which accounts for both species abundance and evenness (Tuomisto, 2010). However, most garden beds showed a high plant species richness with different crops grown in close proximity. This likely reflects the fact that beds typically service the needs of an individual grower.

Table 1
Descriptive information about sites included in this study and their correlating plant biodiversity.

Site	1	2	3	4	5	6	7	8	9	10	11	12
Size (m^2)	680	210	880	15,000	2100	710	700	1000	2400	600	300	1100
Year established	2011	2010	2005	1907	1992	2003	2014	2010	1994	2016	2012	2011
Previous land use	Plant nursery	Parkland	School yard	Paddock	Factory	Tennis court	Car park	Parkland	Paddock	Junkyard	Vacant lot (former blacksmith)	Bowling area
Surrounding land use	Residential allotment and park	Park	Residential allotment	Residential allotment and park	Residential allotment	Residential allotment and community center	CBD	Residential allotment and park	Residential allotment and park	Park	Residential allotment	Residential allotment
Number of gardeners	30	20	35	3	25	20	10	35	30	6	2	45
Use of raised beds	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes
On-Site composting	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Livestock	No	No	No	No	Bees and Chickens	No	Chickens	No	No	No	No	No
Plant richness per site	14	16	13	1	12	19	13	18	21	4	7	11
Plant richness per bed mean (median)	6.75 (6.5)	6.25 (6.5)	4 (3.5)	1 (1)	5 (5)	5.75 (7)	4 (4)	6.75 (7)	6.25 (6.5)	2 (2)	2.25 (2.5)	3.25 (2.5)
Dominant species	Cabbage	Beans	Onions	Vine	Beans	Garlic	Parsley	Onions	Onions	Onions	Onions	Onions
Shannon-Index H	9.1	11.1	9.7	2.0	8.4	14.9	10.6	13.7	15.0	2.0	6.2	4.3

3.2. Potentially toxic metals

In an attempt to identify potential contamination of these urban soils (referred to as garden beds) and in the underlying soil (sampled from between the beds, using soil layers 0–10 and 10–30 cm, referred to as 'underlying soil 10' and 'underlying soil 30'), soil elemental concentrations were compared to *National Environmental Protection Measure Health Investigation "A" Guideline Levels* (NEPM-HIL) as stated by NEPM (1999) (Table 2). Across all sites, the concentrations of As, Cu, Cd, Mn, Ni, Pb and Zn were well below the NEPM-HIL A guideline levels, indicating that minimal risks to human health are posed by the soil either in the beds or the underlying soils (see Table 2). Importantly, for As and Cd, concentrations were below detection limits (0.028 mg kg^{-1}) in the majority of samples and were therefore omitted from statistical analysis.

One of the motivations for undertaking production in raised beds was a perceived risk that there may be contamination in soil at the site(s), as a legacy of previous land use (e.g. industrial or unknown) at the site. To explore this concern, the results were compared for concentrations of Cu, Mn, Ni, Pb and Zn between sample locations (garden beds, underlying soil 0–10 and 10–30 cm) using the sites as replicates (Fig. 1). Whereas this analysis revealed significantly higher concentrations of Ni in the underlying 10–30 cm soil layer than in the garden beds ($p = 0.04$), there were no significant differences between the sampling locations for Cu, Mn, Pb and Zn. Variability within sites was high with a number of outliers identified (see Supp. Fig. 3).

Sites were further classified on the basis of their prior land use; industrial ($n = 3$) or non-industrial ($n = 9$) (Fig. 1). When comparing metals in the garden beds at sites with industrial versus non-industrial land use histories, there were no significant differences detected. However, for the underlying soil layers (0–10 and 10–30 cm, respectively), there were significant differences for Cu, Ni, Pb and Zn, with the industrial sites having higher concentrations than the non-industrial ones (see Fig. 1 and Supp. Fig. 3).

3.3. Phosphorus and nitrogen

Concentrations of plant-available (Colwell) P in the garden beds ranged from 36 to 1265 mg kg^{-1} soil and showed high variability within and between sites. Most of the garden beds contained relatively high concentrations of plant-available (Colwell) P (median = 442.5 mg kg^{-1} soil), exceeding the critical concentration of plant-available (Colwell) P for most horticultural crops (e.g. lettuce = 115 mg kg^{-1} soil, Hartemink (2000)) (see Fig. 2A). Only sites 4 and 10 differed significantly from all other sites, with these beds having significantly lower concentrations of plant-available (Colwell) P than at all other sites. Concentrations of total P in the soil were also measured and were significantly higher in the garden beds than in the underlying soil layers (0–10 and 10–30 cm) (Supp. Fig. 4). A regression analysis between concentrations of total P and plant-available (Colwell) P in the garden beds resulted in a positive, albeit moderate, correlation ($R^2 = 0.43$).

Total nitrogen (N) in the soil collected from the garden beds was generally high (median = 0.7%). Mineral N in the garden beds was comprised from an approximate equimolar ratio of ammonium and nitrate (median = 6.2 mg kg^{-1} and 6.1 mg kg^{-1} soil, respectively), and did not differ significantly between sites (Table 3 and Fig. 2B). However, variability within sites was high; for example, at site 5 mineral N ranged from 7.6 to 26.5 mg kg^{-1} soil. Total N in the underlying soil (0–10 cm) was lower than in the beds (median = 0.4%). Mineral N in the underlying soil was dominated by ammonium rather than nitrate (median = 4.3 mg NH_4-N kg^{-1} soil and 0.6 mg NO_3-N kg^{-1} soil).

Principal component analysis (PCA) revealed that sites 4, 5 and 11 had distinct physico-chemical soil characteristics (see Supp. Fig. 5A), while the 95% confidence limits of the remaining sites overlapped and were thus more closely related to each other. The variation within sites was often small, such as for sites 7, 10 and 12. The first two principal components explain about 56% of the variance of the data set.

3.4. Bioassay

Of the 90 plants included in the bioassay, 13 died within the first 14 days after transplantation, with symptoms of tomato stem rot evident on those seedlings. One seedling was omitted from further analysis due to a mutated growth phenotype. Of the 76 remaining plants, 27 were inoculated with the AMF *R. irregularis*.

Plants growing in the indigenous soil without the *R. irregularis* treatment showed a mycorrhizal root colonization between 3 and 56%. Inoculation with *R. irregularis* increased average colonization significantly from 26 to 31% (Fig. 3B). Altogether, 17 samples had increased colonization, three samples had a neutral response and seven were negatively affected. This change in root colonization was highly variable between samples collected from beds within a given site. For example, two separate beds within site 4 showed the greatest increase (4/B1) and decrease (4/B3) in mycorrhizal colonization with inoculation with *R. irregularis* (Fig. 3A).

Correlation between plant-available (Colwell) P and mycorrhizal root colonization was low ($R^2 = 0.08$), and some samples with high concentrations of plant-available (Colwell) P showed a strong increase in mycorrhizal root colonization with inoculation (e.g., samples 2/B4 or 6/B4). The abundance of AMF spores in the tested subsample ranges from 3 to 44 spores g^{-1} dry soil with a mean of 11 spores (Fig. 3A).

Shoot biomass varied greatly between samples, similar to the measured variability of soil mineral N and plant-available (Colwell) P. However, shoot biomass was significantly lower in the *R. irregularis* inoculated plants (mean = 0.8 mg kg^{-1}), than in the non-inoculated control (mean = 1.0 mg kg^{-1}) (Fig. 3C).

Table 2
Summary description of soil metal concentrations of all collected samples (beds and natural soil).

Summary statistic	As [mg kg^{-1}]	Cd [mg kg^{-1}]	Cu [mg kg^{-1}]	Mn [mg kg^{-1}]	Ni [mg kg^{-1}]	Pb [mg kg^{-1}]	Zn [mg kg^{-1}]
Sample size (n =)	1	9	133	133	123	133	133
Below detection limit/NAs	132	124	–	–	10	–	–
Detection limit	0.028	0.012	0.1	0.1	0.028	0.1	0.1
Minimum	0.6	0.01	0.3	0.1	0.5	0.1	0.6
Median	0.6	0.08	25.2	168.7	7.8	30	103.1
Mean	0.6	0.13	32.6	213.1	8.6	45.8	114.9
Max	0.6	0.38	183.4	750.1	32.6	267.7	661.7
SD	–	0.1	29.3	134.3	4.6	46.9	91.5
HIL-A Guidelines (NEPM)	100	20	6000	3800	400	300	7400

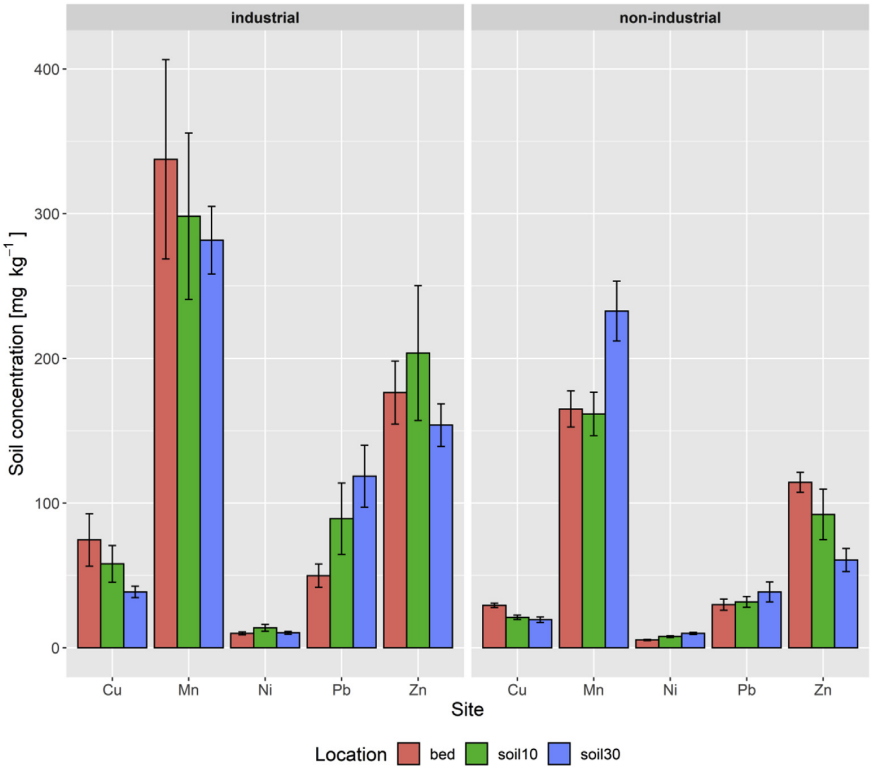

Fig. 1. Soil concentrations of tested heavy metals between sites with industrial and non-industrial history and the different sampling locations 'garden bed' (red bar), 'underlying soil 0–10 cm' (green bar) and 'underlying soil 10–30 cm' (blue bar). Values are mean ± SE, N = 133. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Shoot P concentrations were significantly higher in the *R. irregularis* treatment (mean = 4.0 mg kg^{-1}) than in the non-inoculated treatment (mean = 3.3 mg kg^{-1}). Conversely, concentrations of Fe were lower in the *R. irregularis* treatment (mean = 0.05 mg kg^{-1}) than in the non-inoculated control treatment (mean = 0.07 mg kg^{-1}). There were no significant differences for Zn (Supp. Fig. 6). Regression analysis between concentrations of P, Mn and Zn in the plant tissue and soil resulted in $R^2 < 0.01$ for P and Zn and $R^2 = 0.55$ for Mn.

The PCA showed that shoot biomass was most closely correlated to soil total N, total P, Colwell P, and total C (see Supp. Fig. 5B). Strong negative correlations were found between shoot biomass and mycorrhizal root colonization and, to a lesser degree, soil nitrate.

4. Discussion

The sites included in this urban agriculture study ranged in size, number of participants, and their focus (commercial and community gardens). The nature of most sites was relatively uniform with plants being grown in raised beds with relatively high plant biodiversity compared to conventional agriculture systems. While concentrations of potentially toxic metals in soils were well below guideline levels, they were higher on sites with a history of industrial land use. Whereas systems had relative low levels of mineral N and adequate levels of total N, plant-available (Colwell) P was very high. Collected soils were abundant in AMF spores and a greenhouse bioassay showed high mycorrhizal root colonization, even in soils with high P concentration. Following, these results are discussed in the context of soil health and safety as well as their significance towards sustainable and clean food production.

4.1. Site characterization

There were two broad types of sites identified in this study: community gardens and commercial sites. Both types differed in their configuration, farming methods and plant biodiversity. All community gardens showed a strong multifunctional character by combining mainly social and ecological functions. As such, they allocated more space to non-production areas and wheelchair accessible pathways to allow social gatherings for the community. Food production in most community gardens took place in raised beds, while both commercial sites were growing plants in the natural soil. The decision to use raised beds and imported soil was in many cases due to perceived concerns around potential soil contamination and was in some cases mandated by local government. In general, plant biodiversity in the community garden was higher than in the commercial sites and included many ornamental plants and perennials such as *Rosmarinus officinalis* or *Physalis peruviana*. The higher diversity of crops grown in the community gardens is likely due to using the garden as a kitchen garden, whereas the commercial sites put an emphasis on producing saleable amounts of product. Those results suggest that especially the community gardens present a big potential for urban biodiversity conversation and provide important ecosystem functions (Goddard et al., 2010). The sustainable character of the commercial

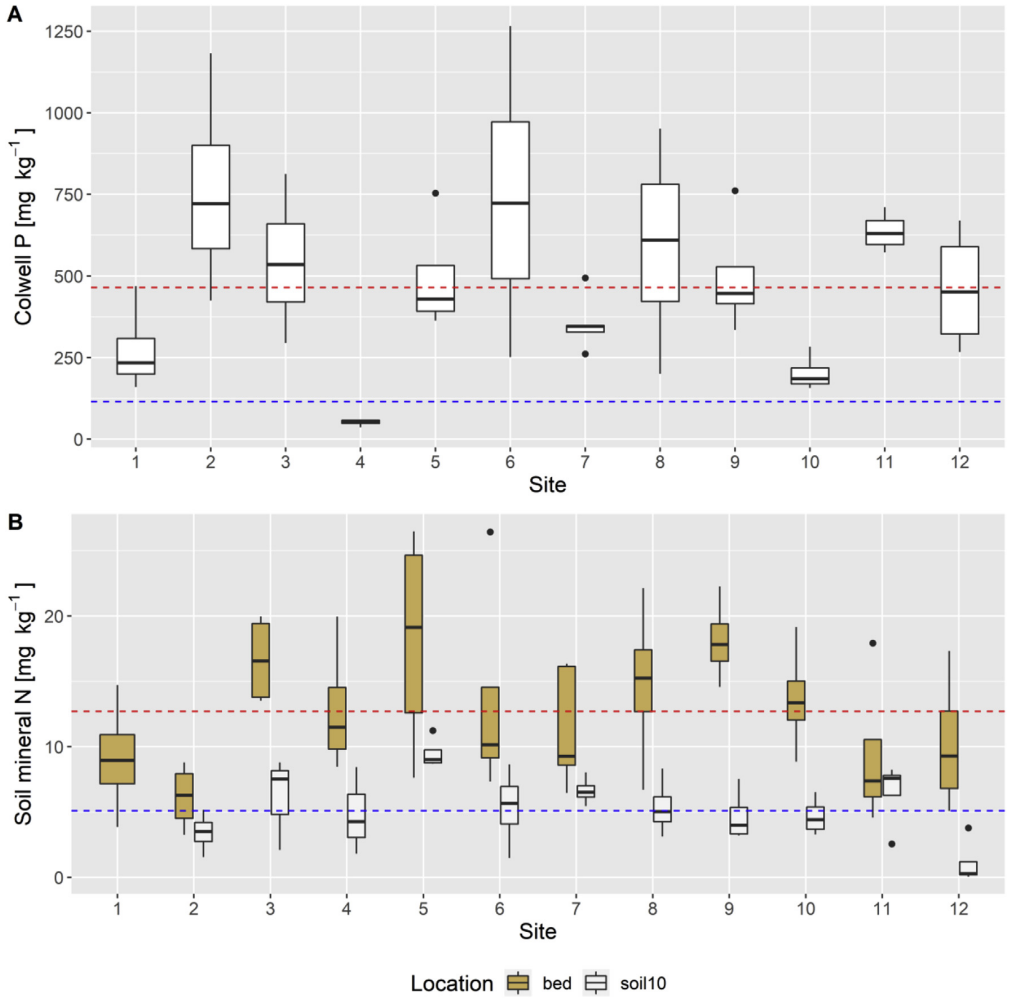

Fig. 2. 2A: Plant-available (Colwell) P concentration of garden beds over all tested sites. Dashed lines indicating critical Colwell P of 115 mg kg^{-1} for lettuce (blue) as a reference and the median of all samples (red). 2B: Mineral-N concentrations of garden beds (brown) and underlying soil (white) over the tested sites. Dashed lines indicating median for location 'garden bed' (bed) (red) 'underlying soil 0–10 cm' (soil10) (blue). N = 80. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sites lies mainly within their focus on food production, combined with their proximity to the consumers and short transportation routes. Although not part of this study, it is likely that food produce of both commercial sites is associated with less greenhouse gas emissions than conventionally produced food (Lee et al., 2015). Both the community gardens and the commercial sites made efficient use of valuable urban space in a densely populated area. Their actual configuration is a reflection of their surroundings and the needs of the local residents and they all followed a strong multifunctional character (Lovell, 2010). This multifunctionality allows all sites to mitigate various challenges that arise from expanding cities (Mahendra and Seto, 2019).

4.2. Potentially toxic metals

One of the main reasons for the use of raised beds in urban environments are concerns over possible soil contamination. Previous studies showed that those concerns are justified and concentrations of metals in urban agriculture soils (Mitchell et al., 2014) and products (Sung and Park, 2018) can exceed regulated guideline concentrations. Anthropogenic input of metals into the soil occurs through various mechanisms such as atmospheric deposition, runoff from metal surfaces, bonfires, burial of metal-containing waste, pesticides, or fertilizers (Alloway, 2004). All samples in this study were below the NEPM HIL-A guidelines for the tested metals, however, sites with industrial historical land use had significant higher concentrations of Cu, Ni, Pb and Zn in the underlying soil layer than sites with non-industrial history. In contrast, there was no significant difference in concentrations of metals in soils from gardening beds when sites with and without industrial land use histories were compared. The use of raised beds with introduced soils appears to have been an effective way to safely (from a metal perspective) undertake food production in sites with industrial histories. Although it is unlikely for developed countries to undertake any form of food production in areas with known soil contamination, raised beds represent one option to help

Table 3
Summary description of Colwell P, total P, Ammonium, Nitrate, total C, total N, C/N, pH and EC of all collected samples (beds and underlying soil).

	Location	Sample size	Min	Median [mg kg^{-1}]	Mean [mg kg^{-1}]	Max [mg kg^{-1}]
Colwell P [mg kg^{-1}]	Garden bed	49	36.3	442.6	465.3	1265.7
Total P [mg kg^{-1}]	Garden bed	50	338.8	1866.0	2296.0	6490.0
	Natural soil 0-10	48	0.6	665.3	852.5	4859.0
	Natural soil 10-30	35	191.1	372.8	561.9	1440.0
Ammonium [mg kg^{-1}]	Garden beds	49	1.2	6.2	6.2	15.8
	Natural soil 0-10	41	0.1	4.3	4.4	9.6
Nitrate [mg kg^{-1}]	Garden beds	49	0.2	6.1	9.1	16.2
	Natural soil 0-10	42	0.1	0.6	1.0	4.6
Total C [%]	Garden beds	49	1.5	7.3	7.6	16
	Natural soil 0-10	49	0.2	5.5	7.5	29
Total N [%]	Garden beds	49	0.03	0.7	0.7	1.3
	Natural soil 0-10	49	0.03	0.4	0.5	1.8
C/N	Garden beds	49	8.3	10.9	12.8	50
	Natural soil 0-10	49	1.5	14.5	16.5	130
pH	Garden bed	50	6.4	7.1	7.1	7.9
	Natural soil 0-10	42	6.5	6.9	7.0	7.8
	Natural soil 10-30	35	6.3	7.1	7.1	7.9
EC [mS]	Garden bed	50	75	341	402	2057
	Natural soil 0-10	43	29	261	288	1234
	Natural soil 10-30	35	31	154	156	528

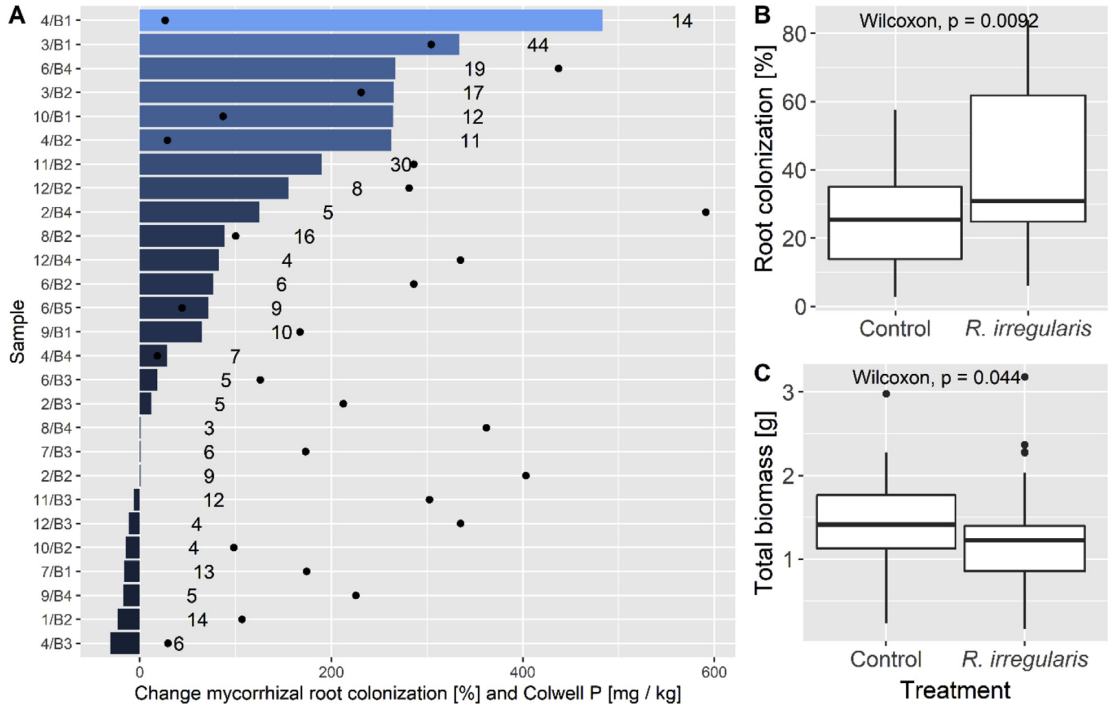

Fig. 3. 3A: Change of mycorrhizal root colonization after addition of R. irregularis in percentage, number of spores present per gram of dried indigenous soil (numbers) and corresponding plant-available Colwell P (dots). 3B: Mycorrhizal root colonization between indigenous soil (Control) and treatment with R. irregularis. 3C: Shoot biomass between non-inoculated soil (Control) and R. irregularis treatment.

ensure a safe and secure food supply system, in countries facing food shortages (Kessler, 2013).

Concentrations of Zn in site 11 (mean = 258 mg kg^{-1}) were well above the typical levels of about 57–100 mg kg^{-1} in organically managed soils (Noulas et al., 2018). This finding might not only be caused by its industrial history, but also the use of Zn-based pesticides or the application of municipal composts (Heiger-Bernays et al., 2009). While speculative, this highlights the need to consider potential introduction of heavy metals, and indeed other contaminants, with external inputs. These levels of Zn are of interest from an agricultural perspective but are still within the critical guideline levels by a factor of 28. Although the re-use of urban waste products comes with certain reservations, it did not negatively affect the sites included in this study (from a metal perspective). On the contrary, it is likely that the use of organic amendments from urban waste streams saved a substantial amount of energy due to the omission of mineral fertilizer (Favoino and Hogg, 2008), however, that was not a focus in this study.

4.3. Phosphorus and nitrogen

With the exception of two sites, plant-available (Colwell) P in the soil collected from the garden beds was very high, and well in excess of required levels for horticultural production (Hartemink, 2000). High levels of plant available P in these soils is likely a reflection of easily accessible nutrient sources that are high in P, such as horse manure (Airaksinen et al., 2001), coupled with the highly immobile nature of P in the soil (Hartemink, 2000). Similar results were found in various urban agriculture projects in Portugal (Arrobas et al., 2017) and the Netherlands (Wielemaker et al., 2019), where the nutrient inputs would even exceed the fertilizer application limits of conventional farming. Nitrogen analysis of the collected garden beds revealed similar and low concentrations of ammonium and nitrate. However, most plant N is derived (following mineralization) from organic forms in the soil which is also represented in the total N analysis. Concentrations of total N in the soil from the garden beds ranged from 0.03% to 1.3% with a median of 0.7%. When comparing those values against the critical concentrations for wheat (0.1%) (Hartemink, 2000), most garden bed soils can be considered adequately supplied with N. This divergence between high amounts of total N and low amount of mineral N might be caused by the highly dynamic cycling of N in soils which is affected by many environmental factors (Hartemink, 2000). All things considered, nutrient management in urban agriculture systems is characterised by an over-supply of urban waste products which leads to excess or imbalanced soil nutrient concentrations. Such imbalances between nutrient inputs and outputs should be closely monitored to avoid build-up in the soil. Excess nutrients may pose a risk due to run-off or can interfere with the uptake of other plant nutrients (Fageria, 2001). However, the use of mainly organic urban waste products also resulted in high total N concentrations which is a significant parameter for good soil health (Hartemink, 2000). One solution to counteract excess or imbalanced nutrients in the context of urban agriculture is to either reduce nutrient inputs or to use a blend of different organic materials with different nutrient profiles. For example, after communicating the issue of high P concentrations to participants of the study, one community garden incorporated spent coffee ground as nutrient source which has a broad N:P ratio of about 30:1 (Liu and Price, 2011). Other common composting materials with high N:P ratios are straw (N:P = 8:1) or wood chips (N:P = 7:1) (Wurff et al., 2016). The results of the PCA revealed that all sites which used raised beds with introduced soils shared a close relationship. This indicates that most soils and composts originate from a similar source, probably due to its easy accessibility. However, most developed cities provide a variety of freely available organic materials with different nutrient profiles. In order to use this resource in a sustainable way, it is necessary for gardeners to familiarize themselves with the principles of balanced nutrient management.

4.4. Bioassay

Mycorrhizal fungi were present in all soils collected in this survey. On average, 11 AMF spores g^{-1} dry soil were present in the samples that were used in the bioassay. Such spore abundance is similar to organic agriculture soils where up to 14 AMF spores g^{-1} soil were found (Oehl et al., 2004). The true mycorrhiza potential of the soil samples is probably still higher, as root pieces or extraradical hyphae in the soils act as another inoculum source but were not measured in this study. The mycorrhizal potential is also reflected by the high percentage root colonization of plants without *R. irregularis* inoculation. The inoculation with *R. irregularis* suggested that most soils have higher mycorrhizal potential and can support higher root colonization. In that way, the addition of *R. irregularis* further bolstered the mycorrhizal root colonization for most samples which might be explained by the fast-growing nature of this AMF species (Malbreil et al., 2014). Interestingly, site 4 showed a high variability in its response to inoculation with *R. irregularis* as those samples showed either a positive, neutral or negative response. This response to inoculation cannot be explained within the methodology of this study and might be linked to other microbial processes that impact mycorrhizal growth (Miransari, 2011). Such a spatial variability of soil microorganisms has been reported previously by Štursová et al. (2016). To this date it is not possible to compare the AMF spore numbers of this study with other urban agriculture sites, as no such data are available.

Given the ample supply of plant nutrients at most sites, it is surprising to find such an abundance of AMF in the soil. Most scientific literature even described an inhibition of mycorrhizal development at high levels of soil P. The results of this study might suggest that nutrient uptake is not the major driver behind mycorrhizal symbiosis in urban agriculture soils, or, is at least redundant from a nutrient perspective. Still, shoot P concentrations in the bioassay were higher in the *R. irregularis* treatment than non-inoculated and significantly exceeded values reported by Watts-Williams and Cavagnaro (2012). This discrepancy between the studies is likely due to the far higher soil P concentration in the urban agriculture soils. Plant shoot weight was decreased in the *R. irregularis* treatment which is commonly found in plants with high mycorrhizal root colonization (Johnson et al., 1997) when compared to non-mycorrhizal control plants. However, it is important to consider that the importance of AMF in ecosystems should not be questioned over the decrease of shoot and root dry weights with higher root colonization. As mentioned by Rillig et al. (2019), AMF provide a broad range of services that positively affect sustainability in food production.

Similarly, the natural establishment of AMF in urban agriculture soils is likely because they provide important ecosystem functions besides nutrient uptake, such as disease resistance or improved soil structure. Direct inoculation of AMF was not practised at any of the sites, suggesting that common management practices of urban agriculture, such as high plant biodiversity and principles of organic farming, lead to high levels of AMF propagules. If needed, most soils could sustain even higher levels of mycorrhizal root colonization after inoculation with a fast-growing mycorrhizal species such as *R. irregularis*. Those result suggest that, although urban agriculture soils are prone to excess soil nutrient concentrations, they are managed sustainably from a microbial perspective. Direct inoculation of urban agriculture soils with mycorrhizal inoculum is not necessary and common urban agriculture practices are naturally selecting for an abundant mycorrhizal assemblages (Verbruggen and Toby Kiers, 2010). Although not addressed in this study, it is likely that this process provides a variety of functions to the host plants and soils that goes beyond the uptake of plant nutrients. If gardeners seek to accelerate the establishment of mycorrhizal communities, it is possible to use small amounts of soil from an established garden bed as inoculum source for new garden plots. If need be, inoculum could even be produced on-site using a variety of organic substrates (Douds et al., 2010). Those options provide low-cost and sustainable alternatives to commercial mycorrhizal inoculants that have been shown to be of variable quality (Salomon et al., unpublished).

4.5. Urban soil health framework

Healthy soils are the foundation of urban green spaces, regardless of whether those spaces are intended for leisure or food production. As such, protecting urban soils from anthropogenic influences and improving soils wherever possible should be a

priority in every urban planning framework. The following preliminary framework outlines the main steps involved in managing urban soil health based on the results of this study and with an emphasis on urban agriculture and urban green spaces (see Supp. Fig. 7).

The basis of this framework is to minimize the impacts of anthropogenic activities on urban soils, for example through environmental policies (De Kimpe and Morel, 2000). Future urban development is then classified as "hazardous" or "safe" depending on the expected effect on the surrounding soil. Hazardous activities are such that are likely to result in adverse soil properties that can only be fixed at high cost (e.g. organic soil pollutants or potentially toxic metals). Activities that only have limited effects on soil health or effects that can be overcome in the context of urban green spaces are considered "safe". Most community gardens in this study were operating on "safe" zones, where soil compaction due to previous urban development was an issue that could be overcome by using raised beds.

The soil health of the urban surrounding is mapped according to the two categories (De Kimpe and Morel, 2000). Where soil contamination is of no concern, urban green spaces are encouraged, for example, through the use of government initiatives such as increased funding (van den Nouwelant et al., 2015), and environmental policies which support community gardens and other environmentally focused communities (Middle et al., 2014). Hazardous areas that are conveniently located for green space development are prioritized for remediation efforts (Yao et al., 2012). Hazardous areas that are unattractive for green spaces are used for clustering activities that are hazardous to soil health. Where urban soils need to be improved, for example in the context of urban agriculture, municipal compost is made available. Such compost blends should be nutrient-balanced and free of contaminants. The composts would ideally be based on a variety of high-quality urban waste streams which are collected city-wide to close the nutrient-loop and increase the city's self-sustainability (Farrell and Jones, 2009).

"The 30-Year Plan for Greater Adelaide" is a critical component of the planning strategy for South Australia, established by the Development Act 1993 (Government of South Australia, 2010). The 30-Year Plan identifies specific goals which are consistent with the goals of this proposed framework, such as increasing the liveability of Adelaide by planting 20 million trees by 2020 and transforming Adelaide into a "green liveable city". However, the importance of healthy urban soil is only briefly mentioned in the Plan and is not bolstered by any specific strategies to achieve this goal. This framework could be implemented as an addition to the Plan to solve this deficit and ultimately improve the overall soil quality of Adelaide for future generations.

5. Recommendations and conclusions

The urban agriculture sites in this study provided multiple benefits towards the local community which included social services, eco-biodiversity, food production and recycling of urban waste streams. All sites showed strong multi-functional characteristics that allowed for efficient space use in a densely populated area. All soil samples were within the national guidelines for concentrations of potentially toxic metals, although higher concentrations were observed in industrially affected soils than in non-industrial soil. The use of raised beds and introduced soil was a successful method to offset those differences caused by the previous industrial legacy. Soils that were used for plant production had an adequate supply of N and very high levels of plant-available P, which mostly stemmed from freely accessible urban waste streams rather than mineral fertilization. One case example showed that organic amendments can be sourced from different urban waste streams with different nutritional values to avoid such imbalanced nutrient concentrations. Although most soils had imbalanced concentrations of plant nutrients, they were managed sustainably from a microbial perspective and contained a high abundance of mycorrhizal propagules. This naturally developed mycorrhizal assemblage is likely to provide important ecosystem functions in the context of urban agriculture. The findings of this study were incorporated into a preliminary framework for the management of urban soil health. This framework aims to facilitate the planning and implementation of urban green spaces by mapping the soil health of urban areas.

CRediT authorship contribution statement

M.J. Salomon: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **S.J. Watts-Williams:** Conceptualization, Validation, Writing - review & editing, Supervision. **M.J. McLaughlin:** Conceptualization, Validation, Resources, Writing - review & editing, Supervision. **T.R. Cavagnaro:** Conceptualization, Validation, Resources, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2020.122900.

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4.6 Supplementary material

FIGURE 4.4: *Representative picture of a sampled community garden. Production predominantly in raised beds and introduced soil as well as fruit trees. Garden is located in an urban settlement with private housing. Picture taken in September 2017.*

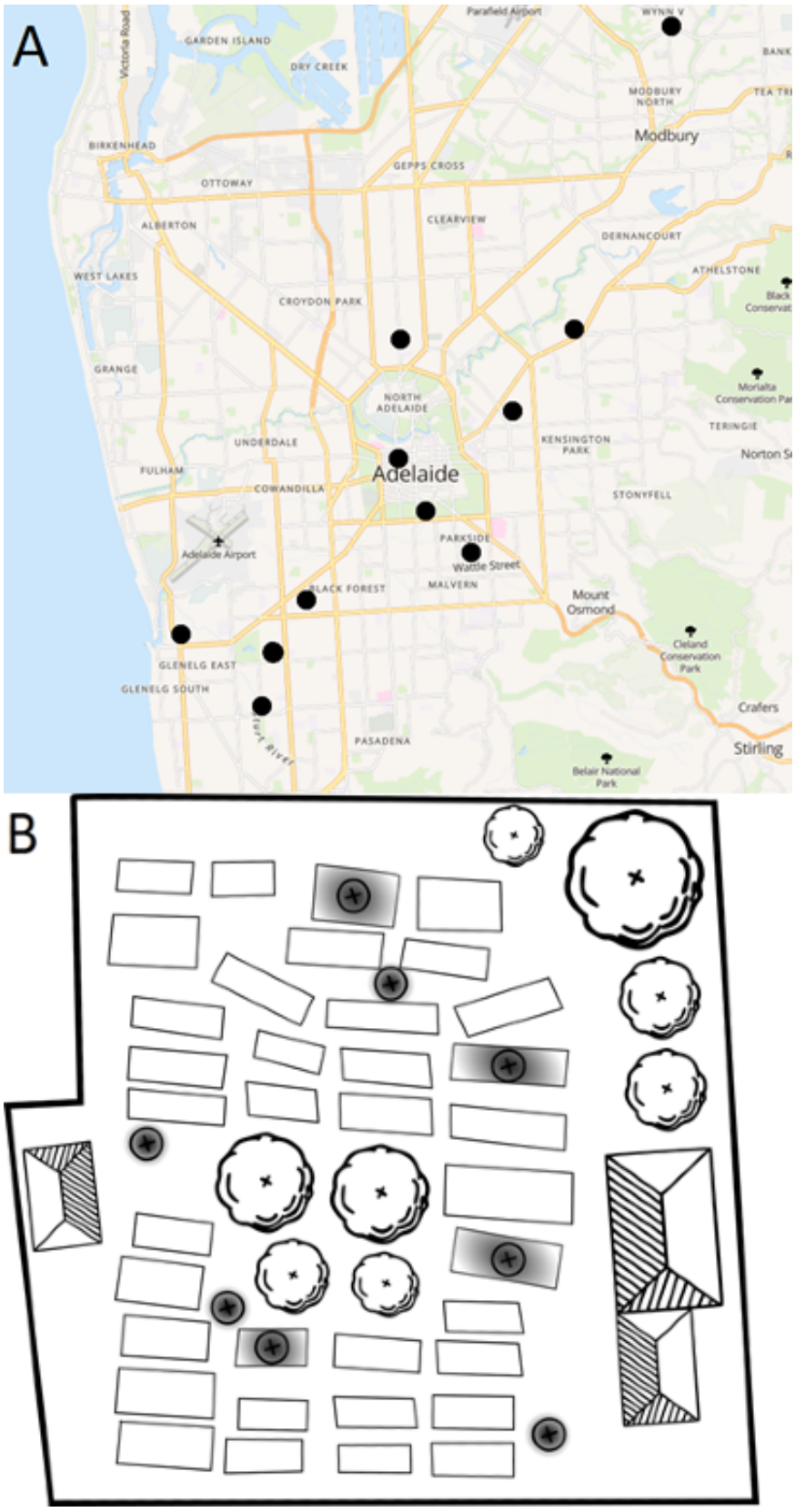
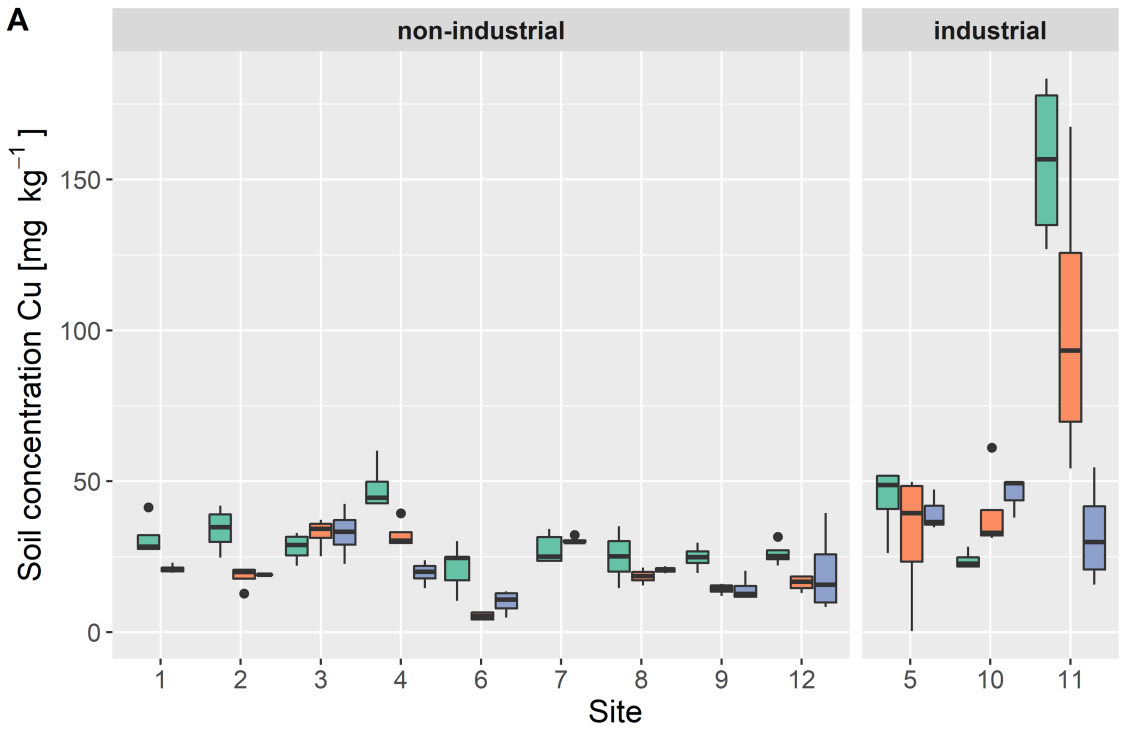
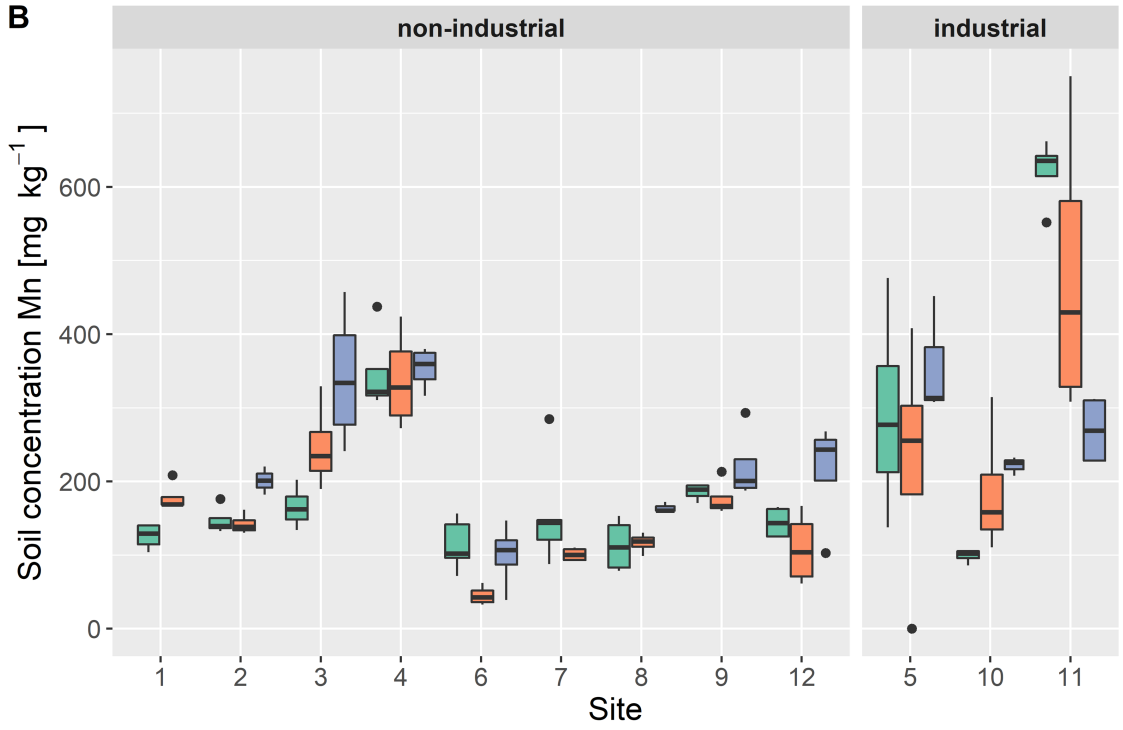


FIGURE 4.5: *A: Approximate location of the 11 (out of 12) surveyed sites that agreed to be acknowledged in this study. Map provided by © OpenStreetMap contributors. B: Sketch of a community garden, indicating raised beds (squares), buildings (shaded squares), trees and sampling locations (circled crosses with dark background).*


A
non-industrial
industrial
Soil concentration Cu [mg kg^{-1}]
150
100
50
0
1
2
3
4
6
7
8
9
12
5
10
11
Site


B
non-industrial
industrial
Soil concentration Mn [mg kg^{-1}]
600
400
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7
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5
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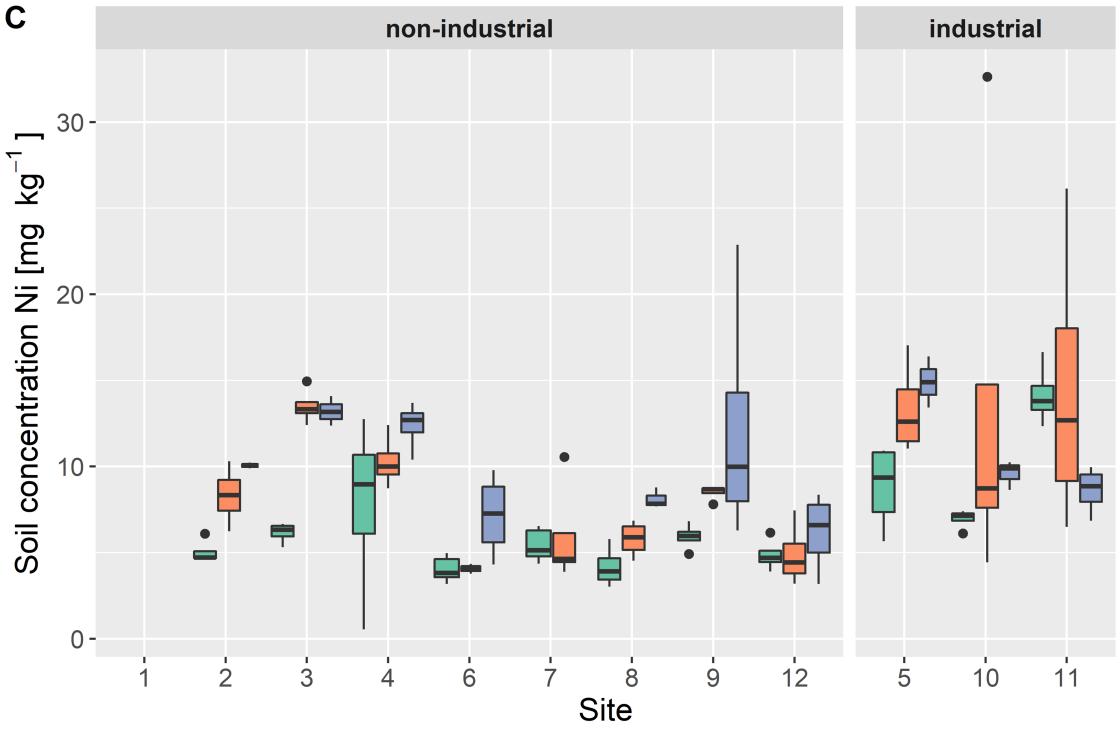
11
Site


C
non-industrial
industrial
Soil concentration Ni [mg kg^{-1}]
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10
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30
1
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7
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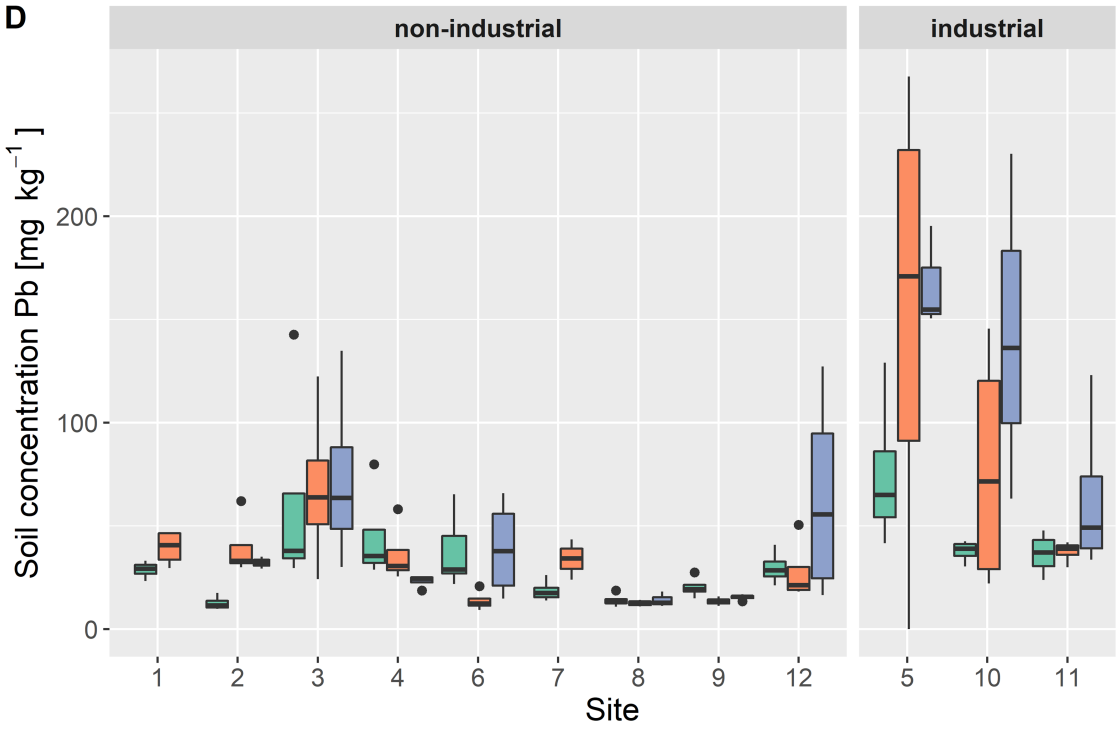
11
Site


D
non-industrial
industrial
Soil concentration Pb [mg kg^{-1}]
0
100
200
1
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6
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8
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12
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11
Site

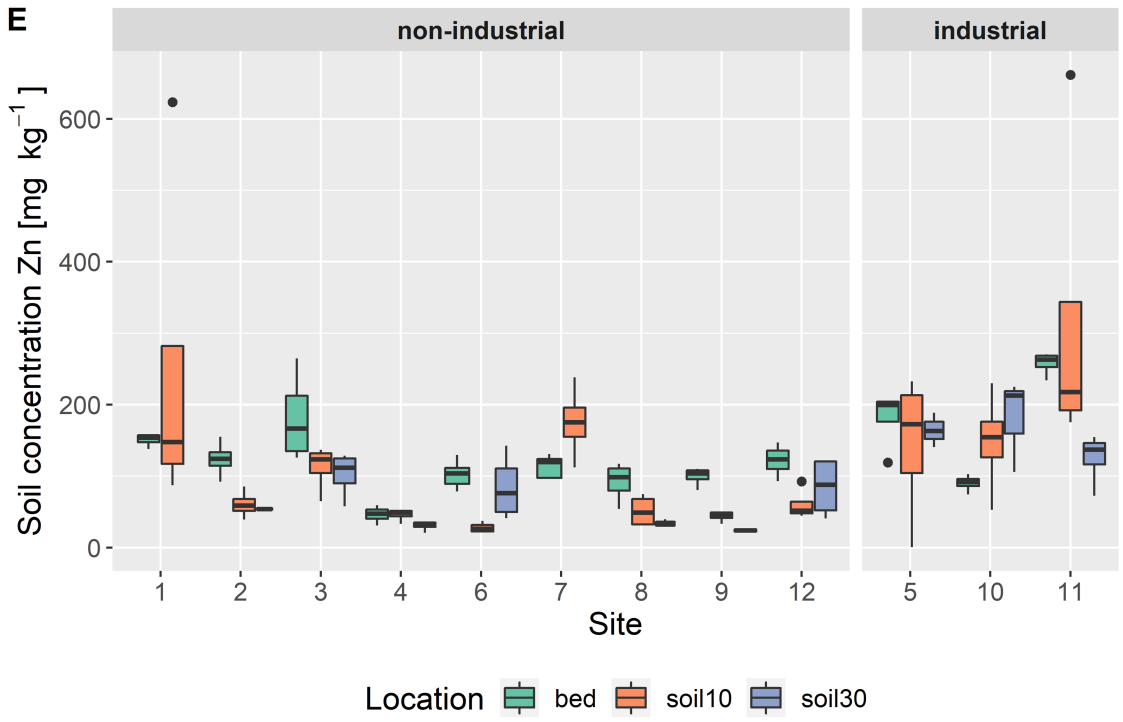

FIGURE 4.6: *Concentrations of Cu (A), Mn (B), Ni (C), Pb (D) and Zn (E) between sites with and without industrial history and the sampling locations bed (green bars), underlying soil 10 cm (orange bars) and 30 cm (blue bars). N = 133.*

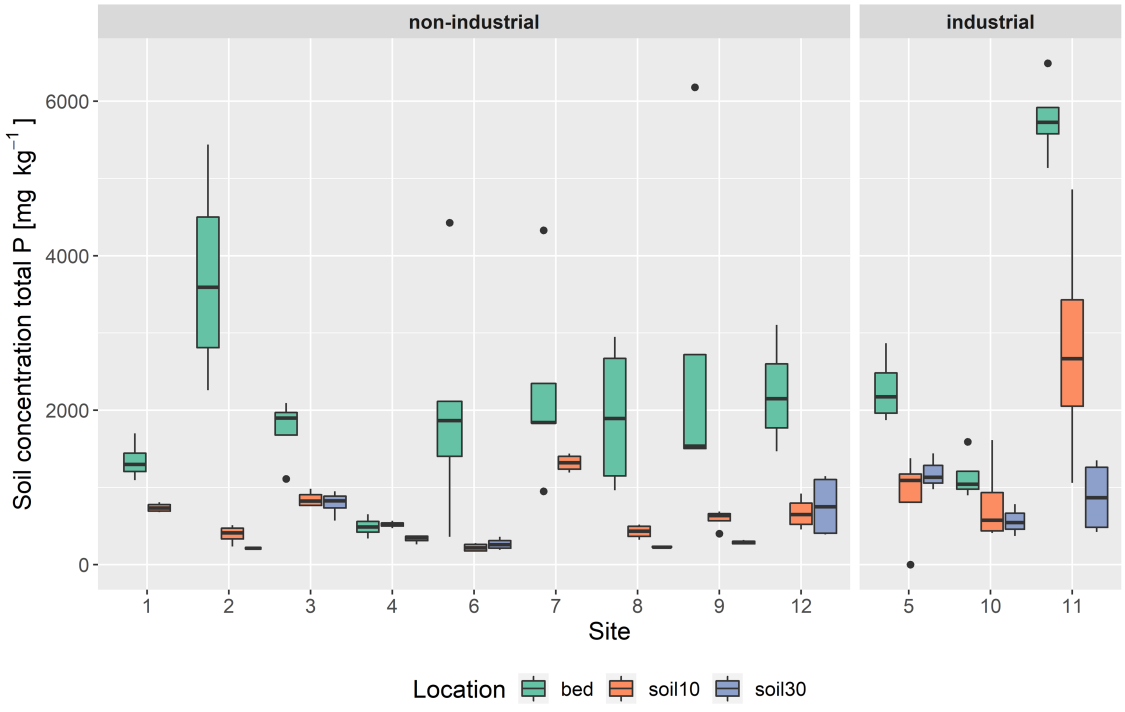

FIGURE 4.7: *Concentrations of soil total P between sites with and without industrial history and the sampling locations bed (green bars), underlying soil 10 cm (orange bars) and 30 cm (blue bars). N = 133.*

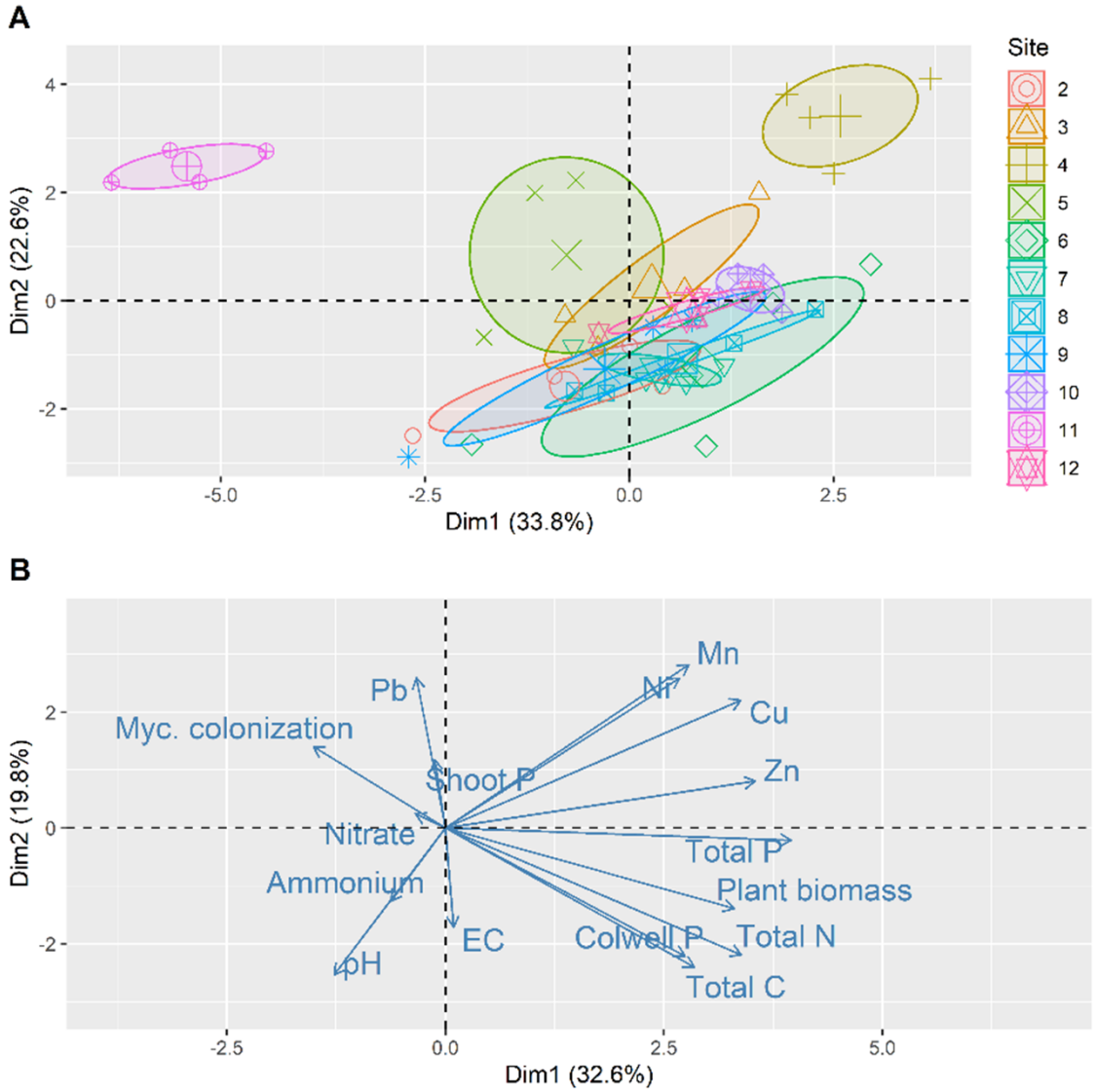

FIGURE 4.8: *A: Clustered Principal Component Analysis (PCA) of physico-chemical soil parameters of the analysed sites, circles indicating 95% confidence limits. B: Vectorized PCA of physico-chemical soil parameters.*

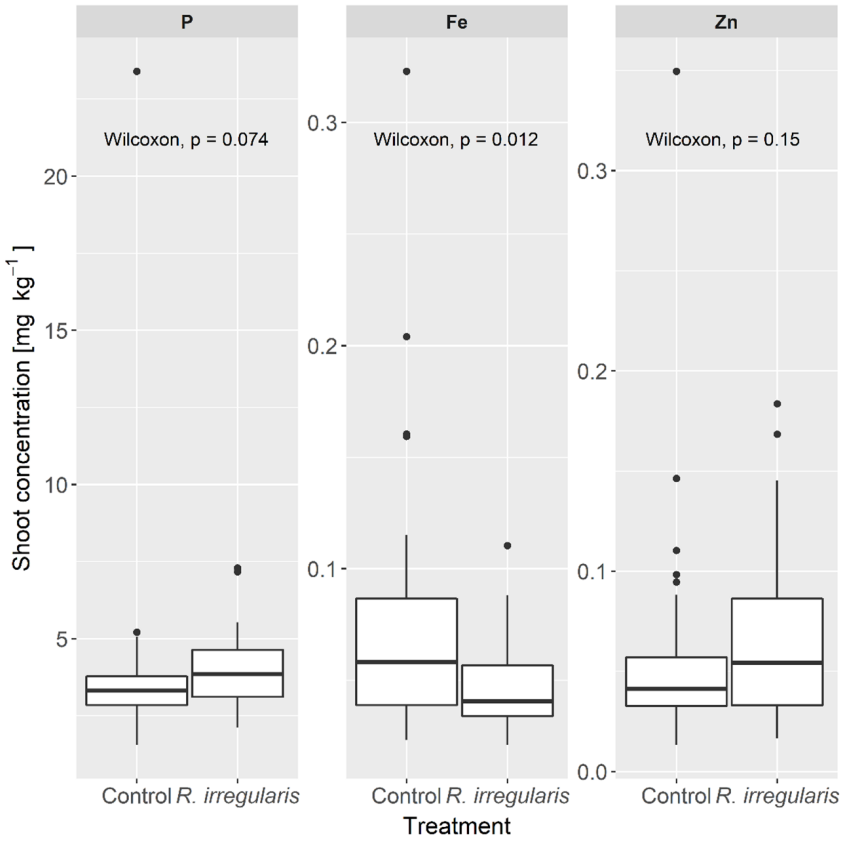

FIGURE 4.9: *Concentrations of shoot P, Fe and Zn between non-inoculated and inoculated (R. irregularis) plants of the greenhouse bioassay (tomato, Lycopersicon esculentum). N = 70.*

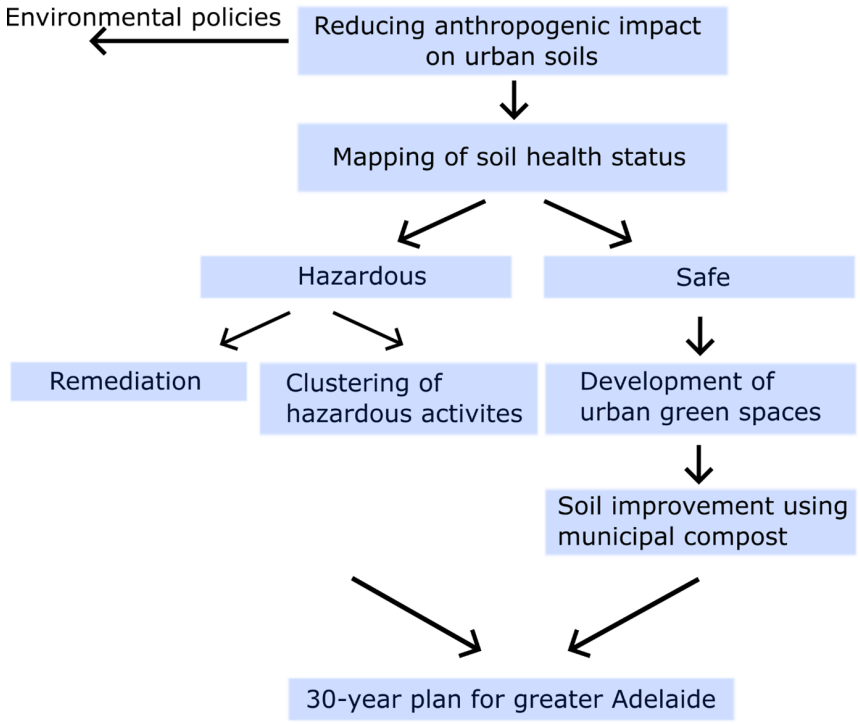

FIGURE 4.10: *Preliminary framework for urban soil health which builds on the 30-year plan for greater Adelaide.*

Chapter 5

Spatiotemporal dynamics of urban agriculture soil health

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Overall percentage (%)	90		
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ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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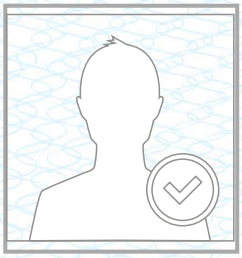

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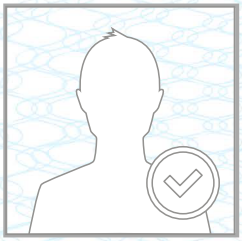

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Abstract

The Food and Agriculture Organization of the United Nations (FAO) has stated that "a greater focus on the Urban Food Agenda is long overdue". With half of the world's population now living in cities, a number projected to increase to 68% by 2050, Urban Agriculture has been flagged as a key strategy for achieving global food security. However, there is a paucity of data on the state of urban agriculture soils. In order to develop efficient management practices, it is necessary to understand the seasonal dynamics of the soil health of these systems. This study sampled two community gardens, and one commercial, urban agriculture site on a monthly basis over the span of one year. The dynamic analysis examined soil nutritional, chemical and microbial properties. Plant biodiversity was significantly higher in community gardens compared to commercial sites. Analysis of soil nutrients revealed fluctuations of mineral nitrogen with seasonal conditions and consistently high concentrations of plant-available phosphorus. We identified gradually decreasing soil total nitrogen and carbon concentrations throughout the year. Soils were abundant in arbuscular mycorrhizal fungi spores. Soil metabarcoding using 16S and ITS amplicons revealed a seasonal gradient of the microbial diversity and changes after the application of organic fertilizer. Soil-borne potential human pathogens were also detected in the soils. The results of this study give conclusions about soil management principles in urban agriculture systems with direct implications for increased sustainability in urban agriculture.

Significance statement

Urban areas are growing at unprecedented rates and are projected to continue to do so in the future. This development intensifies issues regarding global food security and available cropland. Urban agriculture is a promising method to increase urban sustainability and resilient communities through local food production. To optimize this highly adaptive food production system, it is necessary to analyse the seasonal dynamics of important soil health parameters in urban agriculture systems. This knowledge is essential to identify constraints that could reduce the efficiency and sustainability of these systems.

5.1 Introduction

Previous decades of demographic development were characterized by an emerging trend towards urban residency. Since the year 2007, more people reside in urban, rather than rural, areas. This shift towards urbanization is expected to increase in the coming decades, leading to a predicted 6.7 billion people that live in metropolitan areas and a further 3.1 billion people that live in rural areas by the year 2050 (Nations, 2019). In terms of land consumption, the extent of urban land is predicted to double between the years 2000 and

2030 (Seto et al., 2012). A significant area of cropland will be lost due to this urban expansion, thereby threatening global food security (Bren d'Amour et al., 2017). This development coincides with plateauing agricultural yields, which will not meet the global food demand for the year 2050 (Lobell et al., 2011; Ray et al., 2013). Innovative solutions are required to break this self-perpetuating cycle which is driven by increasing population growth and urbanization. As such, cities should not only be viewed as pivotal problems, but potential hotspots for sustainable environmental change (Grimm et al., 2008). One compelling method to increase food security and urban sustainability is urban agriculture.

Urban agriculture offers new frontiers for the efficient land use of urban spaces. It is a multifunctional approach that improves various constraints of urban expansion. Urban agriculture provides essential ecosystem services for urban biodiversity and improves food system sustainability, thereby contributing to food security and resilient communities. It is considered to improve the general wellbeing of citizens by providing social inclusion and therapeutic elements. People are more likely to improve their diet by connecting with the environment and the concepts of food production. With regards to climate change, urban green cover is of special importance to improve the surrounding microclimate. Urban agriculture also allows recycling of urban waste streams, for example in form of composts, thereby increasing resource use efficiency of cities (Orsini et al., 2020). Undoubtedly, the importance and efficiency of urban agriculture is demonstrated in most developing countries where urban expansion is always accompanied by local food production for means of food security and income (Lee-Smith, 2010). The global COVID-19 pandemic revealed the fragility of cities and the food supply chain, which fosters the Urban Food Agenda, an initiative to enhance food security in urban areas (FAO, 2019).

The implementation of urban agriculture practice manifests in various shapes and forms. Its most sophisticated variants use advanced technologies, such as vertical farming, to maximize efficiency of space use. Although enormous yield increases have been reported in these systems, they are currently not operating economically (Asseng et al., 2020). To date, soil-based urban agriculture systems are most commonly used all around the world in urban spaces such as vacant lots, parklands or roof-top gardens (Skar et al., 2019). The urban surroundings and the use of imported soils and organic amendments warrant a research focus on soil health in these systems. Previous research indicates that natural urban soils are often not suitable for plant production and need intensive improvement. Urban soils have often undergone alterations which result in soil compaction, reduced plant growth and low soil carbon contents (Beniston & Lal, 2012). However, urban agriculture soils are often amended with composts and organic fertilizer, leading to an over-abundance (Wielemaker et al., 2019) or imbalance (Arrobas et al., 2017) of plant nutrients (Salomon et al., 2020). These food production systems can achieve high yields, but require expertise to do so sustainably (McDougall et al., 2019). Studies

suggest that within low-density cities, residential lawns could be converted to gardening beds to become self-sufficient for their vegetable demand. The area of lawn that needs to be converted is highly dependant on the projected yields (Hume et al., 2021). Soil health is contributing significantly towards crop yields, however, information on the status of urban agricultural soils is lacking in many regards.

In order to reach the full potential of urban agriculture on its many social, economic and ecological levels, it is important to understand the status of the soil base upon which urban agriculture depends. Importantly, soil conditions can vary greatly between sites and individual gardeners (Salomon et al., 2020), but also over seasons. If urban agriculture is to achieve its potential, these dynamics must be better understood. This understanding allows knowledge-based decision making towards sustainable gardening practices and eliminates potential plant growth constraints. Previous literature on soil health in urban agriculture systems focused on potential soil contamination and also to a lesser degree on plant-nutrient availability.

Here we present results of a study in which we focused a broad range of soil health indicators and plant biodiversity in urban agriculture. We tracked the seasonal dynamic by undertaking repeated monthly samplings over the course of a whole year at two community gardens and one commercial urban agriculture enterprise in the city of Adelaide, Australia. Furthermore, we investigated changes within the microbial community, including the presence of potential human pathogens, through amplicon-based metabarcoding. The results of this study provide novel insights into the soil health of urban agriculture which can be used for improved soil management strategies.

5.2 Material and Methods

5.2.1 Site description

All three sites were located in the metropolitan area of Adelaide (Australia) within a radius of less than 9 km around the CBD. The City of Adelaide (138.5999594, -34.9286212) is the capital of South Australia with a population of approximately 1.3 million people (ABS, 2021) and distinct characteristics of suburban sprawl (Davison, 1997). All three sites participated in an earlier study (Salomon et al., 2020), and were selected due to their different management systems (see below) and high commitment of community members to participate in further research.

The first site was a community garden with 20 active gardeners, surrounded by a community centre and residential housing. It was created in 2003 and previously used as a tennis court. Gardening took place in raised beds which were filled with municipal potting soils, derived from local organic waste recycling facilities. This substrate was further amended with compost and organic manures, predominantly horse manure.

Garden beds were watered with dripping hoses in each garden bed and controlled *via* an evaporative-based irrigation device.

Site number two was a community garden with a size of about 2100 m^2 and 25 active gardeners. It was created in 1992 and the land was historically used as a jam factory with some of its original fruit trees still remaining on site. Production took place in the original soil which was further amended with on-site produced compost. Livestock was also kept on this site, namely honeybees and chickens, with the latter being completely integrated into the garden's nutrient cycle. Each plot was irrigated manually using a watering hose.

The third site was a commercial farm with the products being sold at farmer markets or directly to restaurants. The farm was founded in 2012 on a vacant housing plot which is currently under development and is maintained by a single gardener since. Although, the site is located in a residential area, it has a history of industrial use (metal processing). Plants were grown directly in the natural soil which has been amended with organic manures, commercial and on-site produced composts as well as inorganic soil improvers like sand or rock dust. The available space was used very efficiently, meaning a high plant density and minimal pathways between the garden beds. Irrigation was *via* sub-surface drip irrigation controlled *via* a water timer.

All sites were committed to principles of organic farming, however none of the sites were certified organic. While the commercial site focused on year-round food production with seasonal crops, both community gardens provided additional social functions. This is also represented in the design of the sites, where the community gardens allot more space for pathways and meeting areas, compared to the commercial site.

5.2.2 Sampling

Sampling took place once per month from July 2018 to July 2019 (inclusive). The surveyed sites can be grouped into community gardens (n = 2) and commercial sites (n = 1). Four garden beds were selected at each site to be included in the study. The selection criteria for the community gardens were an active vegetation with high plant richness and a commitment of the gardeners to report any major gardening activities (e.g. application of fertilizers). For the commercial site, four areas the size of an average raised bed (2.5 x 0.8 m) were selected and used for sampling purposes. One garden bed of each site was selected for ongoing soil and gas sampling, while information about plant biodiversity was collected from all four beds. The garden bed selected for continuous soil and gas sampling was divided into four equally sized quadrants for replication.

In each quadrant, samples were collected as follows. Three soil cores were taken from the 0 - 10 cm layer, mixed and stored in air-tight zip-lock bags. The soil samples were kept cool until return to the laboratory and processing. Gas samples were collected by setting up one litre sized portable incubation chambers in each quadrant and sealing them airtight to the ground. 5 mL of head gas were extracted at time zero (blank) and 60 minutes and transferred to evacuated and He-filled glass vials.

5.2.3 Plant biodiversity

Plants that were growing at the time of each sampling day were morphologically identified and indexed to a species level. Plant biodiversity was calculated as species richness and the effective number of species (ENS). The ENS is calculated as "e" raised to the power of the Shannon-Weaver-Index H (Jost, 2006).

5.2.4 Soil chemical properties

Upon returning to the laboratory, soil samples were carefully homogenized and sieved to <2 mm. Subsamples of the sieved soils were collected for further analysis. The first subsample was used for determination of soil gravimetric water content after drying at 105 °C for 24 hours. The second subsample was extracted in 2M KCl for colorimetric determination of NH_4^+ and NO_3^- as described in (Cavagnaro, 2016). Every three months, a third subsample was taken for the measurement of potentially mineralizable nitrogen (PMN), whereby soil was covered in 10 mL of reverse osmosis (RO) H2O and incubated anaerobically for 14 days at 37 °C. The soils were then extracted in 4 M KCl and analysed following the NH_4^+ protocol (Drinkwater et al., 1997). The amount of PMN was calculated as the difference between NH_4^+ of the fresh soil (second subsample) and that after 14 days of incubation. A fourth subsample of soil was taken and stored at -20 °C for soil microbial metagenomic analysis (see below). The remaining soil was dried at 40 °C and used for the following chemical analysis: soil pH and EC was measured in a 1:5 water extract using a TPS WP-81 pH, TDS, Temperature & Conductivity Meter (EnviroEquip Biolab, Australia). Plant-available (Colwell) P was extracted in an alkaline 0.5 M sodium-bicarbonate solution for 16 hours (Colwell, 1963) and measured colorimetrically using the Murphey & Riley colour reagent (Murphy & Riley, 1986). Dumas soil total carbon and nitrogen was analysed by the Australian Precision Ag Laboratory (http://www.apal.com.au/).

5.2.5 Soil biological properties

Soil microbial diversity was analysed by amplicon-based metabarcoding. Four sampling dates (September, December, March, June, corresponding to Austral Spring, Summer, Autumn and Winter, respectively) of two sites (1 and 3), including all four replications of each sampling date, were selected for this analysis (n = 32). DNA extraction, PCR amplification and Illumina MiSeq sequencing were conducted on whole soil samples by the Australian Genome Research Facility (AGRF) as described in **Smith**. Bacterial and fungal communities were identified using the primers 27F-519R (16S) and ITS1F-ITS2 (internal transcribed spacers) on ribosomal RNA. The data was provided as paired-end and FastQ-formatted sequencing files with 300 base pairs (bp) read length.

Soil respiration was measured as CO_2 flux using an infrared gas analyser (IRGA, Model 6262, Li-Cor, Lincoln, NE, USA). 5 mL of each gas sample were transferred from the vial to the gas analyser using air-tight syringes. The concentration of CO_2 per second

was interpolated and the content calculated in R (version 3.6.3) by integrating the area under the curve.

Spores of arbuscular mycorrhizal fungi were sampled from dry soil samples at two time points, then extracted and counted. To do this, 100 mL of dried soil was rewetted and stirred for 30 minutes. The soil was then wet-sieved on 250 µm and 53 µm sieves and the 53 µm extract further purified in a centrifuge with 50% sucrose-gradient (Merryweather & Moyersoen, 1997). The supernatant was washed three times with RO water and transferred to a Petri dish. Spores were counted using a dissecting microscope (Olympus SZ-PT) between 40 - 60 x magnification.

5.2.6 Data analysis

Data was analysed in R (version 4.0.0) and visualized with "ggplot2" (v. 3.3.2) with loess curve fitting (Wickham, 2016). For soil chemical and physical analysis, a principal component analysis (PCA) was performed using the function "prcomp" and visualized with the package "factoextra" (v. 1.0.7) (Kassambara & Mundt, 2017). Statistically significant differences were determined using a repeated measures two-way analysis of variance (ANOVA) with the categorical variables "Site" and "Month". To describe the repeated measure analysis, a mixed effect model was performed using the function lme() in the package "nlme" (v. 3.1-148) (Pinheiro et al., 2020). Data assumptions were confirmed using the Shapiro-Wilk and Levene's test on the residuals of the model. Where significant differences were identified, multiple comparisons were undertaken using the least square means function lsmeans() from the package "lsmeans" (v. 2.30-0) (Lenth, 2016). Significant letters were assigned with the package "lsmeans" and a Sidak-adjusted comparison with $\alpha = 0.05$.

Bioinformatic analysis was conducted by pre-processing the paired-end reads as follows: Residual primers were identified and removed using "Cutadapt" (v. 2.8) (Martin, 2011). Illumina Nextera adapters were identified and removed using the Cutadapt-wrapper "TrimGalore" (version 0.6.5) (Krüger, 2021). Sequence files were then processed in R (v. 4.0.0) using the dada2 pipeline (v. 1.16) (Callahan et al., 2016). Sequences were quality filtered according to the maximum numbers of expected errors and the 16S sequences trimmed to 250 bp (phred score of 30). ITS sequences were not trimmed due to their variable sequence length. The "dada2" (v. 1.16.0) inference algorithm was applied to the filtered data and the forward and reverse reads merged (Callahan et al., 2016). Amplicon sequence variant (ASV) tables were constructed and chimeras removed. Taxonomic classifications were assigned using the idTaxa classifier of the "DECIPHER" package (v. 2.16.1) (Murali et al., 2018). SILVA SSU r138 (2019) and UNITE 2020 (February 2020) were used as training sets for classification. Microbial diversity was visualized using phyloseq (v. 1.32) (McMurdie & Holmes, 2013).

5.3 Results

5.3.1 Plant biodiversity

Over the course of the whole year, both community gardens had significantly higher species richness and effective number of species (ENS) than the commercial site (see Suppl. Figure 5.6). The plant species of the community gardens would often include ornamental plants or perennials, such as capers (*Capparis spinosa*) or gooseberry (*Physalis peruviana*). In most cases, the predominant plant species were crop plants typical for the seasons on Adelaide's mediterranean climate (see Suppl. Table 5.2).

5.3.2 Soil nutrients

The mineral forms of nitrogen (NH_4^+ and NO_3^-) displayed great variability throughout the year (see Figure 5.1 A and B). Individual samples ranged from being undetectable in the soil extract (below 0.1 mg L^{-1}), up to 123 mg kg^{-1} (NH_4^+) and 185 mg kg^{-1} (NO_3^-). Concentrations of mineral N were lowest in the months before and after summer, with a slight increase towards winter. Site number three showed a strong increase in mineral N after applying fertilizer between October and November 2019. Statistical analysis showed no significant differences between sites 1 and 3, whereas site 2 had lesser soil concentrations of mineral N (see Suppl. Table 5.4). Potentially mineralizable nitrogen (PMN) was measured seasonally and could supply significant amounts of N (see Suppl. Figure 5.7).

Plant-available (Colwell) P was very high at all three sites and consistently exceeded the critical concentration for a common horticultural crop like lettuce, which is 121 mg kg^{-1} (Robertson and McPharlin, 1997). Compared to mineral N, the concentrations of Colwell P in the soils showed less variability throughout the year (see Figure 5.1 C). Still, statistical analysis showed a strong temporal effect on concentrations of plant-available (Colwell) P (see Suppl. Table 5.4).

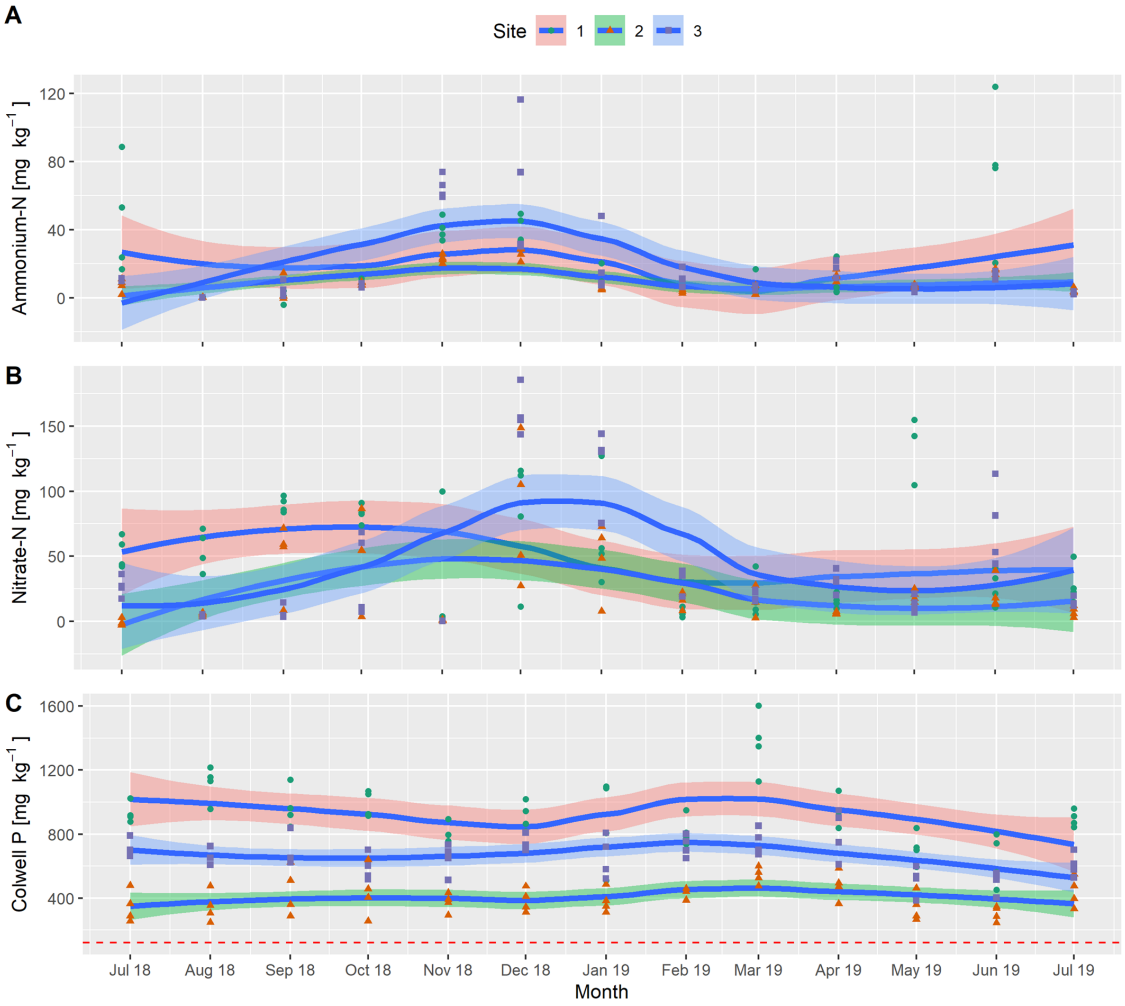

FIGURE 5.1: *Annual soil dynamics of ammonium (A), nitrate (B) and plant-available (Colwell) P (C) of garden beds from three urban agriculture sites. Curve fitting using a loess regression, coloured area displays 95% confidence intervals.Red-dotted line indicating critical concentration of Colwell P for lettuce (Lactuca sativa).*

The dynamics of soil total C at sites 1 and 3 followed a very similar pattern (see Suppl. Table 5.4). Both sites showed a negative, almost linear, decrease over the 12 month period (Figure 2A and B). A similar pattern is observable for total N, however, with significant differences between sites 1 and 3. For these two sites, concentrations of total C in soils were approximately 33% lower at the end of the study than in the previous year. Site 2 had significantly less total C/N than the other sites, however, it remained more consistent throughout the year. Despite these changes in total C and N, the ratio of C:N remained relatively constant (see Figure 5.2).

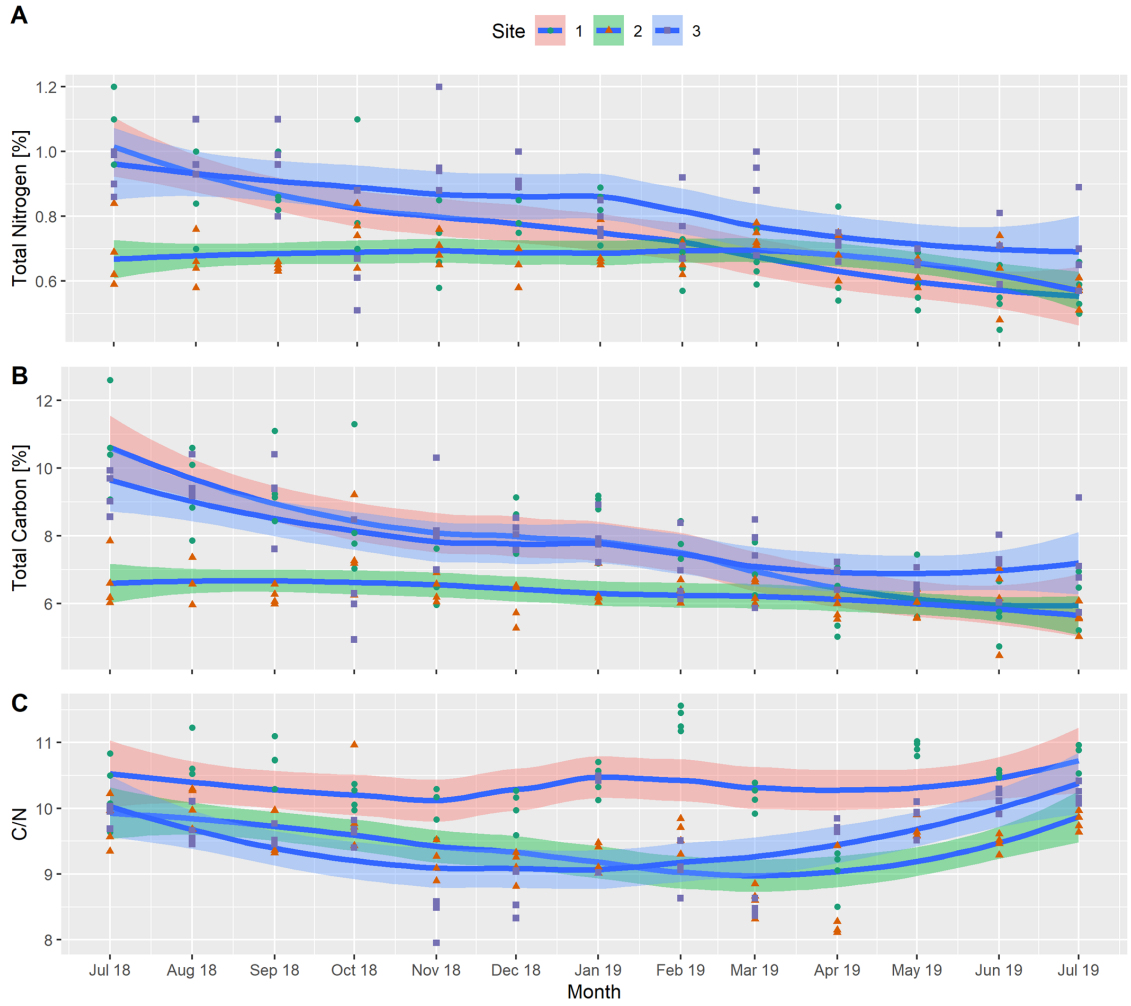

FIGURE 5.2: *Annual dynamics of total nitrogen (A), total carbon (B) and C-N ratio (C) of soil in garden beds from three urban agriculture sites. Curve fitting using a loess regression, coloured area displays 95% confidence intervals.*

5.3.3 Soil physicochemical properties

When looking at the soil physicochemical dynamics, all sites differed significantly from each other, whereas site 3 showed the most variable pattern (see Suppl. Figure 5.4 A and Table 4). The pH of sites 1 and 2 stayed on average within 7 and 7.5, whereas Site 3 showed a much stronger decrease to almost pH 6.5.

The EC of Sites 1 and 2 showed only little divergence from each other (see Suppl. Figure 5.4 B). The soil EC of Site 3 started and ended at a similar level than in the other two sites but showed a much stronger maximum during January 2019. At Site 3, the pattern of soil EC was almost the inverse of the soil pH.

5.3.4 Soil microbiome and biological activity

All three sites showed very similar soil respiration dynamics (see Suppl. Figure 5.9 A). Between all three sites, the average soil respiration at the beginning of the study was 980 mg C-CO_2 m^{-2} d^{-1}, compared to 1350 mg C-CO_2 m^{-2} d^{-1} at the end of the study. The soil respiration peak for all sites was between December 2018 and January 2019 during Austral Summer. The similarity between sites is confirmed by statistical analysis, where soil respiration is the only factor with no significant differences between the sites.

The AMF spore count at two different time points revealed an abundance of AMF spores (see Suppl. Figure 5.9 B). All sites pooled together had an average of 4 spores mL^{-1} soil. The number of spores was relatively consistent between the two sampling dates and significantly increased only for Site 3 between October 2018 and April 2019.

Soil metabarcoding of bacteria and archaea (16S rRNA) revealed a total of 41,005 taxa between all samples, of which the most prevalent phylum was the Actinobacteriota (see Suppl. Table 5.3). Alpha diversity showed divergence between Sites 1 and 3 in December (see Figure 5.3 A). The principal coordinates analysis (PCoA) shows that the 16S microbial profile was highly diverse between Sites 1 and 3 throughout the whole year and without any overlaps. Site 1 was more densely clustered together than Site 3 (see Figure 5.3 B). When looking at the microbial abundance, only Site 1 showed a strong change, specifically as an increase in June 2018. That increase is mainly due to the genera *Marmoricola spp.*, *Methyloceanibacter spp.* and *Pedomicrobium spp.* Both sites are made up of similar genera and of similar distribution (see Figure 5.3 C). Various samples contained sequences of soil-borne potential human pathogens, such as *Clostridium spp.* and *Streptomyces spp.* (Bultman et al., 2013). There were also a variety of plant growth promoting bacteria present, mainly bacteria with the potential to solubilize phosphorus (Kalayu, 2019) (see Suppl. Table 5.4).

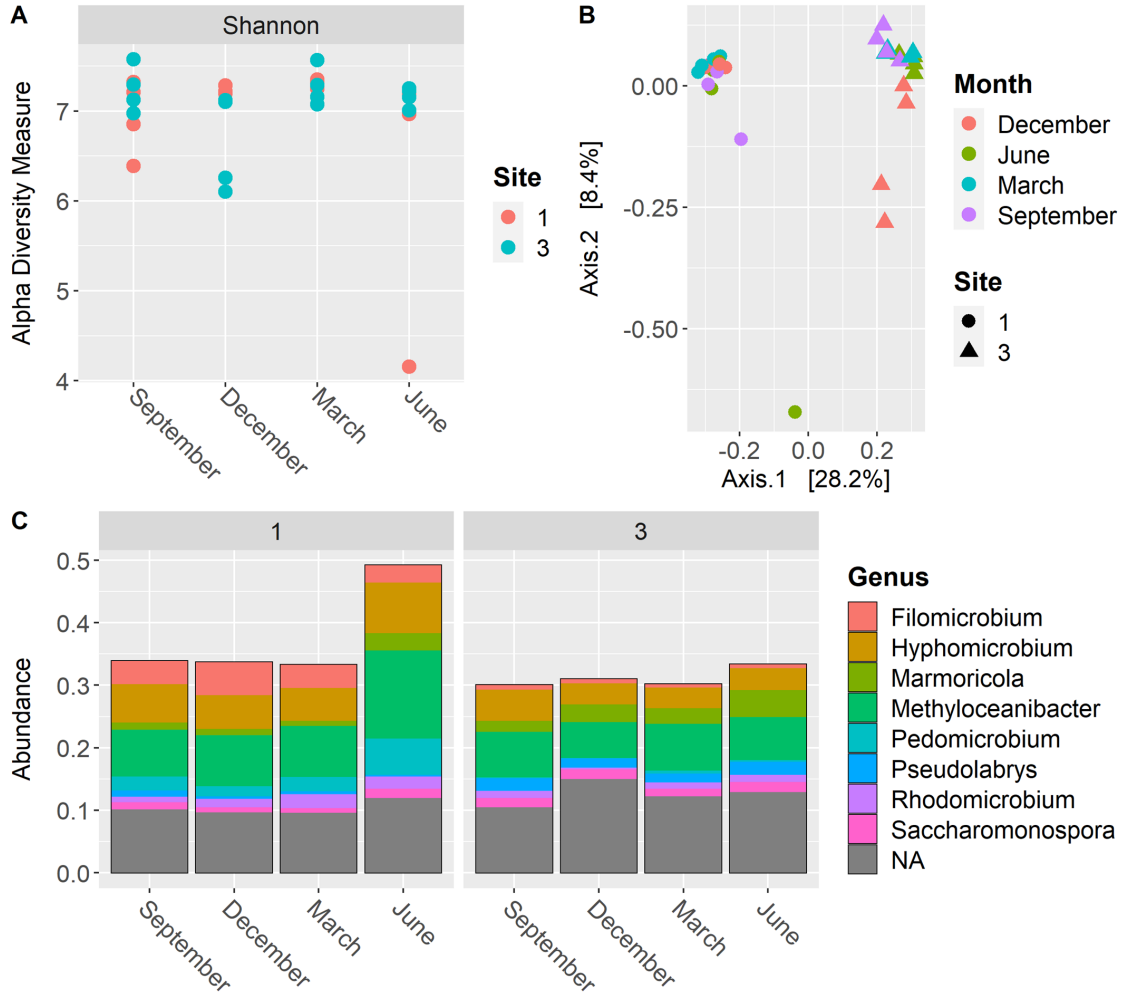

FIGURE 5.3: *16S metabarcoding of soil samples from two urban agriculture sites taken at 3-monthly intervals. Data displayed as alpha diversity (A), PCoA biplot (B) and relative abundance of genera (C).*

Soil metabarcoding using ITS revealed a total of 2765 taxa between all samples, of which Ascomycota is the most prevalent phylum (see Suppl. Table 5.3). The alpha diversity of Site 1 is higher than in Site 3, especially during December and March and with some overlaps during September and June (see Figure 5.4 A). When represented as a PCoA, the ITS microbial profile between the two sites is equally diverse from one another as seen in the 16S microbial diversity. Site 3 showed a vertical gradient throughout the four sampling dates with strong differences between September and December 2018. Samples of Site 1 are stronger clustered and do not show such clear differences between the sampling dates (see Figure 5.4 B). There is a clear difference between the genus composition of both sites. For example, *Mortierella spp.* are not present in soil from Site 1 and Preussia ssp. are not present at Site 3. Also, Site 3 showed a much stronger change in microbial abundance between the months, whereas Site 1 was relatively constant (see Figure 5.4 C). Various samples contained sequences of AMF which were described as Glomeraceae or *Funneliformis spp.* The number of AMF reads does not correlate with the high spore concentration present in the soils. Further fungal organisms with potentially

beneficial effects on plant growth were classified, such as *Trichoderma spp.* or *Rhodotorula kratochvilovae* (Kalayu, 2019, Deng and Cao, 2017). Similar to the 16S metabarcoding, multiple sequences of potential human and plant fungal pathogens were identified (Bultman et al., 2013) (see Table 5.1).

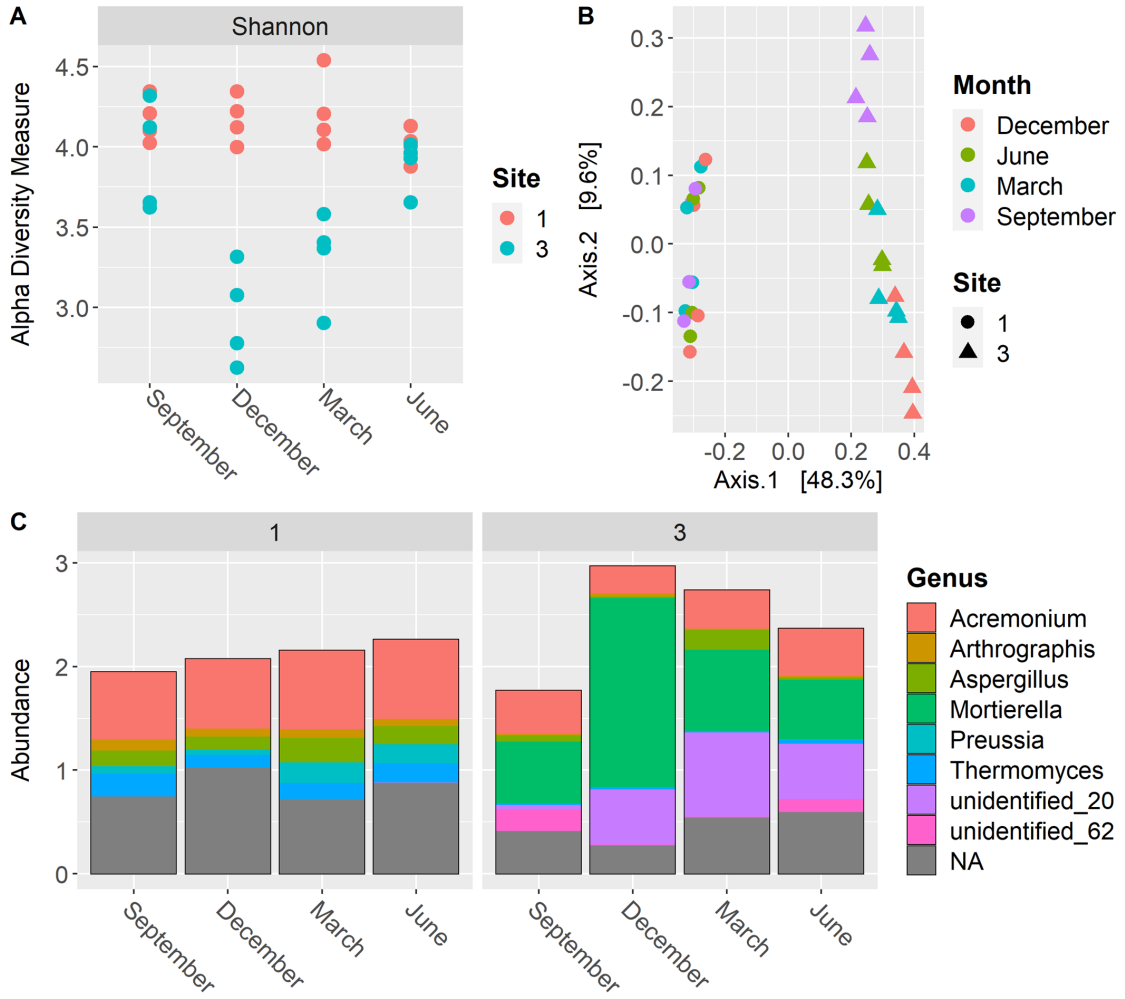

FIGURE 5.4: *ITS metabarcoding of soil samples from two urban agriculture sites taken at 3-monthly intervals. Data displayed as alpha diversity (A), PCoA biplot (B) and relative abundance of genera (C).*

TABLE 5.1: *Occurrence of selected genera within the 16S and ITS amplicons of site 1 and 3.*

Amplicon	Classification	Genus	Occurrence
16S	Potential human pathogen	*Actinomadura spp.*	Site 1 and 3
		Clostridium spp.	Site 3
		Coxiella spp.	Site 3
		Mycobacterium spp.	Site 1 and 3
		Nocardia spp.	Site 1 and 3
		Streptomyces spp.	Site 1 and 3
		Thermoactinomyces spp.	Site 1 and 3
	Potential plant beneficial bacteria	*Mesorhizobium spp.*	Site 1 and 3
		Bacillus spp.	Site 1 and 3
		Pseudomonas spp.	Site 3
	Potential indicator of herbicide residues (Chloridazon)	*Phenylobacterium spp.*	Site 1
ITS	Potential human pathogen	*Aspergillus spp.*	Site 1 and 3
	Potential plant beneficial fungi	*Aspergillus spp.*	Site 1 and 3
		Penicillium spp.	Site 1 and 3
		Trichoderma spp.	Site 1 and 3
		Arthrobotrys spp.	Site 1
	Plant beneficial fungi	*Rhodotorula kratochvilovae*	Site 1
		Funneliformis spp.	Site 1 and 3

5.4 Discussion

5.4.1 Plant biodiversity

In general, the two community gardens showed a higher and more variable plant biodiversity than the commercial site. This situation is likely the result of the commercial garden focusing on larger yields of a (smaller) selection major seasonal cash crops. This difference between the community gardens and the commercial site is not surprising when considering the motivations of urban gardeners in developed countries (Lovell, 2010). That way, community gardens might improve urban biodiversity (Goddard et al., 2010). The possible contribution of urban farms towards food security has been analysed by (McDougall et al., 2019), who found yields around double the amount of typical commercial farms. Such analysis was not the focus in this study; however, we can assume higher caloric efficiency in the commercial site based on the selection of plants and plant density. Approximately two months after the last sampling date, most plants of the commercial site were removed and replaced with garlic and sweet corn in an effort to

reduce root-knot nematodes. That event is not represented in the data set, but it reflects the ability for urban agriculture to respond to certain issues (root-knot nematodes) and requirements (food security vs. recreational benefits) in a flexible and dynamic way.

5.4.2 Soil nutrient concentrations

The concentrations of mineral N (NH_4^+ and NO_3^-) followed a similar pattern at all sites and was highly variable throughout the twelve-month sampling period. Those results are of special interest since all sites followed different gardening practices and used natural or imported soils with or without raised beds. It demonstrates the influence of environmental variables, such as temperature and precipitation on the N dynamic of urban agriculture systems. The PCA shows air and soil temperatures were positively correlated with mineral N (see Suppl. Figure 5.10). It is generally considered that increasing temperatures combined with sufficient soil moisture lead to higher net mineralization rates of N. The pool of mineral N is then either taken up by plants, or lost through leaching, gaseous emissions, and/or bound in the soil organic matter (Hartemink, 2000). These mechanisms can also be observed in the mineral N cycle of all three sites, where highest concentrations were available during the summer months and then decreased significantly through autumn and winter where nitrate-N was probably washed out due to higher precipitation. The lowest availability of N in the year coincided with the planting of typical Austral winter crops, some of which have high N demands, such as cabbage or broccoli (Karthika et al., 2020). Although low concentrations of mineral N were found at this time, plants had access to an abundant supply of PMN. Within this study it is not possible to judge whether crops were N-limited, as that would require tissue analysis.

Soils of all sites had an ample supply of total N, although it constantly decreased throughout the year for two sites. For comparison, typical total N concentrations in agricultural soils are around 0.2% (Chen et al., 2009), compared to 0.7% in this study. The concentration of total C in the soil showed a similar decrease for all sites. The reason for declining C and N is best explained by management practices rather than biological reasons. It is likely that gardeners applied less compost than in previous years and that the nutrient demand of plants and nutrient losses were higher than the inputs. The C:N ratio for all sites was constantly below the recommended rate of 15, which is often linked to increased N mineralization and thereby N losses (Brust, 2019). One way to increase the C:N ratio is through the application of composts or organic mulches with a high C:N ratio, such as straw (C:N = 110:1) (Gaind et al., 2009).

The concentration of plant-available (Colwell) P in the soil was mostly stable across all sites. From an agricultural perspective, the measured concentrations are considered very high and far in excess of those recommended for typical vegetable crops. Similar results have been found in previous research at the same (and other) sites (Salomon et al., 2020) and in urban farms in Portugal (Arrobas et al., 2017) and Netherlands (Wielemaker et al., 2019). The main reason for this global trend is an over-application of freely

available composts and organic amendments. This problem is further aggravated by the immobile nature of P in soils where it is rather stable compared to C and N (Hartemink, 2000). The main source of nutrients in this study was local horse manure (Site 1), self-made compost and chicken manure (Site 2) and commercial and self-made compost (Site 3). Horse and chicken manures have been analysed with total P concentrations of 9 and 21 g kg^{-1} manure (Pagliari & Laboski, 2012). Excessive concentrations of soil P should be avoided as this condition can cause environmental damage, such as *via* runoff or dispersal of P into ground waters (Cavagnaro et al., 2015). It can also inhibit the uptake of essential plant minerals such as zinc due to nutrient antagonism (Rietra et al., 2017). One way to reduce soil concentrations of P is to change the composting regime to ingredients with lesser amounts of P, such as spent coffee grounds (N:P = 30:1) (Liu & Price, 2011).

5.4.3 Soil physicochemical properties

The seasonal dynamics of soil pH and EC are very similar for Sites 1 and 2. Site 3 noticeably diverged from the other two sites, which can be linked back to the fertilizer applied between October and November 2018. During this time, the sampled garden bed was completely renewed by applying a layer of compost on the topsoil. This event was followed by a sharp increase of soil EC, of up to 3000 mS cm^{-1}. Most crop plants do not tolerate such high salinity and show depressed growth (Maas et al., 1986). However, no new crops were planted into this garden bed until March 2019 due to high summer temperatures. The soil pH followed the exact inverse pattern of the soil EC and decreased to 6.5 in January 2019. This change in pH would be less detrimental to plants than the measured values of EC, and a pH between 6.5 and 7.0 is in fact recommended for most horticultural crops (Jones Jr, 1985). High EC in composts is derived from ions which are either important plant nutrients, such as K^+, or Na^{2+} which can interrupt the water uptake of plants. The salinity of composts is mostly determined by its feedstock and decreases over time. This is mostly due to leaching and precipitation, and to a lesser extent due to volatile organic sulphur compounds or microbial consumption (Gondek et al., 2020). Excess application rates can thereby lead to reduced plant growth, reduced seed germination or the inhibition of microbial activity. As seen in this study, the EC value decreased over time, most likely due to leaching (Hargreaves et al., 2008).

5.4.4 Soil microbiome and biological activity

Soil respiration (CO_2) at all three sites was not significantly different from each other and showed similar trends throughout the sampling period. This indicates that, like mineral N, soil respiration is strongly influenced by external factors. The PCA confirms that the most positive correlated factors were soil and air temperature (see Suppl. Figure 5.10). Another reason for the lack of significant site-effects can be found in the high variability of the individual results. In this study, the mean soil respiration between all three sites was 1.34 g CO_2-C m^{-2} d^{-1} which is close to what has been measured in conventional agricultural systems (0.95 g CO_2-C m^{-2} d^{-1}) (Ding et al., 2007). Higher rates of 3.0 to 7.8 CO_2-C

m^{-2} d^{-1} were measured in urban forests and parklands during summer (Weissert et al., 2016), compared to 1.7 g CO_2-C m^{-2} d^{-1} found in this study during summer. This discrepancy might be due to increased microbial activity in leaf litter (forests) or grass clippings (parklands) (Cornwell et al., 2008; Raciti et al., 2011). Soil respiration in this study culminated towards 975 g CO_2-C loss through respiration per raised bed and year, which would, theoretically, require 14 kg of soil to replace (average raised bed area = 2 m^2, soil C = 7%). In practice, there are other carbon inputs, for example through the application of mulch, compost or due to plant growth. Inputs were not sufficient to counteract the CO_2 efflux, as seen in the decreasing soil C concentration.

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts that colonize about 80% of all terrestrial plants and most crop plants. AMF are widely studied for their positive effects on plant nutrition. Given the high soil fertility reported in this study, AMF might be considered redundant from a plant nutritional perspective. However, AMF are associated with a wider range of beneficial plant and ecosystem services, such as protection against soil-borne pathogens or the immobilization of contaminants (Harrier & Watson, 2004). Previous work on urban agricultural sites in Adelaide revealed that all three sites contain an abundance of AMF propagules (Salomon et al., 2020). In this study, the occurrence of AMF was again confirmed by means of spore counts. Soils contained on average 4 spores mL^{-1} which is similar to agricultural soil under crop rotation practices (Oehl et al., 2003). The mycorrhizal abundance can be explained by organic farming practices, reduced soil disturbance (no-dig gardening) and high plant biodiversity. All these methods have been shown to bolster the presence of AMF (Bowles et al., 2017).

Previous research using 16S and ITS amplicons identified soil pH (Kaiser et al., 2016) and nutrient availability (Thomson et al., 2015) as the predominant predictors of microbial community composition. Similar results were observed in this study where Site 3 showed a drop in 16S alpha diversity in December 2018, probably due to a decrease in pH. Similarly, one sample of Site 1 showed a strong decrease in alpha diversity in June 2019. Although no changes in pH were observed for this month, it is likely due to the application on fresh chicken manure in some areas of the bed. The availability of fresh manure and changes to the C:N ratio could have favoured certain bacteria over others, thereby decreasing the alpha diversity (Urra et al., 2019). This might also explain the higher abundance of genera in June 2019 for Site 1. Such changes in the microbial community might impact ecosystem functions (van der Heijden & Wagg, 2013). The distribution of genera between both sites and months is very similar, although different soil types were used (natural vs. introduced soil). This could either be a result of analytical bias or due to their greater dispersal potential within the same urban environment.

When looking at the metagenomic analysis of the ITS amplicon it becomes obvious that Site 3 showed a strong seasonal gradient with a decrease in alpha diversity, whereas Site 1 was steadier. The seasonal dynamic of Site 3 could be explained by a variety of

reasons, such as the decrease in pH and C:N towards December 2018, as well as the low plant biodiversity and irrigation during that period (Thomson et al., 2015). Conversely, the steady soil fungal community in Site 1 could be due to more consistent soil moisture and high plant biodiversity. Both sites are made up of mostly different fungal genera and only share *Acremonium spp.* and *Aspergillus spp.* with each other. Previous research also uncovered a heterogeneous spatial distribution of fungi between different maize microcosms (Moll et al., 2016). Those results suggest that unlike bacteria, fungal communities do not follow a cosmopolitan distribution within urban ecosystems. This could be due to difficulties in spore dispersal or the dominance of niche fungal communities. This, however, is speculative and would require further investigation on a broader spatial scale.

Sites contained a variety of both potential beneficial and pathogenic microbial genera. In most cases, OTUs could not be identified to the species level, thereby leaving a margin of error to their real pathogenic potential. For example, the genus *Nocardia* comprises a total of 85 species of which many are non-pathogenic (Kenneth et al., 2010). Most identified (potential) pathogens are considered soil-borne diseases with an almost cosmopolitan distribution (Bultman et al., 2013). The presence of potential human pathogens in soils is a common issue with special importance for food production in combination with organic fertilization. The risk of introducing pathogens can be reduced through a strict composting regime under thermophilic conditions (Qian et al., 2016). Although soil samples from all sites contained an abundance of AMF spores, sequencing only detected a few reads of *Funneliformis spp.* and unclassified Glomeromycota. This can be explained by the non-selectivity of ITS towards AMF which requires the use of more selective primers when being targeted (van Geel et al., 2014).

5.4.5 Recommendations for soil management practices in urban agriculture systems

Overall soil health in the urban agriculture sites was sufficient to grow most food crops, however, potential plant growth constraints were identified. Composts and other organic fertilizers should be balanced with regards to their N:P ratio to avoid excessive build-up of P. One such urban waste product is spent coffee ground which is readily available in most metropolitan areas (N:P = 30:1) (Liu & Price, 2011). Mulch can be applied to counteract a decreasing C-pool due to soil respiration and to improve various other soil health parameters (Lal, 2004). Fertilizer should be applied more frequently according to the current demand of plant-nutrients, rather than to meet the demand of the whole growing season with one application. This way, chemical and microbial soil parameters remain steady and nutrient leaching is minimized. With regards to N, fertilization should be emphasized during times of higher precipitation and when growing crops with high N demands. Principles of organic farming are likely to increase the abundance of AMF which can have positive impact on plant growth and soil ecosystem functions. Gardeners should be aware about the possibility of soil-borne human pathogens and how to reduce this risk factor, e.g. through strict composting regimes and wearing of personal protective

equipment. In order for urban agriculture to contribute to global food security and the Urban Food Agenda, soil management principles should be incorporated for sustainable and long-term soil health.

5.4.6 Conclusion

Soil samples were collected on a monthly basis from three different urban agriculture sites and analysed according to various soil health parameters. Mineral N (NH_4^+ and NO_3^-) showed a great variability throughout the year, ranging from 0 mg kg^{-1} to 123 mg kg^{-1} for NH_4^+ and 185 mg kg^{-1} for NO_3^-. At the start of the analysis, concentrations were between 0.7 and 1.0% for total N and between 6.5 and 11.5% for total C. For two sites, these concentrations were gradually decreasing. PMN was measured every three months which indicated a relatively large pool of N being available for plant-uptake after mineralization. Soil concentration of plant-available (Colwell) P was less variable than N and all sites were well above the critical concentrations for most crops. The dynamics of soil pH and EC were greatly influenced by the application of organic fertilizer, resulting in EC levels of up to 3000 mS cm^{-1} for one site. Soil CO_2 respiration was the only variable with no significant differences between the sites, thereby highlighting the great influence of environmental conditions such as temperature and precipitation on soil respiration. The occurrence of AMF spores was measured as another indicator for soil biological health. Although all sites had very high concentrations of plant-available (Colwell) P, between 3 and 5 spores mL^{-1} soil were found at two different time points. 16S and ITS amplicon based metabarcoding was used to investigate the development of the soil microbial community between two different sites and at four time points (Spring, Summer, Autumn, Winter). Results revealed contrasting microbial communities between the sites with a strong seasonal gradient. The presence of various potential human pathogens, as well as plant-beneficial microorganisms was discovered.

Urban agriculture has been proposed as a multifunctional tool for sustainable food production and resilient communities. Yet, there is a dearth of studies focusing on soil health in urban agriculture systems. Using a dynamic approach, we uncovered potential soil health constraints that could undermine the productivity of these systems. These issues have been addressed with recommendations for soil management practices, focusing on the use of urban waste products.

5.5 Acknowledgements

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5.6 Supplementary material

FIGURE 5.5: *Overview of site 3 in October 2018. Site is located in a developing area and surrounded by residential buildings and remnant industry.*

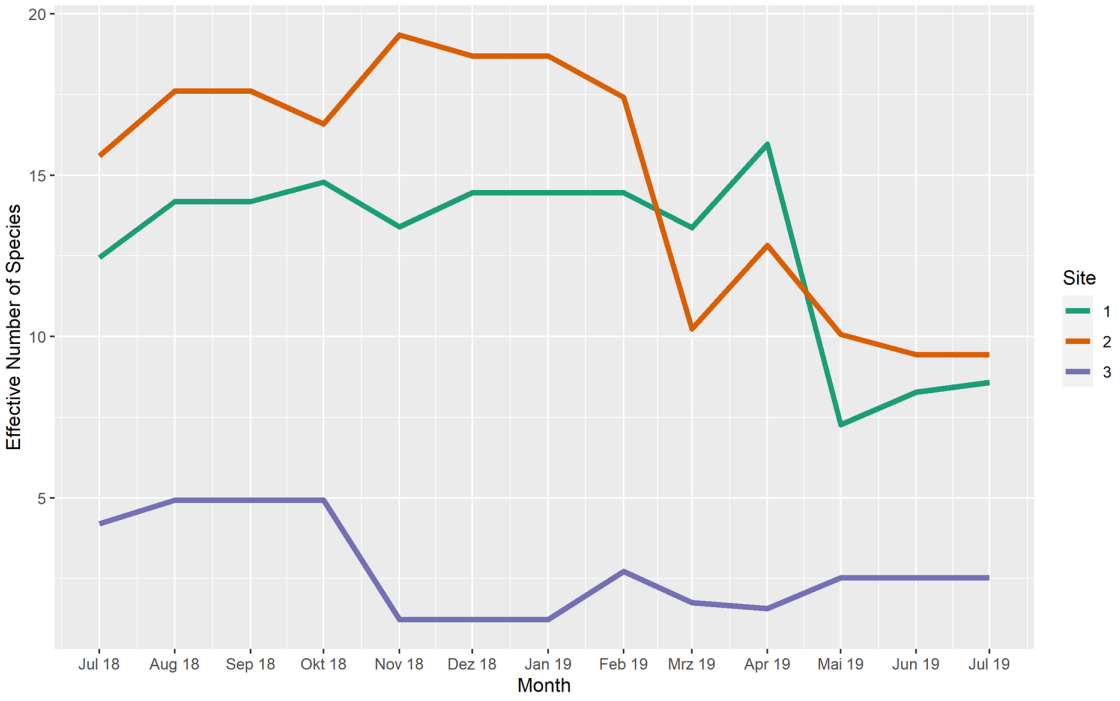

FIGURE 5.6: *Annual dynamic of plant biodiversity sampled from four gardening beds of three urban agriculture sites.*

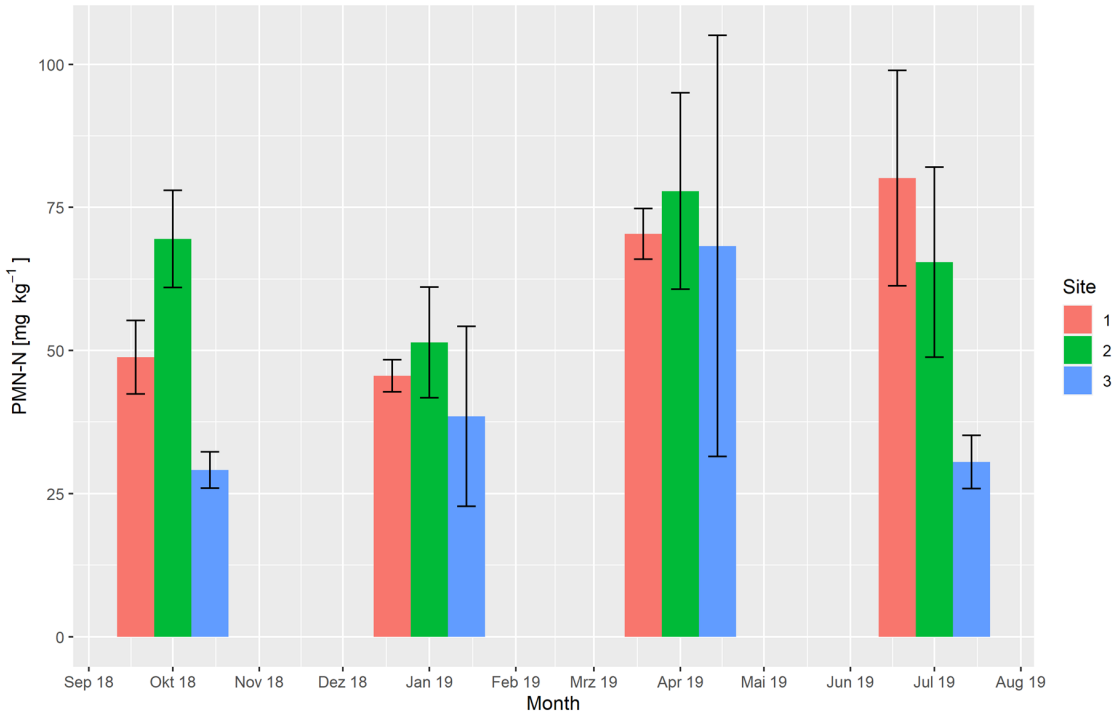

FIGURE 5.7: *Annual dynamic of soil potential mineralizable nitrogen (PMN) of garden beds from three urban agriculture sites. Error bars indicating standard error.*

TABLE 5.2: *Overview of plant biodiversity between sites and sampling dates. ENS = Effective Number of Species.*

Month	Site	Shannon-Index	ENS	Species Richness	Predominant crop
Jul 18	1	2.5	12.4	19	Faba beans
	2	2.7	15.6	27	Brussel sprouts
	3	1.4	4.2	6	Lettuce
Aug 18	1	2.7	14.2	20	Faba beans
	2	2.9	17.6	29	Brussel sprouts
	3	1.6	4.9	6	Lettuce
Sep 18	1	2.7	14.2	20	Faba beans
	2	2.9	17.6	29	Brussel sprouts
	3	1.6	4.9	6	Lettuce
Oct 18	1	2.7	14.8	21	Faba beans
	2	2.8	16.6	25	Garlic
	3	1.6	4.9	6	Lettuce
Nov 18	1	2.6	13.4	18	Strawberry
	2	3	19.4	30	Tomato
	3	0.2	1.2	2	Lettuce
Dez 18	1	2.7	14.5	19	Strawberry
	2	2.9	18.7	29	Tomato
	3	0.2	1.2	2	Lettuce
Jan 19	1	2.7	14.5	19	Strawberry
	2	2.9	18.7	29	Tomato
	3	0.2	1.2	2	Lettuce
Feb 19	1	2.7	14.5	19	Strawberry
	2	2.9	17.4	26	*Helianthus tuberosis*
	3	1	2.7	3	Rhubarb
Mar 19	1	2.6	13.4	17	Strawberry
	2	2.3	10.2	21	*Helianthus tuberosis*
	3	0.6	1.8	2	Rhubarb
Apr 19	1	2.8	16	19	Strawberry
	2	2.6	12.8	20	Broccoli
	3	0.5	1.6	2	Beet root
May 19	1	2	7.3	11	Leek
	2	2.3	10.1	23	Onions
	3	0.9	2.5	3	Beet root
Jun 19	1	2.1	8.3	12	Leek
	2	2.2	9.4	23	Onions
	3	0.9	2.5	3	Beet root
Jul 19	1	2.1	8.6	12	Leek
	2	2.2	9.4	23	Onions
	3	0.9	2.5	3	Beet root

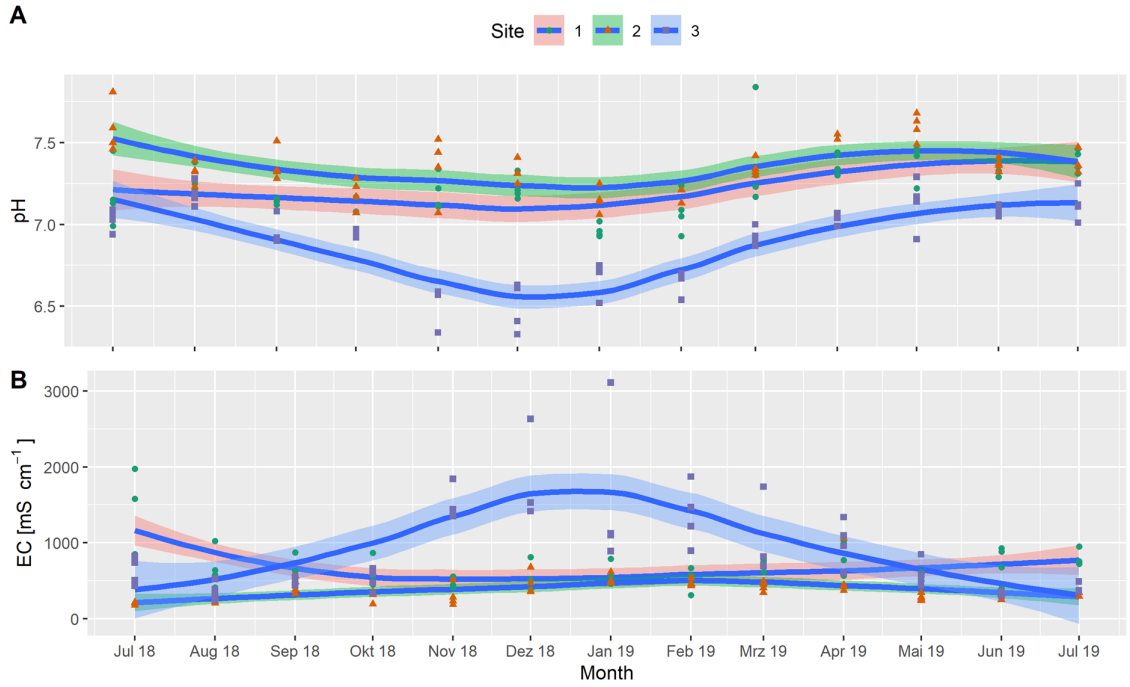

FIGURE 5.8: *Annual dynamic of soil pH and EC of garden beds from three urban agriculture sites. Curve fitting as loess regression, coloured area displays 95% confidence intervals.*

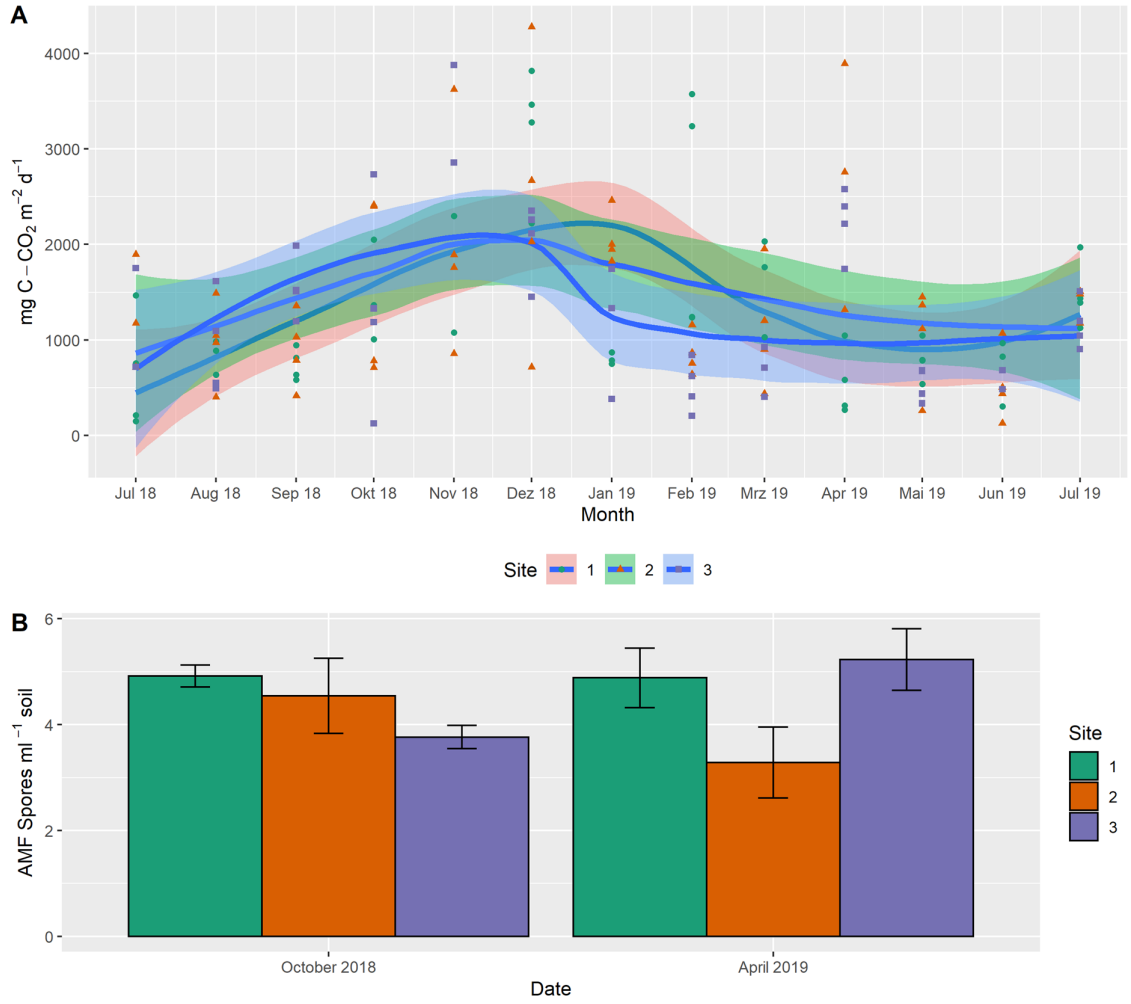

FIGURE 5.9: *A: Annual dynamic of soil respiration (CO_2) of garden beds from three urban agriculture sites. Curve fitting as loess regression, coloured area displays 95% confidence intervals. B: AMF spore richness in collected soil samples.*

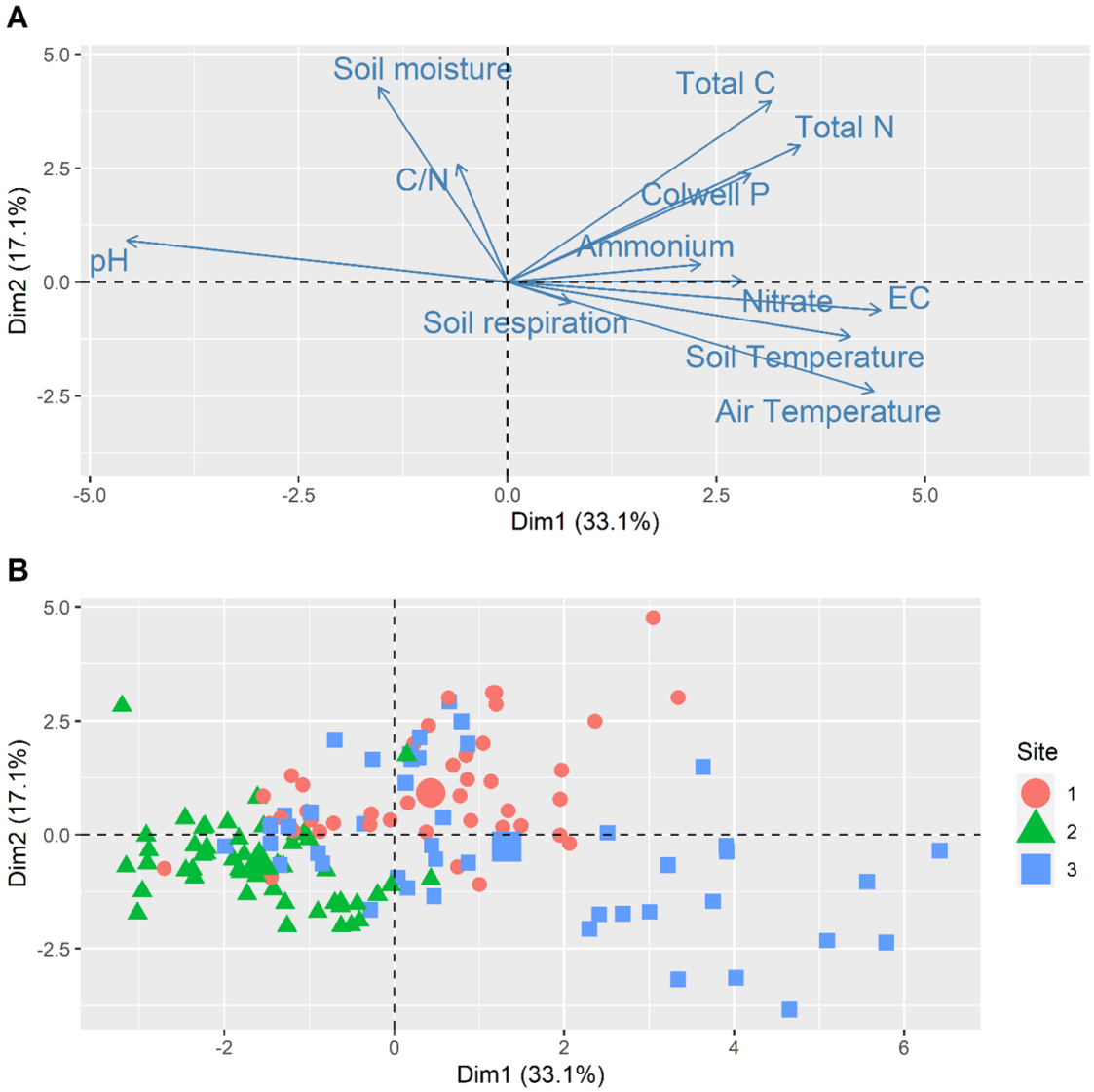

FIGURE 5.10: *Principal component analysis (PCA) of three urban agriculture sites and monthly sampling over one year.*

TABLE 5.3: *Overview of identified taxa and most commonly found phyla and families. Occurrence describes number of identified reads for each phylum or family.*

ITS	2765 taxa in 32 samples		
Phylum	Occurrence	Family	Occurrence
Ascomycota	894	*Aspergillaceae*	84
Basidiomycota	184	*Microascaceae*	61
Mortierellomycota	41	*Chaetomiaceae*	44
16S	**41005 taxa in 32 samples**		
Phylum	Occurrence	Family	Occurrence
Actinobacteriota	11694	*Nocardioidaceae*	1038
Proteobacteria	8683	*Microbacteriaceae*	655
Firmicutes	4101	*Rhodobacteraceae*	415

TABLE 5.4: *ANOVA table of measured soil health properties.*

pH				
	numDF	denDF	F-value	P-value
(Intercept)	1	114	714562.2	< 0.0001
Month	12	114	23.6	< 0.0001
Site	2	114	257.5	< 0.0001
Month:Site	24	114	5.9	< 0.0001
Site	lsmean	Group		
1	7.229	b		
2	7.358	c		
3	6.902	a		
EC				
	numDF	denDF	F-value	P-value
(Intercept)	1	114	191.2	< 0.0001
Month	12	114	6.1	< 0.0001
Site	2	114	51.3	< 0.0001
Month:Site	24	114	7.2	< 0.0001
Site	lsmean	Group		
1	672	b		
2	374	a		
3	906	c		
Colwell P				

	numDF	denDF	F-value	P-value
(Intercept)	1	114	3002.6	< 0.0001
Month	12	114	10.6	< 0.0001
Site	2	114	312.1	< 0.0001
Month:Site	24	114	3.1	< 0.0001

Site	lsmean	Group
1	919	c
2	404	a
3	662	b

Ammonium

	numDF	denDF	F-value	P-value
(Intercept)	1	114	113.1	< 0.0001
Month	12	114	20.7	< 0.0001
Site	2	114	10.8	< 0.0001
Month:Site	24	114	5.4	< 0.0001

Site	lsmean	Group
1	19.31	b
2	9.34	a
3	17.39	b

Nitrate	numDF	denDF	F-value	P-value
(Intercept)	1	114	299.3	< 0.0001
Month	12	114	18.2	< 0.0001
Site	2	114	16.5	< 0.0001
Month:Site	24	114	5.6	< 0.0001

Site	lsmean	Group
1	50.3	b
2	24.5	a
3	41.5	b

Total N

	numDF	denDF	F-value	P-value
(Intercept)	1	114	2511.8	< 0.0001
Month	12	114	11.8	< 0.0001
Site	2	114	37.6	< 0.0001
Month:Site	24	114	4.2	< 0.0001

Site	lsmean	Group
1	0.745	b
2	0.67	a

3	0.823	c	

Total C

	numDF	denDF	F-value	P-value
(Intercept)	1	114	2260.4	< 0.0001
Month	12	114	12.6	< 0.0001
Site	2	114	46.9	< 0.0001
Month:Site	24	114	3.6	< 0.0001

Site	lsmean	Group
1	7.71	b
2	6.31	a
3	7.78	b

C/N

	numDF	denDF	F-value	P-value
(Intercept)	1	114	147256.5	< 0.0001
Month	12	114	22.5	< 0.0001
Site	2	114	139.1	< 0.0001
Month:Site	24	114	7.4	< 0.0001

Site	lsmean	Group
1	10.37	b
2	9.44	a
3	9.51	a

Soil respiration

	numDF	denDF	F-value	P-value
(Intercept)	1	100	656.8	< 0.0001
Month	12	100	8.6	< 0.0001
Site	2	100	0.8	0.47
Month:Site	24	100	3.2	< 0.0001

Site	lsmean	Group
1	*NA*	*NA*
2	*NA*	*NA*
3	*NA*	*NA*

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Chapter 6

Global evaluation of commercial arbuscular mycorrhizal inoculants under greenhouse and field conditions

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Abstract

The global market for beneficial microbial inoculants such as arbuscular mycorrhizal fungi (AMF) is rapidly increasing and there is substantial interest to use them for sustainable plant production. AMF are important plant symbionts that associate with most terrestrial plants. They improve plant growth and resistance towards a variety of abiotic and biotic stresses. These abilities have great economical potential which has resulted in an increasing number of commercial AMF inoculants. However, legal quality control procedures to ensure the effectiveness of these products are lacking. Here, we present the results of a global evaluation of AMF inoculants in which we included three independent studies across three continents (Australia, Europe and North America). The Australian and European studies tested 25 different commercial AMF inoculants in sterilized and non-sterilized soils under greenhouse conditions. This is supplemented by the North American study which evaluated the effects of 3 verified commercial inoculants under field conditions. In the greenhouse trials, we observed that 84% of the mycorrhizal inoculants did not lead to mycorrhizal root colonization when added to sterilized soil. In non-sterilized soil, the addition of these inoculants did not bolster mycorrhizal colonization in the presence of indigenous AMF. Metagenomic analysis in the field trial revealed changes in the mycorrhizal community after inoculation. For one inoculant, this was accompanied by increased biomass production. This global evaluation of commercial inoculants raises concerns over unreliable products which do not result in mycorrhizal root colonization when added to sterilized soils. Under field conditions, effects on plant growth are dependent on changes within the mycorrhizal community. The results of this study highlight the need for standardized quality control of AMF inoculants and further research on their establishment under field conditions.

6.1 Introduction

Improving the sustainability of our food production systems is a key issue to maintain human development within its planetary boundaries . To do so, biological fertilizers and microbial inoculants have been proposed as an important technology to reduce our dependency on energy-intensive agrochemicals (Bhardwaj et al., 2014). One such successful example of microbial inoculants is found within the rhizobium-legume symbiosis. The intensive research and development of a well-regulated market for rhizobium inoculants enabled farmers to produce high-protein crops and to reduce the application of mineral nitrogen fertilizer (Bullard et al., 2005). Likewise, many other beneficial microorganisms have been shown to improve soil quality and plant fitness (Abbott et al., 2018). However, despite their potential, the global commercialization of a wide range of microbial inoculations lagged behind the expectations. One reason being diverse regulatory definitions between countries or the absence of mandatory quality control criteria (Du Jardin, 2015).

One particular group of microbes with beneficial effects are the arbuscular mycorrhizal fungi (AMF). Most terrestrial plant species, including most crop species, form symbiotic associations with arbuscular mycorrhizal fungi (AMF) (Smith & Read, 2008). AMF provide valuable ecosystem services, including enhanced plant nutrient acquisition, growth, and stress resistance (Al-Karaki et al., 2004; Pozo & Azcón-Aguilar, 2007; Wu et al., 2008), improved soil structure (Rillig & Mummey, 2006), and reduced soil nutrient loss (Cavagnaro et al., 2015). Up to 80% of plant P and N is delivered to plants by mycorrhizal fungi. It is for these reasons that AMF are considered to play an important role in natural and agricultural systems and are an important target for sustainable land management (see Rillig et al., 2019 for a recent discussion).

The formation of arbuscular mycorrhizas (AM) can be promoted by increasing plant cover, reducing soil disturbance (Bowles et al., 2017) and mineral fertilization (Gryndler et al., 2006), or by altering agricultural management practices (Verbruggen et al., 2010) and crop rotations (Albizua et al., 2015). Where such options have been exhausted or are not feasible, AMF can be reintroduced into soils (Rocha et al., 2019). The number of companies producing AMF inoculants has increased substantially (Vosátka et al., 2008), and the global market value of microbial inoculants (of which AMF are an important category) is projected to reach US$ 3.622 billion by 2024 (Research & Markets, 2017). While inoculation of soils with AMF, especially under low P conditions, has been shown to increase mycorrhizal colonization and to improve plant growth in some studies (Bender et al., 2019; Cely et al., 2016; Hijri, 2016; Hoeksema et al., 2010; Köhl et al., 2016; Lekberg & Koide, 2005; Pellegrino et al., 2015; Zhang et al., 2019), the production and distribution of such inoculants is challenging and quality control is often missing (Herrmann & Lesueur, 2013; von Alten et al., 2002). Moreover, the addition of fertilizers to some inoculants may potentially mask any effects of AMF on plants, further complicating efforts to assess their impact on AMF. Thus, with increasing interest in the use of AMF inoculants, there is a need for an independent, critical and broad evaluation of the reliability and efficacy of commercially available AMF products.

Here we present results of a global evaluation of commercially available AMF inoculants. We performed three independent studies across three continents (Australia, Europe and North America), in which we assessed 28 different commercial AMF inoculants in terms of their mycorrhizal inoculation potential (MIP) and their plant growth responses under a broad range of different growing conditions. The three experiments were independently performed by three research groups and started due to concerns over unreliable commercial inoculants. These concerns are mostly based on anecdotal knowledge within the research community (Pickles et al., 2020) as well as outdated and limited scientific research on specific products (Corkidi et al., 2004; Tarbell & Koske, 2007). The Australian and European studies involved two greenhouse bioassays each, in which the

inoculants were tested under AMF-favourable conditions using sterilized soil and non-sterilized soil. The North American study complements both greenhouse studies by testing three commercial inoculants under field conditions and evaluating their impact on the AMF community using metagenomics analysis (see Figure 6.1).

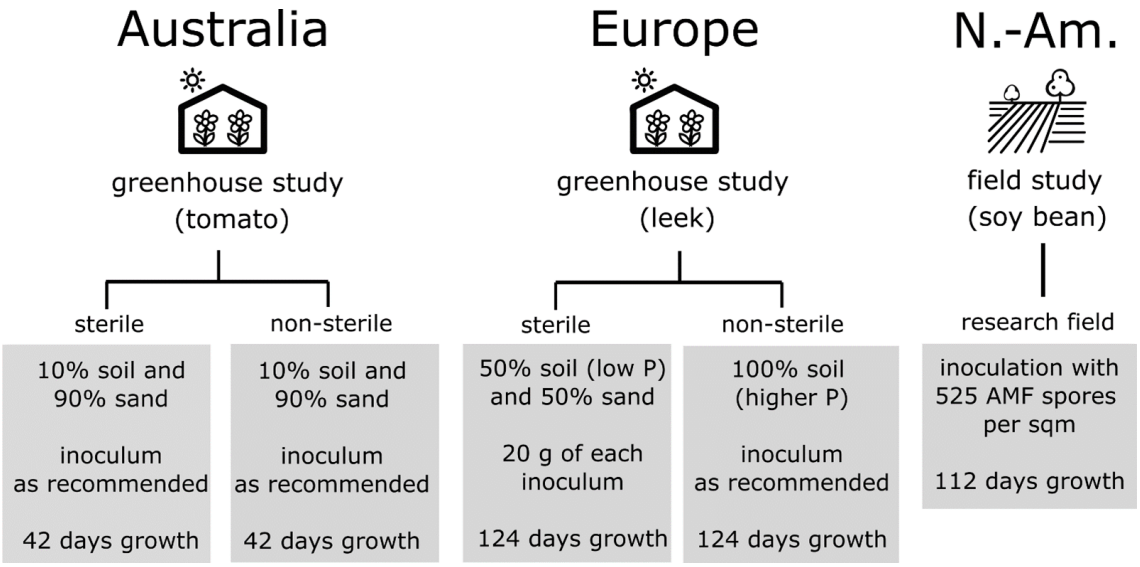

FIGURE 6.1: *Overview of all three studies and their corresponding research approach.*

6.2 Material and Methods

This study analysed a total of 28 commercial AMF inoculants, 10 were tested in Australia, 15 in Europe and 3 in North America. All inoculants were commercially sourced and have been de-identified for the purpose of this study. The inoculants were evaluated regarding their potential for mycorrhizal inoculation and mycorrhizal growth response. Plant biomass and mycorrhizal root colonization data were gathered from two separate bioassays using sterile and non-sterile soil (Europe and Australia) and a field study (North America). AMF spore abundance in the different inoculants was measured by means of spore extraction. Furthermore, in the Australian study the inoculants were analysed for their concentration of available plant nutrients (P and N) (see Suppl. Table 6.2).

6.2.1 AMF inoculants

To justify inclusion in this study, inoculants needed to contain at least one isolate of AMF and be labelled for commercial agri- or horticultural applications. Inoculants of the North American study were selected based on previous confirmation of viability and successful root colonization under controlled conditions. Using online search engines and knowledge of commercial producers, a total of further 25 different inoculants were purchased in the Australian and European study; none were produced by the same company. Across all inoculants, 12 contained additional plant growth promoting microbes and a total of four inoculants also contained ectomycorrhizal propagules. None of the products had

exceeded their expiration dates (if one was provided). Most inoculants used ground clay or other inert substrates as carrier materials. Inoculant G (Australia) contained high concentrations of plant available nutrients (above 5% NPK) (see Suppl. Table 6.2).

Laboratory-cultured isolates of *R. irregularis* were used in the Australian and European studies as a positive control. These isolates were subcultured on Marigold (*Tagetes patula nana*, Australia) and *Plantago lanceolata* (Europe) in a closed pot system (Walker & Vestberg, 1994). Previous studies demonstrated the effectiveness of these isolates and high mycorrhizal root colonization rates after inoculation (Bender et al., 2019; Köhl et al., 2016; Watts-Williams et al., 2019).

6.2.2 Greenhouse experimental designs (Australia and Europe)

In order to quantify the MIP of the different inoculants, the Australian and the European studies conducted two separate greenhouse bioassays, using non-sterilized and sterilized soils. In the first bioassay, sterilized soil was used to give information about the inoculant's maximum MIP in an artificial environment, while the second bioassay would create a more similar testing environment to natural conditions in agricultural applications. In the Australian study and the first greenhouse bioassay of the European study, all inoculants were applied according to the recommendations on the product label or in the more detailed description that could be found online on the websites of the different companies. The second greenhouse bioassay of the European study used higher application rates in order to apply the same amount of inoculum across all treatments and as a way to verify the presence of viable AMF propagules. All inoculants were either applied directly into the planting hole, mixed into the whole substrate, or applied as solution, as per manufacturer instructions.

Australian greenhouse study

In the Australian study, tomato (*Lycopersicum esculentum* L.) cv. 76R was used as a host plant. With the exception of the soil, the experimental design and measurements taken from both bioassays were identical. The first bioassay (chronologically the second bioassay) was conducted in October 2018 with double-autoclaved soil that was collected from the Waite Arboretum 2017 (latitude 34°58'07.4" S, longitude 138°37'56.5" E) in August 2018 (Austral winter). The second bioassay was conducted in March 2018 with a non-autoclaved soil (see below) that was collected from the same location in January (Austral summer). The location of the collected soil was in close proximity to a mature *Pyrus amygdaliformis* tree and otherwise dominated by grassland. Soil was collected from the 0 – 10 cm soil layer. Soil from a similar location had already been used in previous experiments and was found to support good plant growth as well as a high AMF colonization by indigenous and introduced AMF. The non-autoclaved Arboretum soil contained on average 19 indigenous AMF spores g^{-1}. The soil was classified as an Urrbrae red-brown earth (Alfisol) (Cavagnaro, 2016) and before the soil (collected in January 2017) was mixed

with sand (see below), the soil had a pH of 6.5 (1:5 water extract), a plant-available (Colwell) P concentration of 10.5 mg kg^{-1}, and a DTPA-extractable Zn concentration of 28 mg kg^{-1}.

The bioassays included one non-inoculated control and eleven treatments of which ten were commercial inoculants and one positive control of *R. irregularis* (WFVAM10). For the bioassay with sterilized substrate, the control plants were inoculated with 10% (w/w) pot volume AMF-free mock substrate to account for non-mycorrhizal soil microorganisms. The mock inoculum was cultured under similar conditions as the *R. irregularis* inoculum (see above) but lacked AMF propagules in the soil.

Each pot contained 1,500 g of substrate which was prepared from one part soil and nine parts washed fine sand (w/w). Additionally, 20 mg kg^{-1} P in the form of calcium phosphate dibasic ($CaHPO_4$) was added. Previous studies demonstrated that this concentration of P was sufficient to support good plant growth and to maintain high mycorrhizal root colonization in the tomato variety 76R (Watts-Williams & Cavagnaro, 2012). Depending on the treatment and the amount of inoculum added, the substrate was adjusted to a final weight of 1,500 g. To assess the potential for plant nutrient deficiencies or toxicity as a result of the autoclaving process, the growth substrate was analysed for various nutrients (see Suppl. Table 6.3).

Tomato seeds (76R genotype) were surfaced-sterilized in a 6% sodium hypochlorite (NaOCl) solution for 15 minutes, then rinsed and pre-germinated on moist filter paper. After germination, the seeds were planted into double-autoclaved sand for two weeks and transplanted into the final substrate after the first true leaves emerged. For both bioassays, plants were grown for 42 days in an environmentally controlled greenhouse and randomized weekly. Two 1000 W metal-halide growth lamps were used to provide supplemental light for 16 hours per day. Average greenhouse temperatures for the first bioassay in October averaged at 23 °C during the day and 11.2 °C at night. For the second bioassay in March, 22.9 °C during the day and 12.7 °C at night were recorded. The plants were watered once per week with 10 mL of a modified Long-Ashton solution minus P (Cavagnaro et al., 2001) and watered daily to 10% gravimetric moisture content using reverse osmosis (RO) water, reaching the substrate's field capacity. The substrate of all pots was covered with an air-permeable white fabric to reduce evaporation and avoid cross-contamination between treatments. Plants were destructively harvested after 42 days. The roots were carefully washed free of soil and separated from the shoots. A subsample of fresh roots was collected and stored in 50% ethanol for subsequent staining and mycorrhizal root colonization measurement. Shoots and roots were dried at 60 °C for 48 hours, and the dry weights recorded.

European greenhouse study

The European study used leek (*Allium ampeloprasum*) cv. Longton as host plant for the greenhouse bioassays. Both bioassays were conducted in a similar way and mainly differed in the used soil and the inoculant application rate. Minor changes were made regarding the pot and soil volume between the first and second bioassay. The first bioassay was performed in July 2018 (chronologically the second bioassay) and used an equal mix of autoclaved field soil and sand. The loess field soil was provided by the Research Institute of Organic Agriculture (FiBL) and sieved to <3 mm before it was mixed with quartz. Both substrates were separately autoclaved at 121 °C for 90 minutes before mixing. The soil mixture had a pH of 8.5, total P of 492 mg kg^{-1} and total nitrogen of 0.006% in the mixed soil (see Suppl. Table 6.4). Pots with 1.3 L capacity were used and filled with 1 L of soil; 20 g of inoculum were used for all treatments, regardless of manufacturers' recommendations.

The second bioassay was conducted in June 2018 and used non-sterilized field soil with inoculum application rates as recommended by the manufacturer. The used field soil was a silt-dominated Cambisol and collected from an "integrated production"-certified farm in the Canton Aargau, Switzerland (latitude 47°32'17.2" N, longitude 8°17'25.3"E). The previous crop rotation of this soil included winter wheat and bush beans. Soil analysis was performed by Agroscope Reckenholz and measured a pH of 7.5, total P of 809 mg kg^{-1} and total nitrogen of 0.15% (see Suppl. Table 6.4). The soil was collected from the 0-30 cm layer and sieved to <5 mm before usage. 900 mL soil were filled into 1,000 mL-sized pots without further treatments added to the soil. Inoculum of each treatment was applied as recommended by the manufacturer.

Leek seeds were surface sterilized in 2.5% NaClO for 20 minutes and propagated for 26 days before being transplanted into the final substrate in the greenhouse. A sterilized mix of 70% sand and 30% expanded clay was used as propagation substrate.

For both experiments, four leek seedlings per pot were planted for the first three weeks and were then reduced to three plants per pot afterwards. Pots were covered with grit to minimize soil evaporation and contamination between treatments. Average greenhouse conditions were 25 °C during the day and 18 °C at night. Plants were watered to field capacity with rainwater and randomized every two weeks. Fertilizer was applied twice during the last two months of each bioassay using a modified Hoagland solution containing one fourth P (= 0.125 mM P as $NH_4H_2PO_4$). Due to the different soil volumes between the bioassays, different amount of fertilizer have been applied as follows: For the first bioassay, 60 mL were applied in October and November. For the second bioassay, 47 mL of modified Hoagland solution were applied in September and October. Both bioassays were destructively harvested after 124 days. Roots were cleaned of soil and separated from the shoots. Subsamples of fresh roots were stored in 70% ethanol to determine the mycorrhizal colonization of roots. Root and shoots were then dried at 60°C

for 48 hours and the dry mass was recorded.

Field study experimental design (North America)

The North American study examined the effects of three commercially available mycorrhizal inoculants on the mycorrhizal root colonization and biomass of field-grown soybean plants (cultivar AG1234). The field experiment was conducted at the Aurora Research Field Station, South Dakota State University. The field soil had a pH 5.6, plant-available (Olsen) P of 12.4 mg kg^{-1}, NH_4^+ 1.5 mg kg^{-1} and NO_3^- 10.3 mg kg^{-1} (see Suppl. Table 6.5). The experiment was conducted in a randomized block design with five plots per treatment and a plot size of 9 m^2. Plant density was at 395,000 seeds ha^{-1} which resulted in four plant rows per plot. Only the two inside rows were used for the analysis, while the two outside rows were treated as border rows. Two weeks after seeding, the commercial inoculants were applied at a rate of 525 AMF propagules m^{-2}. The fungicide Topsin M (negative control) was also applied two weeks after seeding and every two weeks thereafter at a rate of 1.25 g m^{-2}. Plant shoots and roots were destructively harvested after 16 weeks and dried for 48 hours at 70 °C. Fresh subsamples of the roots was stored in 50% ethanol for subsequent mycorrhizal root colonization measurement.

6.2.3 Mycorrhizal root colonization measurement

In all studies, roots were stained according to (Vierheilig et al., 1998). For this, plant roots were first cleared in a 10% KOH solution before being stained using an ink and vinegar solution. Mycorrhizal root colonization was calculated according to the intersect grid method (McGonigle et al., 1990), counting at least 100 intersects per sample under a dissection microscope (60 to 80 X magnification).

6.2.4 Inoculum analysis

AMF spores of the inoculants were extracted using the wet-sieving and decanting technique (Gerdemann & Nicolson, 1963). AMF spores were then collected between a 38 and 500 μm sieve and further purified in a sugar gradient centrifugation (Brundrett et al., 1996). The supernatant was transferred to a Petri dish and AMF spores were counted under a dissection microscope.

Inoculants of the Australian study were further analysed for their physiochemical composition and concentration of plant nutrients. Three sub-samples of each inoculant were taken and analysed as follows. One sub-sample was extracted in water (1:5) and pH and EC were measured using a TPS WP-81 pH & conductivity meter (EnviroEquip Biolab, Australia). Another sub-sample was extracted using 2 M KCl as described in (Cavagnaro et al., 2006) and used for colorimetric determination of mineral N (NH_4^+ and NO_3^-). The last sub-sample was extracted in 0.5 M sodium-bicarbonate solution at pH 8.5 and used for colorimetric determination of plant-available (Colwell) P, using the Murphy & Riley colour reagent (Murphy & Riley, 1986).

6.2.5 Metagenomic analysis (North American study)

Metagenomic analysis using the AMF-specific primers AMV4.5NF – AMDGR (van Geel et al., 2014) was conducted on root samples after DNA extraction and primer-based metabarcoding. Five soil samples of each treatment were collected, and the roots extracted. The root samples were washed free of soil and stored at -80 °C. DNA was extracted and 100 ng sample RNA was used for the cDNA preparation with the TruSeq DNA LT sample kit (Illumina, San Diego, CA, USA). The concentrations of the final cDNA libraries were measured with the HS dsDNA kit in a Qubit 3 (Thermo Fisher Scientic, Waltham, MA, USA). The average size of the library was determined by the DNA nano kit in a BioAnalyzer 2100 (Agilent, Santa Clara, CA, USA). The final libraries were diluted to 4 nM individually and pooled together for library denaturation, and a final concentration of 1.8 pM was loaded and sequenced by Illumina NextSeq 500 sequencing with an output of 2x151 pair reads. The sequencing data were analysed using Mothur (v1.42.3) (Schloss et al., 2009) as follows: Contigs (contiguous fragments) were assembled and low quality and duplicate contigs removed. Chimeras were removed and the remaining contigs were clustered into OTUs which were then classified according to MaarjAM database version 8 (2019-06-05) (Öpik et al., 2010). Following analysis was then performed on the taxonomic assignment rather than OTUs. 10,454 unique OTU sequences were identified which were assigned to 12 different genera. Five of these genera were assigned as 'unclassified' at a higher taxa level, potentially encompassing several genera. Downstream analysis was done in R (v. 4.0.0) and phyloseq (v. 1.32.0) (McMurdie & Holmes, 2013) and included only the three replicates with the highest reads. This was done in an effort to normalize library size between treatments due to uneven read numbers. The abundance values of OTUs in the dataset were transformed to relative abundances and filtered to keep only those with a variance greater than 1e-4. The workflow was verified by including a sample of *R. irregularis* as positive control (data not presented here).

6.2.6 Statistical analysis

The Australian study used a randomized complete block design with a repetition of six pots per treatment. Pots were randomized weekly. In the first bioassay, a total of seven plants were omitted: Two plants showed the same symptoms of mutated growth as in the first bioassay and another five plants from treatment G died between weeks four and six. For the second bioassay, one pot was omitted from statistical analysis due to mutated growth and lack of sympodial branching.

Data from both bioassays was skewed and non-normally distributed. The non-parametric Kruskal-Wallis one-way analysis of variance with Bonferroni correction was then used to discover differences within groups. Where significant differences were identified, Fisher's Least Significant Difference was used as a post hoc test. All data was analysed

with R (version 3.5.0) and the package 'Agricolae' (version 1.2).

The European study used a randomized complete block design with seven pots per treatment in the first bioassay and six pots per treatment in the second bioassay. Each pot was planted with four seedlings and reduced to three plants at week four. Due to the uneven numbers of alive plants per pot, the mean value of each alive plant in a pot was used as a statistical replicate. Inoculant 11 was omitted from both bioassays as it resulted in complete plant loss, even for replanted seedlings. Similar effects appeared for treatments 5, 10 and 15 in the first bioassay, which were therefore omitted as well. Treatment 1 was not available in sufficient quantities for the first bioassay (which was chronologically the second bioassay).

Data from the European study followed a non-normal distribution. Similar to the Australian study, the non-parametric Kruskal-Wallis one-way analysis of variance with Bonferroni correction was used to identify differences within groups. Fisher's Least Significant Difference was then used as post hoc test. All data was analysed with R (version 3.5.0) and the package 'Agricolae' (version 1.2).

The North-American study used a randomized block design with five plots per treatment. Plants of the two middle rows of each plot were harvested and each plot was treated as a replicate. Biomass data showed a normal distribution pattern and was analysed using analysis of variance (ANOVA). Multiple comparison was then performed using the function glht() in the 'multcomp' package (version 1.4) and R (version 3.5.0). Mycorrhizal root colonization was non-normally distributed and was therefore analysed as above, using the non-parametric Kruskal-Wallis one-way analysis of variance with Bonferroni correction and Fisher's test of Least Significant Difference.

6.3 Results

6.3.1 Validation of commercial inoculants in sterilized soil (Australia and Europe)

We first performed a range of experiments to test whether commercial inoculants contained viable propagules and promoted plant growth in sterilized substrate with tomato (Australia) and leek (Europe) as host plants. Plants inoculated with a validated laboratory strain (*Rhizophagus irregularis*) were heavily colonized by AMF (48% in the Australian study and 77% in the European study), while the control plants remained practically uncolonized (see Figure 6.2 A). The very low percentages ($< 2\%$) of root colonization in the control treatments could be caused by non-AMF root colonizing fungi. For the commercial inoculants, only 4 of the 25 treatments resulted in a distinct formation of AM (which we define as >20% root length colonized) - all of which were in the European study. These products, (2, 6, 7 and 12) resulted in an AMF colonization between 82 and

89%. Lower ($\leq$ 12%). Highly variable colonization percentages were found for two other inoculants (3 and 13) in the European study. None of the commercial inoculants included in the Australia study resulted in distinct formation of mycorrhizas (all $\leq$ 6% colonization).

In the European experiment, treatments 2, 6, 7, 12 and the positive control (the laboratory strain of *R. irregularis*) led to significant positive growth responses of leek in sterile soils (see Figure 6.2 B). In the Australian study, only inoculant G (which contained high measured concentrations of NPK) resulted in a higher plant biomass than the non-inoculated control. In some treatments (1, 5, 10, 11 and 15) of the European study, plant biomass data are unavailable because all plants died after inoculation (see Discussion and supplementary material 6.4.4 for further explanation).

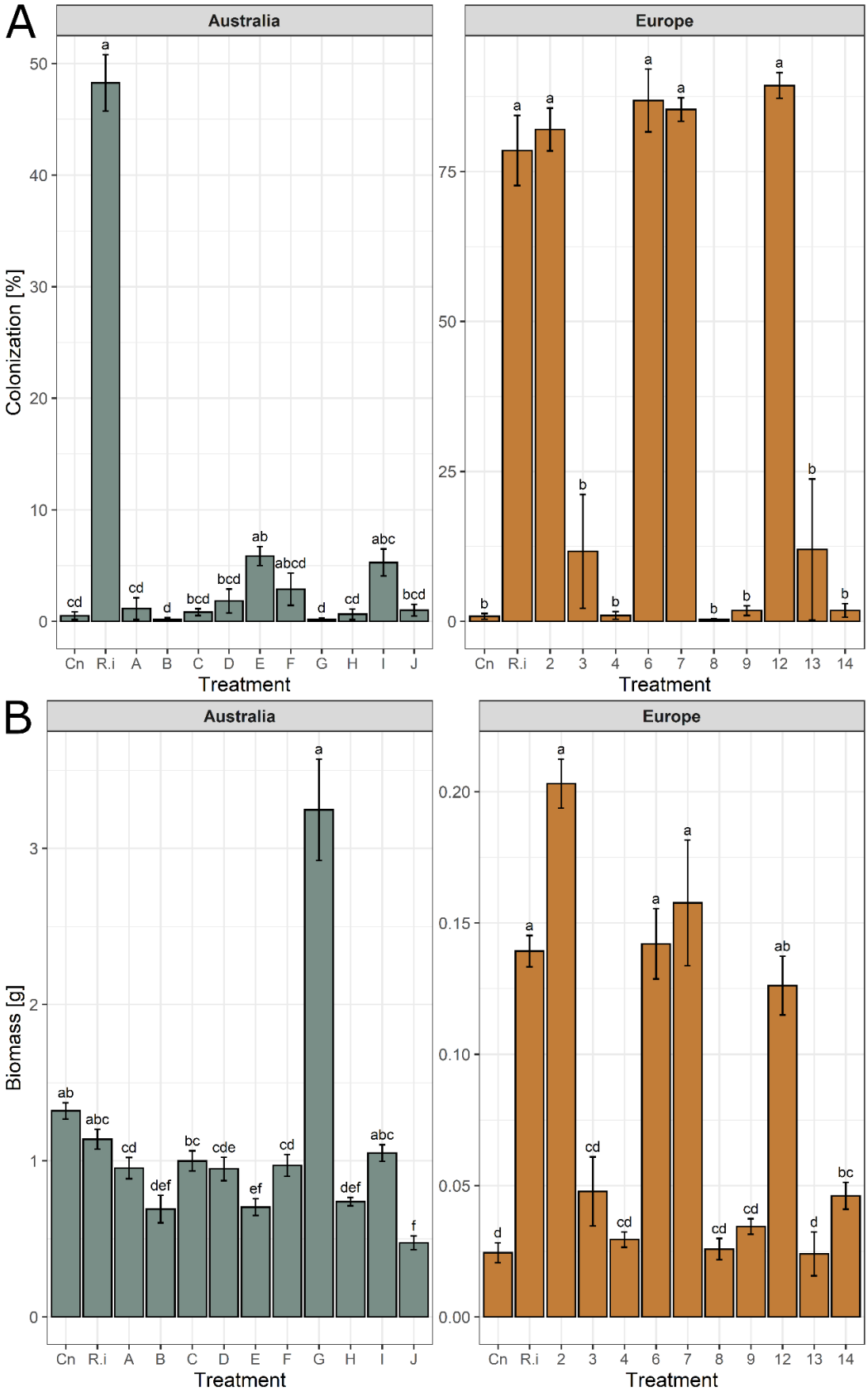

FIGURE 6.2: *A: Mycorrhizal root colonization of tomato (Australia) and leek (Europe) in the greenhouse bioassays using sterilized soil. B: Total plant biomass of tomato (Australia) and leek (Europe) in the greenhouse bioassays using sterilized soil. Error bars indicating SE. Letters and numbers refer to the different commercial inoculants tested. Cntr= control treatment without AMF addition; R.ir = Rhizophagus irregularis (positive control).*

6.3.2 Effect of commercial inoculation on root colonization and plant biomass in unsterilized field soil

We then tested whether commercial AMF inoculants enhanced root colonization and promoted plant growth in unsterilized field soils. Roots in all treatments were colonized by AMF (see Figure 6.3), reflecting the natural AMF potential in the soil under AMF-favourable growing conditions. The natural root colonization levels for the control were highest in the Australian study (tomato; 56%), followed by North America (soybean; 49%) and Europe (leek; 17%). The addition of a pure laboratory culture of *R. irregularis* enhanced root colonization around 3-fold in the European study but remained unchanged in the Australian study. No pure laboratory culture was used in the North American study.

Across all three studies, only one out of the 28 commercial inoculants resulted in significantly higher root colonization (treatment 13 in the Europe study) than in the respective control. None of the commercial inoculants in the Australian and North America studies bolstered mycorrhizal root colonization in the presence of indigenous AMF. In one case, AM colonization was inhibited (inoculant G in the Australian study). The fungicide control treatment in the North America study significantly reduced mycorrhizal root colonization compared to the other treatments.

Total plant biomass did not correlate with mycorrhizal root colonization when plants were growing in non-sterilized soil, or field soil (see Figure 6.3 B). For example, plants treated with inoculum A in the North America study had significantly higher total biomass compared to the other treatments, although there were no differences in the mycorrhizal root colonization. Significant positive growth responses were observed using inoculants that contained fertilizer, such as treatment G in the Australian study, as well as 10 and 15 of the European study. Other treatment effects in the greenhouse bioassays were variable and again not correlated to mycorrhizal root colonization.

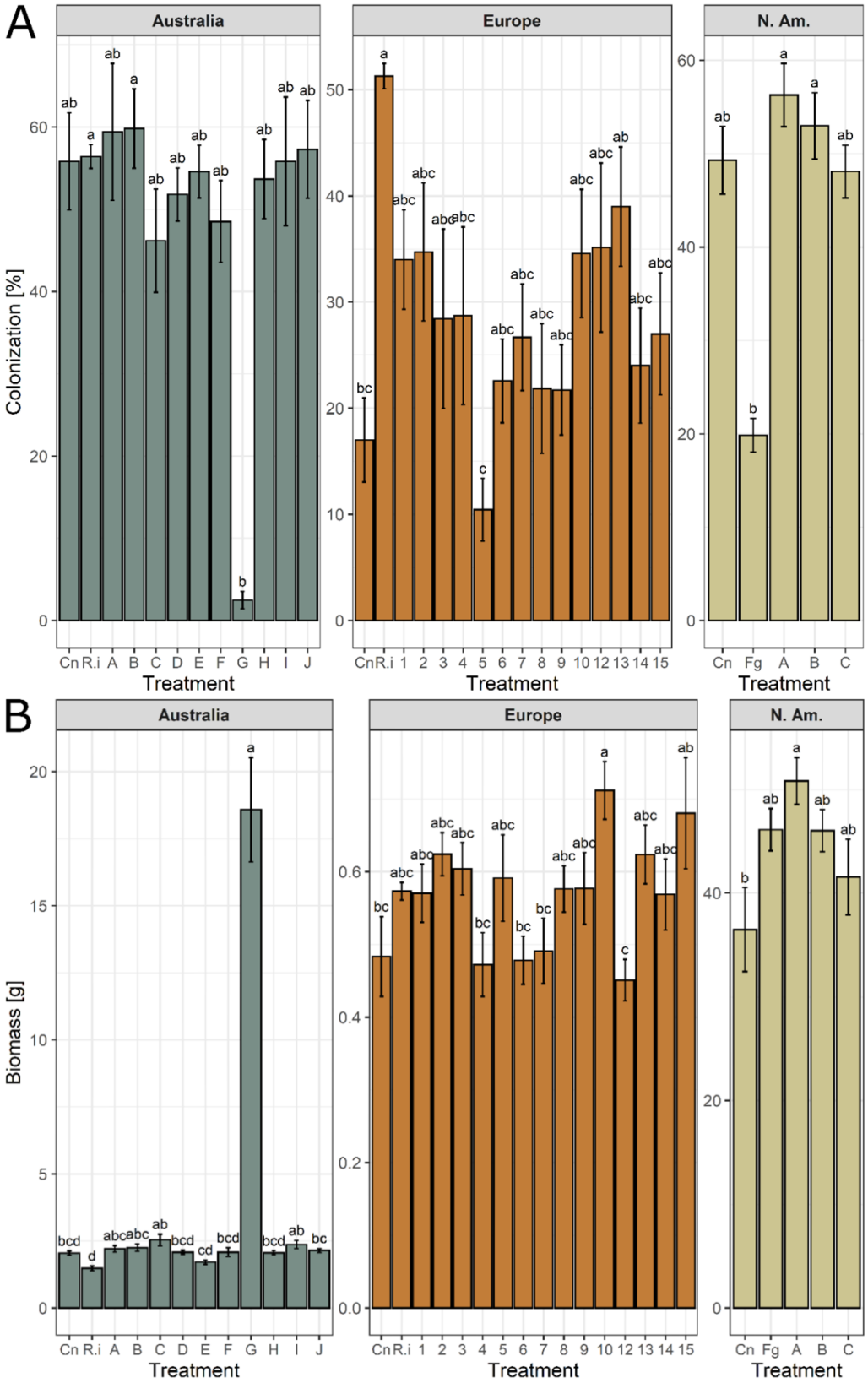

FIGURE 6.3: *A: Mycorrhizal root colonization of tomato (Australia) and leek (Europe) plants in greenhouse bioassay using non-sterilized soil and of soybean plants (N. America) under field conditions. B: Total plant biomass of tomato (Australia) and leek (Europe) plants in greenhouse bioassays using non-sterilized soil and of soybean plants (N. America) under field conditions. Error bars indicating SE. Letters and numbers refer to the different commercial inoculants tested. Cntr= control treatment without AMF addition; R.ir = Rhizophagus irregularis (positive control); Fngc = fungicide treatment (negative control).*

The metagenomic evaluation of colonized roots in the North American study targeted mycorrhizal communities using AMF-specific primers (AMV4.5NF – AMDGR). Alpha diversity (Shannon) was highest in the control, followed by the fungicide treatment. The addition of either of the three inoculants reduced AMF diversity, especially for inoculant B (see Figure 6.4). The AMF profile of the control group consisted of various genera within the Glomeromycota. Many genera disappeared in treatments A and B which were then dominated by AMF that matched the content of the inoculants (see Suppl. Figure 6.6).

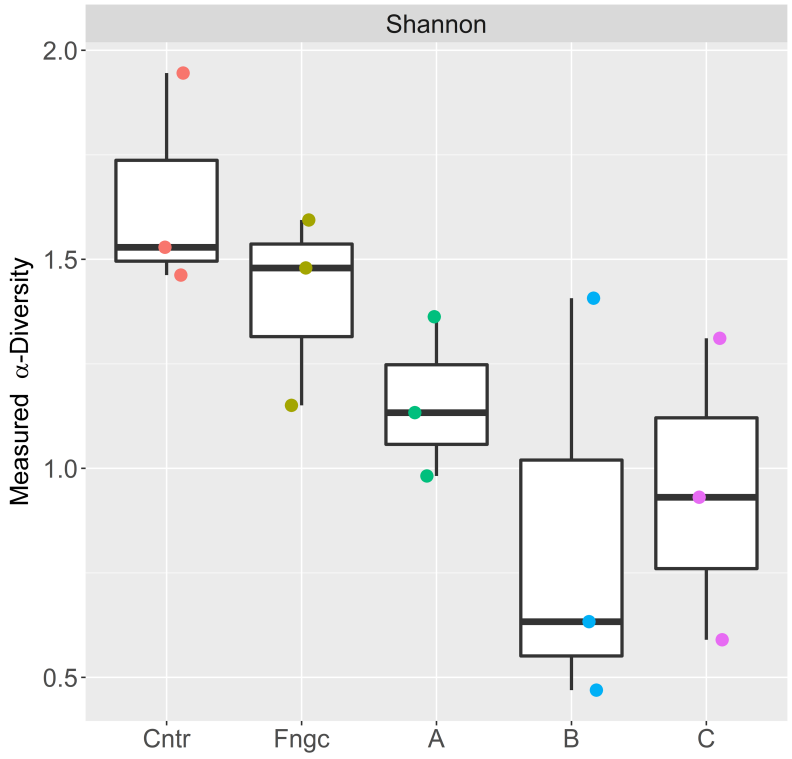

FIGURE 6.4: *Shannon alpha diversity of the AMF communities in colonized soybean root pieces from field soil in the North American study. Cntr = Control treatment without AMF addition, Fngc = fungicide treatment (negative control), A-C = inoculation treatments using commercial AMF inoculants.*

6.3.3 Inoculants spore count

The spore concentration of the inoculants showed big variations within each study and ranged from 1 spore g^{-1} (G) to 1715 spores g^{-1} (H) (see Table 6.1). No spores were found in inoculant 10 of the European study. The inoculants were applied as recommended in both Australian bioassays and the second bioassay of the European study. Following those recommendations, inoculants with already small spore concentration were further diluted, such as inoculant J (Australia) which was diluted from 22 spores g^{-1} to 4 spores pot^{-1}. Inoculants that resulted in mycorrhizal root colonization in the European study had either a high concentration of spores or were applied at a high dosage (2, 7, 12).

However, many inoculants with high spore concentrations or application rates did not result in mycorrhizal root colonization.

6.4 Discussion

6.4.1 Product quality of mycorrhizal inoculants

It is estimated that the world's population will exceed nine billion by the year 2050. Thus, global agriculture will have to face the task of almost doubling food production, while reducing the dependence on agrochemicals, and reducing environmental costs linked to the excessive use of fertilizers and pesticides (Berruti et al., 2016; Tilman et al., 2002). There is an increasing interest in using biofertilizers and developing soil microbial inoculants for sustainable food production. As a consequence, industry has boosted investments into the development of microbial inoculants (Kaminsky et al., 2019) with an expected market value of US$ 3.622 billion by 2024 (Research & Markets, 2017). Those inoculants can be segmented into AMF inoculants, as they are discussed in this study, and other microbial inoculants which are increasingly used to promote plant growth like nitrogen fixing bacteria (Figueiredo et al., 2011), *Trichoderma sp.* (Stewart & Hill, 2014) or mixtures of various microbes with presumed beneficial effects for plants or ecosystem processes (de-Bashan et al., 2012).

This study, in which a total of 28 different products over three continents were tested, demonstrates that the product quality of arbuscular mycorrhizal inoculants is highly variable when analysed as percentage root colonization. Out of the 25 commercial inoculants that were tested in sterile soil and under AMF-favourable conditions, only four (all found in the European study) resulted in distinctive mycorrhizal root colonization. Those results demonstrate that about 84% of the tested inoculants did not contain viable propagules, as defined by the ability of propagules to develop arbuscular mycorrhizal symbiosis.

The reasons for failed mycorrhizal colonization are likely due to one or a combination of the following factors: I: Insufficient amount of AMF propagules in the inoculum; II: AMF propagules were not viable at time of production or propagules did not survive packaging; III: AMF propagules were dormant or inactive due to prolonged storage; IV: Recommendations linked to storage or application of the product were sub-optimal; V: The added AMF strains were not adapted to the soil or the host plants used in this study; VI: The AMF strains are not compatible with other materials (e.g. nutrients) included in the inoculum. Irrespective of the causes, there is clearly a need for more rigorous testing to verify spore abundance, germination and viability of the products at time of production, and after their distribution and/or storage.

TABLE 6.1: *AMF spore count analysis of the tested inoculants. NA = Not enough inoculant available and not possible to re-order; DB = re-ordered and analysed from a different batch. Numbers in brackets indicating standard error.*

Australia			**Europe**			
Inoculant	Spores g^{-1}	Spores applied per pot (average)	Inoculant	Spores g^{-1}	Spores applied per pot (average)	
		Bioassay 1 and 2			Bioassay 1	Bioassay 2
A	9 (±0.4)	17	1	131 (±35)	2620	131
B	7 (±2.9)	3	2 (DB)	55 (±3.6)	1100	2140
C	189 (±46.5)	783	3	*NA*	*NA*	*NA*
D	77 (±10.7)	1452	4	20 (±5.3)	400	20
E	30 (±1.3)	38	5	110 (±6.6)	2200	350
F	5 (±1.2)	19	6	*NA*	*NA*	*NA*
G	1 (±0.5)	30	7	24 (±1.4)	480	640
H	1715 (±132.5)	7718	8	NA	NA	NA
I	14 (±0.4)	47	9	22 (±3.3)	440	42
J	22 (±0.4)	4	10	0 (±0)	0	0
North America			11	*NA*	*NA*	*NA*
Inoculant	Spores g^{-1}	Spore applied m^{-2} (average)	12 (DB)	267 (±23.8)	5340	267
A	280 (±32.3)	525	13	24 (±6.3)	480	28
B	2744 (±85.5)	525	14	*NA*	*NA*	*NA*
C	93 (±18.7)	525	15 (DB)	13 (±2.2)	260	13

Most of the inoculants contained AMF taxa that colonize a broad range of host plants and soil types (e.g. *Rhizophagus irregularis*; *Funneliformis mosseae*). For instance, 75% of products contained strains of *Rhizophagus irregularis* (previously known as *Rhizoglomus irregularis* or *Glomus intraradices*; (Redecker et al., 2013)) a fungus with a worldwide distribution (Öpik et al., 2006) and ability to colonize a wide range of host plants (Smith & Read, 2008), including many crops such as wheat, maize, rice, potato, tomato and grasses (Hoeksema et al., 2010). In view of its broad habitat preference and host range, *Rhizophagus irregularis* is a suitable candidate for commercial products.

Another element to consider are additives to the inoculum, and whether they are labelled or not. AMF activity is often inhibited by fertilizer application (Smith & Read, 2008) and the mixing of fertilizer and AMF propagules in some products (as suggested by high levels of measured NPK) could have contributed to the reduced efficiency of these products. The nutrient analysis of the Australian study showed that many inoculants contained high levels of plant nutrients, although not labelled as such (see Suppl. Table 6.2). Some inoculants also contained a variety of plant growth promoting microorganisms which could be parasitic to AMF (De Jaeger et al., 2010).

6.4.2 Root colonization in non-sterilized soil

Inoculants of all three continents were tested in non-sterilized soil under greenhouse conditions (Australia, Europe) and field conditions (North America). This approach is a realistic scenario in which producers or growers would add the inoculum to field soil in an effort to bolster the natural mycorrhizal inoculation potential.

The results of the North American field study are consistent with previous studies (Schlaeppi et al., 2016). The establishment of inoculants in field soils and under field conditions with indigenous AMF populations is more difficult to predict than in sterilized soil. For instance, Kokkoris et al., 2019 found that the establishment of an inoculated fungus was not related to cropping or inoculation practices and was site specific. Moreover, indigenous AMF might be better adapted to the local conditions and outcompete some of the inoculated fungi (Oehl et al., 2010). Alternatively, inoculated fungi might replace native AMF (Bender et al., 2019; Schlaeppi et al., 2016) and may invade new areas with unintended ecological consequences (Schwartz et al., 2006) such as the observed pine invasions in areas where the fungal symbionts (ectomycorrhizal fungi) of pine trees were originally absent (Nuñez et al., 2009; Policelli et al., 2019). Similarly, soil inoculation in the North American field study resulted in lower alpha diversity, probably due to the displacement of native AMF and the reduction of species richness. Although we cannot determine within the scope of this study whether the introduced AMF species will persist in the field soil or not, it is a possibility that AMF inoculants can become invasive (Thomsen & Hart, 2018). This is an issue that deserves attention, especially if non-native AMF are distributed.

The non-inoculated control plants of both greenhouse studies revealed the presence of indigenous AMF propagules and subsequently displayed relatively high natural mycorrhizal root colonization. Plants in the Australian study were equally colonized with no significant differences between the treatments. Only inoculant G of the Australian study suppressed root colonization by AMF, probably due to its high fertilizer content. Increases in mycorrhizal root colonization were not expected after the inoculants failed in the first bioassay with sterilized soil. Furthermore, the equally high root colonization between the control and *R. irregularis* treatment led to the conclusion that full mycorrhizal potential in the soil had already been reached due to the presence of native AMF propagules.

In the European study, mycorrhizal root colonization was more than doubled in the treatment with *R. irregularis* compared to the control. However, among all the tested commercial inoculants, only treatment 13 was able to significantly increase root colonization. Even treatments that proved effective in the sterilized soil failed to lead to significant changes in the mycorrhizal colonization in field soil. This might be a reflection of the smaller amount of inoculum that was used, the presence of an indigenous AMF community that could impair establishment or outcompete colonization by introduced AMF, or other soil characteristics (e.g. higher P content) of the field soil in this study. Alternatively, inoculated AMF might replace other fungi without altering the overall level of mycorrhizal colonization (Schlaeppi et al., 2016). Our results also suggest that dosage recommendations of some of the companies are either too conservative, or the spore viability is not sufficient.

6.4.3 Mycorrhizal growth response (MGR)

Generally, inoculants with high nutrient concentrations (e.g. G in the Australian study) can lead to biomass increases in non-sterilized soil. However, caution is required, as extremely high nutrient concentrations can be harmful and cause plant mortality (see Suppl. 6.4.4) as has been observed before (Hardesty, 1967). The nutrient analysis of all Australian inoculants revealed high concentrations of mineral nitrogen or plant available phosphate in some inoculants, although not identified on the product labels (see Suppl. 6.2). Treatment effects in the Australian study were highly variable and not associated with mycorrhizal root colonization. Moreover, the results of the sterilized and non-sterilized soil experiments were inconsistent. The overall biomass was higher in non-sterilized than sterilized soils, highlighting the influence of the natural soil biota on plant growth (van der Heijden et al., 2008). The application of viable commercial inoculants to the field soil demonstrated the interplay between introduced and native AMF species. Although one introduced inoculant provided better above-ground functional traits towards the host plant, it outcompeted native mycorrhizal communities and reduced mycorrhizal diversity.

Although tested under AMF-favourable conditions, most inoculants of the European and Australian studies failed to bolster mycorrhizal root colonization. This situation is not satisfactory in terms of consumer protection, and it discredits a market that might play a key role for future sustainable agriculture. The results gathered in this study warrant the introduction of standardized quality control guidelines for AMF inoculants.

Those guidelines should include: I: Transparent labelling and documentation of the inoculant's content and its production method, including expiration dates and detailed instructions for usage in different soil environments; II: High concentrations of viable spores or propagules that can colonize the target host within an acceptable time frame; III: A selection of microbial strains that are suitable for the proposed environment; IV: Suitable carrier material that facilitates application of inoculum; V: Any additives have beneficial or neutral impact on AMF development; VI: Tests of spore viability and germination rates; VII: Greenhouse tests showing that inoculants colonize plant roots and lead to enhanced plant growth under controlled conditions (see Fig. 6.5). Overall, this work demonstrates that in order for AMF biofertilizers to become a global asset for sustainable agriculture, there is an urgent need for the establishment of global quality standards.

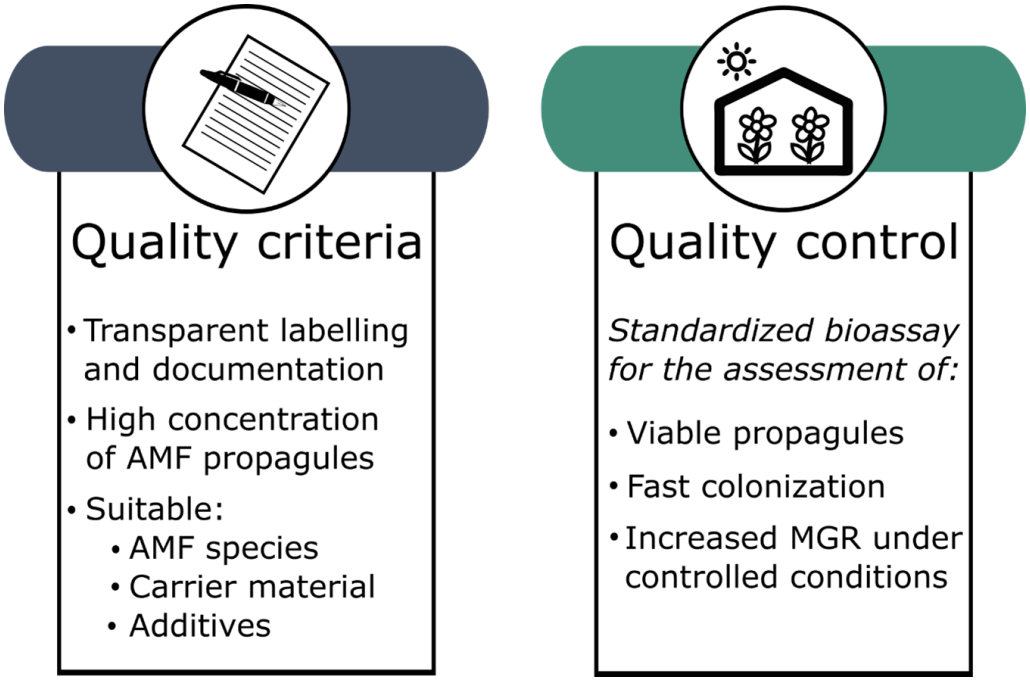

FIGURE 6.5: *Proposed framework for basic testing guidelines and quality control of microbial inoculants containing AMF propagules. MGR = Mycorrhizal growth response.*

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Supplementary material

6.4.4 Plant growth description of the greenhouse bioassays (Australian and European studies)

In both greenhouse studies, high plant mortality was observed in certain treatments, even after the seedlings were already well established or after they had been replanted. This effect was most distinct in the sterilized soils and treatments with high fertilizer concentrations. In the European study, all plants treated with inocula 5, 10, 11 and 15 died in the first bioassay. Furthermore, plants treated with inocula 11 also died in the second bioassay in the non-sterilized soil. Similarly in the Australian study, where several plants in treatment G died between week four and six. Plants treated with inoculants C, F and I started wilting in both bioassays after they were transplanted into the substrate. The inoculants had been applied directly into the planting hole, probably causing fertilizer burn. Their corresponding electric conductivity (EC) was measured as: 10.56 dS m^{-1}, 9.63 dS m^{-1}, and 9.95 dS m^{-1}, respectively (1:5 water extract).

6.4.5 Inoculum nutrient analysis (Australian study)

Mineral nitrogen (N) concentrations determined in the inoculants ranged from 0.4 to 8.6 mg kg^{-1} for most inoculants. Only the laboratory culture of *R. irregularis* and inoculant E had N concentrations below the detection limit. Relatively higher amounts of ammonium and/or nitrate were also associated with higher EC values of up to 12 dS m^{-1}. The inoculants tested showed high variability in concentrations of plant-available (Colwell) P, which ranged from 7.5 mg P kg^{-1} to 3863 mg P kg^{-1} (see Suppl. Table 6.2).

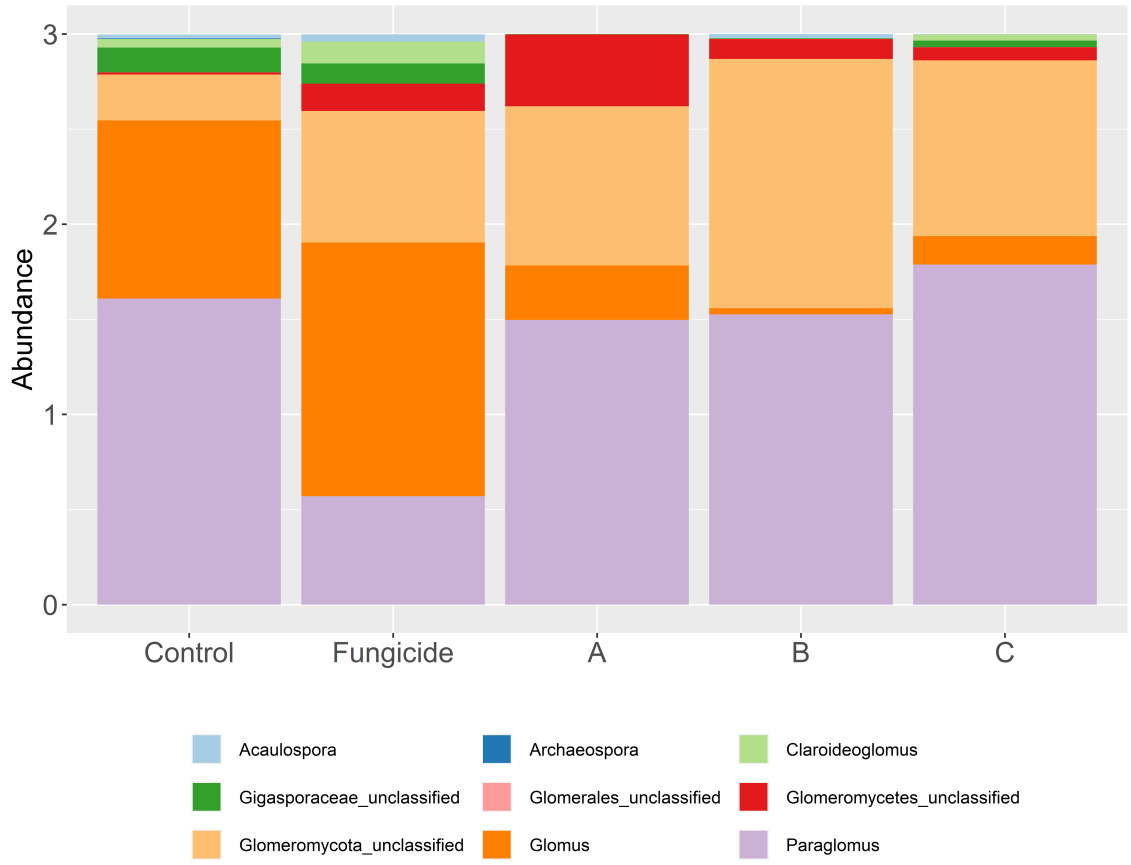

FIGURE 6.6: *Relative abundance of the AMF communities in colonized soybean root pieces from field soil in the North American study. Control = Control treatment without AMF addition, Fungicide = fungicide treatment (negative control), A-C = inoculation treatments using commercial AMF inoculants.*

TABLE 6.2: *Overview of the nutrient analysis of the Australian inoculants. BDL = below detection limit (NH_4^+ = 0.3 mg kg^{-1}, NO_3^- = 0.2 mg kg^{-1})*

Treatment	NH_4^+ [mg kg^{-1}]	NO_3^- [mg kg^{-1}]	Colwell P [mg kg^{-1}]	pH	EC mS cm^{-1}
Control soil	-	-	-	-	-
R. irregularis	BDL	BDL	5.1	6.3	61.4
A	BDL	0.4	NA	5.4	482
B	1.5	0.6	1249	8.5	8,730
C	BDL	4.6	43.3	6.3	11,950
D	5	0.5	23.5	8.6	126
E	BDL	BDL	24.9	5.5	575
F	7.1	8.6	255.5	7.5	10,260
G	4	0.8	3863.1	5.9	> 20,000
H	0	0.7	7.5	7.4	1674
I	NA	NA	NA	NA	NA
J	BDL	0.2	919.7	4.9	290

TABLE 6.3: *Physico-chemical properties of the soil used in the first Australian bioassay.*

Element	Measurement	Unit	Soil:sand = 1:9 (duplicate 1)	Soil:sand = 1:9 (duplicate 2)
pH (1:5 water)	water (1:5)		6.66	6.78
EC (1:5 water)	water (1:5)	mS m^{-1}	39	32
Organic carbon	W&B	%	0.18	0.21
Ammonium	2M KCl	mg kg^{-1}	$\leq$1	$\leq$1
Nitrate	2M KCl	mg kg^{-1}	$\leq$1	$\leq$1
Sulfur	KCl	mg kg^{-1}	4	3.4
Phosphorus	Colwell	mg kg^{-1}	20	21
Potassium	Colwell	mg kg^{-1}	59	55
Boron		mg kg^{-1}	0.1	0.1
Chloride		mg kg^{-1}	25	22
Calcium	Amm-Ac	mg kg^{-1}	216	198
Magnesium	Amm-Ac	mg kg^{-1}	29	25
Sodium	Amm-Ac	mg kg^{-1}	20.4	17.3
Copper	DTPA	mg kg^{-1}	0.11	0.08
Iron	DTPA	mg kg^{-1}	17	9.2
Manganese	DTPA	mg kg^{-1}	9.3	8.6
Zinc	DTPA	mg kg^{-1}	0.31	0.36

TABLE 6.4: *Physico-chemical properties of the soil media used in the first and second European bioassays.*

Element	Measurement	Unit	Loess – sand mix (bioassay 1)	IP-farm field soil (bioassay 2)
pH			8.49	7.46
Organic carbon		%	0.06	1.13
Total carbon		%	0.58	1.5
Total nitrogen		%	0.006	0.15
Sand		%	68.6	19.8
Silt		%	24.6	59.1
Clay		%	6.7	19.2
Humus		%	0.11	1.95
Phosphorus	Ammonium acetate	mg kg^{-1}	15.7	123.9
Phosphorus	CO_2 saturated water	mg kg^{-1}	1.2	12.5
Phosphorus	total	mg kg^{-1}	492.04	809.3

TABLE 6.5: *Physico-chemical properties of the soil used in the North American field study.*

Element	**Measurement**	**Unit**	**Field soil**
pH	Water 1:5		5.64
Phosphorus	Olson	mg kg^{-1}	12.4
Ammonium	KCl	mg kg^{-1}	1.5
Nitrate	KCl	mg kg^{-1}	10.3

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Chapter 7

Establishing a quality framework for commercial inoculants containing arbuscular mycorrhizal fungi

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Abstract

Arbuscular mycorrhizal fungi are obligate plant symbionts that associate with most crops. As part of this symbiosis, they provide multiple ecosystem services such as improved uptake of plant-essential nutrients, increased yield resilience and the reduction of greenhouse gas emissions from soils. These advantageous characteristics are sorely needed to reduce agriculture's footprint and improve food system efficiency. The production of mycorrhizal inoculants has significant economic value that has resulted in a variety of commercial inoculants being released on the market. However, several studies have shown that the lack of an effective regulatory framework for this industry may have contributed to unreliable inoculants. A recent global analysis of inoculum efficiency showed that over 80% of the tested products contained no viable propagules when tested in sterilized soil. We propose a framework that can be used to assess the quality and reliability of arbuscular mycorrhizal inoculants. This framework can be used by regulatory agencies and industry for the evaluation of commercial inoculants of arbuscular mycorrhizal fungi and for the introduction of certification labels. Such measurements contribute to the adoption of arbuscular mycorrhizal inoculants by the farming community and increase sustainability in food production systems.

7.1 Introduction

One of the major challenges of the 21st century is the production of food for an ever-growing population, which is expected to reach 9.7 billion people by 2050 (Nations, 2019). While the average yield of food production systems has consistently improved over the last two centuries, most agricultural practices are heavily reliant on pesticides and mineral fertilizers (Liu et al., 2015). However, those products are part of the world's most energy intensive production processes and are often dependent on finite resources such as in the case of phosphorus (P) fertilizers (Woods et al., 2010). The application of mineral fertilizers and pesticides has also been shown to be of low efficiency (Baligar et al., 2001), and negatively affects the surrounding environment, human health (Carvalho, 2017; van den Berg et al., 2020) and food quality (Kim et al., 2017; Reganold & Wachter, 2016). The extensive use of fertilizers in today's food systems is a major factor contributing to agricultural global greenhouse gas emissions (Vermeulen et al., 2012), and can have severe adverse effects on biodiversity and global sustainability (Steffen et al., 2015). Furthermore, there is evidence for a plateauing of productivity in agrochemical-based food production systems (Lobell et al., 2011). Projections show that current development of food production rates will not meet the food demand for future decades (Ray et al., 2013). Other pressing issues include the development of pesticide resistances (Gould et al., 2018), the emergence of new crop pathogens (Fones et al., 2020) and the increasing consumer demand for pesticide-free food (Rana & Paul, 2017). There is rapidly emerging interest to reduce agriculture's footprint and reliance on agrochemicals through the use

of biostimulants, including microbial inoculants (Abbott et al., 2018). Commercial microbial inoculants include the highly successful rhizobia (Howieson & Dilworth, 2016) and selected generalist organisms that seek to improve plant vigour with significant potential to reduce the demand of agrochemicals (Berruti et al., 2016). These microbiome application have the potential to increase farm productivity and yield resilience for sustainable food production (Singh et al., 2020). Their use underpins various global challenges and sustainable development goals, such as food safety, food security and climate change mitigation (D'Hondt et al., 2021).

One group of well-studied symbionts are arbuscular mycorrhizal fungi (AMF) which colonize roots and provide nutrients in exchange for photosynthates. AMF have been shown to improve the uptake of essential plant nutrients, such as phosphorus and zinc (Smith & Read, 2008; van der Heijden et al., 2015). At the same time, they may increase plant resistance towards pathogens (Jung et al., 2012) and abiotic stress (Plouznikoff et al., 2016) (see Table 7.1). AMF follow a cosmopolitan distribution and can be found in almost all ecosystems (Öpik et al., 2006). However, their natural abundance can be diminished by common agricultural practices, including the application of fertilizers (Cheng et al., 2013), soil disturbance (van der Heyde et al., 2017), or selection of cultivars that do not associate with AMF (Zhang et al., 2019). Conversely, AMF populations can be rebuilt using management practices such as cover crops (Bowles et al., 2017) or principles of organic farming (Verbruggen et al., 2010). In cases where these practices are not applicable, the in situ use of AMF inoculum has been shown to increase mycorrhizal root colonization and yield resilience (Giovannini et al., 2020; Hijri, 2016).

7.2 Status quo

With the global economic value for microbial inoculants expected to reach $11.45 billion USD by 2026 (Consulting, 2018), an increasing number of commercial AMF inoculants has been released on the markets over the last few decades (Benami et al., 2020; Vosátka et al., 2008). Retail markets in most countries offer a variety of commercial AMF inoculants which are available for amateur and professional applications alike (Bitterlich et al., 2020; von Alten et al., 2002). One meta-analysis between 28 AMF manufacturer showed that over 90% of the 68 mycorrhizal products are provided in a solid-state and only 10% as liquid formulation. All products used species within the Glomeraceae, of which *Rhizophagus irregularis* (39%), *Funneliformis mosseae* (21%) and *Claroideoglomus etunicatum* (16%) were most commonly used. Two third of the products used a conglomerate of AMF species rather than a single species. About 20% included other beneficial microorganisms (Basiru et al., 2020).

However, the global market for agricultural microbial inoculants has been dragging behind the expectations that developed from scientific findings in laboratory or controlled environments. One reason being the inconsistent results of microbial inoculants,

TABLE 7.1: *Overview of potential mycorrhizal benefits towards plant growth and ecosystems.*

	Benefits	**Reference**
Plant	Improved uptake of minerals, especially phosphorus, copper, zinc.	(Cavagnaro et al., 2006; Watts-Williams et al., 2013)
	Increased plant biomass and yields.	(Rocha et al., 2019)
	Improved water uptake, osmotic regulation and drought resistance.	(Augé, 2001)
	Improved resistance against soil salinity	(Evelin et al., 2019; Fileccia et al., 2017)
	Increased plant metabolite production.	(Zeng et al., 2013)
	Protective effects towards soil contamination and adverse soil physio-chemical characteristics.	(Gamalero et al., 2009; Lenoir et al., 2016)
	Induction of systemic pathogen resistance.	(Pieterse et al., 2014)
	Protective effects against nematodes and root diseases.	(Harrier & Watson, 2004)
Ecosystem Services	Increased nitrogen fixation in legumes Soil aggregation, improved soil structure and carbon sequestration.	(Kafle et al., 2019; Püschel et al., 2017) (Rillig & Mummey, 2006; Wilson et al., 2009)
	Reduced nutrient leaching.	(Cavagnaro et al., 2015)
	Interaction and driving force of microbial activities.	(Barea et al., 2002)
	Reduced greenhouse gas (N_2O) emissions from soils.	(Bender et al., 2014)
	Common mycorrhizal network between plants for allocation of nutrients, seedling establishment and plant to plant interactions.	(van Der Heijden & Horton, 2009)

including AMF, when applied under various field conditions (Singh et al., 2020). For AMF, this could be caused by environmental factors, such as incompatible symbionts which are not adapted to soil and climate conditions, but also technical reasons, such as poor product quality. For the end consumer it is not possible to verify the quality of AMF inoculants. In addition, many commercial inoculants incorporate a variety of (non-mycorrhizal) plant-growth promoting microorganisms, biological additives, or plant nutrients. Often, these additives are not clearly labelled, and positive plant growth effects might be falsely attributed to mycorrhizal colonization (Salomon et al., under review). The commonly used *in vivo* production method for AMF inoculum may introduce unwanted contaminants such as nematodes, weeds, algae or saprophytes when quality control systems are not in place (von Alten et al., 2002).

Mandatory quality control of commercial mycorrhizal inoculants is sparse or non-existent in most countries. Previous studies from multiple countries that focused on a small number of products showed consistently that ineffective AMF inoculants are a common phenomenon rather than the exception (Faye et al., 2013; Tarbell & Koske, 2007). In a recent study by Salomon et al. (under review), 25 products from Australia and Europe and the USA were tested under greenhouse conditions. Over 80% of the commercial AMF inoculants failed to induce mycorrhizal root colonization in sterilized soils.

One recent approach towards a legislative quality management is the amendment of the EU fertilizer regulation 2019/1009, which took effect in April 2019. To date, the European standardization committee CEN TC 455 "plant biostimulants" is establishing standard methods for the product certification of mycorrhizal inoculants. These standards will be tested and verified in Europe-wide ring tests, performed by independent laboratories. The focus of these methods is on the quantification of viable microorganisms in the products, whereas effectiveness remains a voluntary declaration by the producers.

Earlier quality control mechanisms were established in Japan by the Soil Productivity Improvement Act in 1996 (Saito & Marumoto, 2002). This legislation was implemented as a reaction towards Japan's first wave of agricultural microbiology in the 1990s, during which several agrochemical companies released AMF inoculants. The government approved AMF as the "first and only microbial amendment" alongside official criteria for overseeing the quality of AMF products. A standard bioassay protocol was introduced which governed mandatory testing and labeling guidelines (see Suppl. 7.5.2). Ongoing research confirmed the reliability of domestic AMF producers, indicating that the introduced measurements are efficient (Saito and Ezawa, unpublished).

7.3 Aims, goals and objectives

Similar to the efforts within Japan and the EU, we propose a general quality management framework for commercial AMF inoculants which can then be adopted by regulatory

agencies. We identified essential quality criteria that need to be met by the producers to ensure working AMF inoculants (see Figure 7.1 and Table 7.2). In a first step, we are solely focusing on the most basic quality criteria for AMF inoculants which can be summarized as:

- Occurrence of viable propagules that result in mycorrhizal root colonization under controlled conditions.
- Absence of plant pathogens and contamination
- Facilitated inoculum application (e.g. pure AMF blends, carrier materials or solutions).
- Detailed description of the AMF species, additives, storage criteria and procedures for application.

These criteria are then validated by using a standardized *in vivo* bioassay (see Suppl. 7.5.1). This bioassay is considered a low-cost method for validating spore viability in a plant substrate. It provides additional information regarding potential contaminations with plant pathogens, either through visual symptoms or reduced plant growth. The proposed framework could be adapted by regulatory agencies for product evaluation. Certification labels could be introduced for compliance by commercial AMF manufacturer. Such control measurements lead to increased consumer confidence, thereby supporting the adoption of AMF inoculants by the farming community.

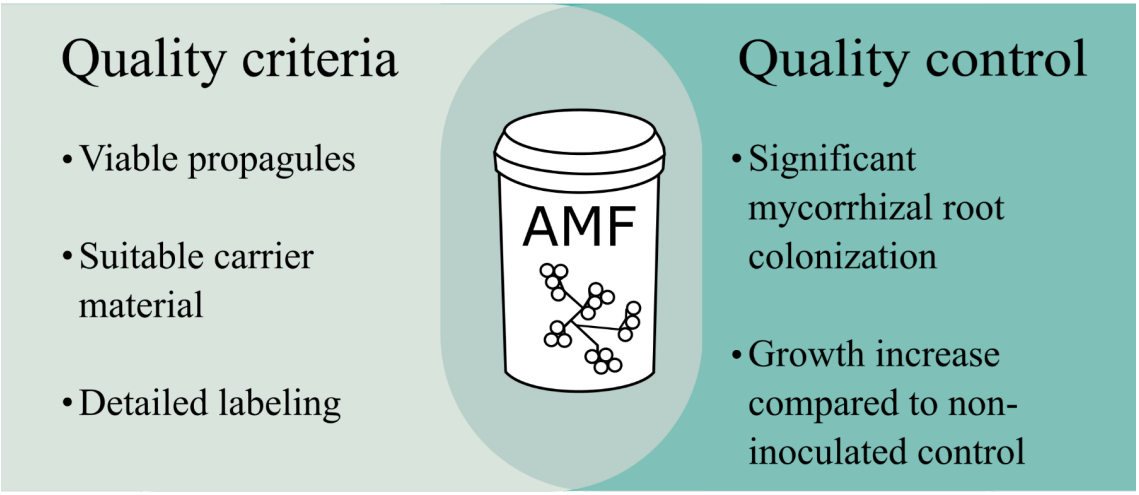

FIGURE 7.1: *Quality framework for the assessment of AMF inoculants.*

7.4 Basic quality criteria

7.4.1 Inoculum composition and viability

The selected AMF species for the inoculum should contain at least one generalist which is able to colonize a broad range of host plants in the desired environment. Such generalist

TABLE 7.2: *Proposed quality criteria and quality control for AMF inoculants that need to be met by producers.*

Quality criteria	
Inoculum composition and viability	- Inclusion of a generalist AMF species (exemption applies for specialized inoculum with specific host) - Free of plant-pathogens - Fast distribution channels to end-consumer (at least one growing season before expiration date)
Carrier material	- Facilitates application of inoculum - Only suitable additives that do not interfere with the mycorrhizal development
Package label	- Propagule composition (AMF isolates) - Carrier material and other additives - Plant-available nutrients (NPK) - Batch number - Production and expiration date - Instructions on storage and application - Documented evidence of root colonization (including picture) and plant growth stimulation on the producer's website
Quality control	
	- Confirmed root colonization in standardized bioassay - Confirmed plant growth stimulation in standardized bioassay

AMF species that are widely used for commercial and scientific purposes are *Rhizophagus sp.* and *Glomus sp.* (Öpik et al., 2006). Exemptions apply for products that target specific host plants and environments and require specialized AMF species.

The inoculant should contain enough viable propagules to achieve mycorrhizal root colonization. High concentrations of viable propagules are particularly important to account for the declining germination rate of AMF propagules during longer storage (Ruiz-Lozano & Azcon, 1996). Consequently, dosage recommendations should account for decreased spore viability over time and therefore contain certain margins. The distribution channel of the inoculant should ensure that the consumer receives the product at least one growing season before the expiration date.

Any AMF inoculum should be free of plant pathogens and other harmful contaminations. To this date, most inoculants are produced *in vivo* on host plants such as sorghum or maize (Berruti et al., 2016). These production systems naturally include a range of microorganisms that are associated with the AMF propagules. However, none of those microorganisms should be pathogenic to the host plant. A variety of commercial tests are available to confirm the absence of plant pathogens (Ophel-Keller et al., 2008). Such testing would not be required for *in vitro* produced propagules. Various advancements have been reported which could facilitate the large-scale production of axenic AMF inoculum in the near future (Gargouri et al., 2021; Ijdo et al., 2011; Sugiura et al., 2020).

The selected isolates should lead to a positive mycorrhizal growth response (MGR) in mycorrhiza-responsive crops such as sorghum (*Sorghum bicolor*), maize (*Zea mays*) or leek (*Allium porrum*) when grown under standardized conditions (see Suppl. 7.5.1) (Tran et al., 2019). This test also uncovers a certain plant pathogens which would negatively impact the MGR. This bioassay is to be done after the selection of the final AMF species and annually thereafter.

7.4.2 Carrier materials

If AMF propagules are dispersed in a carrier material, this should facilitate the application of the inoculant without negatively impacting its viability. Different solutions are available for agricultural applications, such as algal or polymeric beads (Vassilev et al., 2005), liquid solutions (Malusá et al., 2012), biochar (Sashidhar et al., 2020) or as a seed coating (Rocha et al., 2019). Spores can be dispersed in coarse material that makes it easy to handle, such as calcinated clay (Vassilev et al., 2005). The material should be homogenous so that AMF propagules can be dispersed evenly.

If biological or chemical additives are incorporated, these need to work synergistically or at least not affect mycorrhizal root colonization or plant growth. Compounds that have been successfully tested in combination with AMF include various plant-growth promoting microorganisms (Wu et al., 2005) or biological compounds, such as chitin or humic

acids (Gryndler et al., 2003). In any case, additives such as mineral fertilizer should not suppress the development of arbuscular mycorrhizas and need to be labelled appropriately.

7.4.3 Package and labeling

AMF inoculum needs to be stored in a water- and light-proof container. Care must be taken during packaging to ensure that the propagules are undamaged, and that the inoculum viability remains unchanged. The label should describe the included AMF species, any additives, recommended storage criteria and procedures for application. Where the product label does not provide sufficient space, information can be provided *via* additional product sheets or online. The package labeling must include all necessary information about the inoculum content (propagule composition and concentration), production method (*in vivo* or *in vitro*), additives, plant-available nutrients (NPK), batch number, production and expiration date, instructions on storage and application, and information about quality measures.

Inoculum producers should provide the results of the latest standardized *in vivo* bioassays (see Suppl. 7.5.1) and any further privately undertaken quality control measurements. This report should contain: 1) a visual proof of root colonization by AMF; 2) MGRs after the inoculation compared to non-inoculated controls. Such information should be provided on the company's website, and regularly updated.

7.4.4 Quality control: Bioassay

Mycorrhizal inoculants should be tested in a standardized bioassay under controlled conditions (see Suppl. 7.5.1). Rather than focusing on the broader ecological and plant-physiological advantages of AMF, the proposed bioassay is designed to control the minimum requirements for commercial AMF inoculants. The aim of this bioassay is to assess whether inoculants contain viable spores and colonize selected host plants in sterilized soil under controlled conditions. This bioassay provides additional information regarding potential contamination with plant pathogens, which are reflected in the MGR or which can be visually identified. The inoculants are tested under AMF-favorable conditions that include mycorrhizal-responsive host plants (maize, sorghum or leek) which are grown under low concentrations of plant-available P. The desired outcome at the end of the bioassay is a positive growth response and a significant mycorrhizal root colonization which we define as $>$20% colonized root length.

7.5 Conclusion and future perspective

Microbial inoculants are an essential building block for resilient and sustainable food production systems. However, the current market requires drastic changes to break the cycle of unreliable products and skeptical consumers. The here proposed framework covers

quality criteria and quality control measurements that can be used to improve the adaption of AMF inoculants on a broader scale. In its current stage, the framework focuses on minimum requirements with the potential for intensification in the future. This intensification can be guided by scientific research that focuses on the application of AMF inoculants under real-life conditions and its ecological consequences. More research is necessary to fully understand the establishment of introduced AMF under field conditions and its impact on indigenous AMF communities (Hart et al., 2017). The purity of AMF inoculum can be further improved through advancements in the mass production of spores (Gargouri et al., 2021; Tanaka et al., 2020). However, new production methods need to be evaluated towards their impact on AMF functioning and genetic stability (Kokkoris & Hart, 2019).

The here proposed framework is a first step towards the regulatory-backed improvement of AMF inoculum by ensuring basic quality criteria. It could be adapted *via* various pathways, such as an open partnership between companies, regulatory agencies and farming communities. Major AMF producers need to be included during the implementation process to ensure its practicality and widespread adoption. Important discussion points for the legal adaptation include the specific mechanisms of certification, the role of testing organizations and its cost distribution. Companies would then adhere to the proposed requirements and provide transparent information about their production process. These inoculants would be certified, and all essential information provided to the farming communities. In return, the farming communities provide feedback which can be reviewed by the companies for future product development.

Supplementary material

7.5.1 Standard *in vivo* bioassay

Following, we describe a standardized *in vivo* bioassay for the evaluation of AMF inoculum viability and its effect on plant growth. Further specifications for this protocol are given in Table 3. Detailed instructions are available at:
https://dx.doi.org/10.17605/OSF.IO/R9WGN

1. The soil for this bioassay is low in plant-available P, but otherwise not limiting plant growth. A practical solution to reduce the P concentration of the soil is the use of sand-soil mixes and to re-apply nitrogen, potassium and micronutrients in the form of nutrient solutions that are lacking phosphorus (Long Ashton -P, see Suppl. Table 7.3). The soil is sterilized to inactivate any native AMF propagules.

2. The inoculum is tested against a non-inoculated control group to quantify the MGR. Each treatment has a minimum of 5 biological replicates to allow the statistical testing of effects. For the inoculated group, the inoculum is applied as recommended by the manufacturer. Suitable host plants are added, either as seeds or seedlings.

The soil is regularly watered, to near field and the nutrient solution is applied weekly or biweekly. All groups are treated identical in terms of dry soil weights, water and fertilizer applications, and homogenous seedling materials.

3. At the end of the bioassay, plants are destructively harvested by carefully removing the plants from the pots and washing the soil off the roots. A subsample of about 300 mg fresh roots is taken and stored in 50% EtOH. The shoots and roots are separated, dried at 65 °C for at least 48 hours and the dry weights recorded. The MGR can be calculated as followed:

$$MGR = \frac{[Biomass\ (inoculated) - Biomass\ (control)]}{Biomass\ (control)} \tag{7.1}$$

4. The subsampled roots are stained following the ink-vinegar method as described by (Vierheilig et al., 1998) and visualized in the book by (Brundrett et al., 1996). First, the roots are washed with water and cleared in 10% KOH, either at room temperature for 3-4 days or for 10-15 minutes at 80 °C. The exact time depends on the plant species, root thickness and root pigmentation. Roots are fully cleared when only the cell wall and cell membrane remain visible under a dissecting microscope. Roots are washed again with water and stained in a 10% ink and 90% vinegar solution for 15 minutes at 65 °C. After staining, roots are washed under water and de-stained for one day in an acidified water solution, containing 2% household vinegar (approx. 5% acetic acid). Roots are now ready for examination or can be stored in a 50% glycerol solution. The colonized root length can be determined following the grid-line intersect technique described by (McGonigle et al., 1990).

TABLE 7.3: *Formulation for modified Long Ashton nutrient solution lacking P*

Macronutrients		**Micronutrients**	
Potassium sulphate (K_2SO_4)	2 mM	Boric acid (H_3BO_3)	2.86 mg L^{-1}
Magnesium sulphate ($MgSO_4$)	1.5 mM	Manganese chloride ($MnCl_2$)	1.81 mg L^{-1}
Calcium Chloride ($CaCl_2$)	3 mM	Zinc sulphate ($ZnSO_4$)	0.22 mg L^{-1}
Iron (Fe) EDTA	0.1 mM	Cupric sulphate ($CuSO_4$)	0.08 mg L^{-1}
Ammonium sulphate $(NH_4)_2SO_4$	4 mM	Sodium Molybdate ($NaMoO_4$)	0.025 mg L^{-1}
Sodium Nitrate ($NaNO_3$)	8 mM		

TABLE 7.4: *Preparation of 5 L of modified Long Ashton nutrient solution lacking P (Cavagnaro et al., 2006)*

Stock solution	mL in 5 L	Final concentration
250 mM Potassium sulphate	40	2 mM
375 mM Magnesium sulphate	20	1.5 mM
1 M Calcium chloride	20	4 mM
110 mM Iron (Fe) EDTA	5	0.1 mM
2 M Ammonium sulphate	10	4 mM
1 M Sodium nitrate	40	8 mM
1 L Micronutrient solution	5	

7.5.2 Standard bioassay protocol for AMF inoculants in Japan

Excerpt from Soil Productivity Improvement Act (Law No.34 of 1979, amended in 1996) Ministry of Agriculture, Forestry and Fisheries, Japan

1. Preparation of growth medium: Apply a standard amount of product (inoculum) to 50 cm^3 vermiculite and sow seeds of an assay plant.
2. Growth conditions: Grow the plants at 25 °C under a lighting condition of 15,000 – 20,000 lx (16 h light / dark cycle) for 4 weeks.
3. Assessment of mycorrhizal colonization:
   - The roots are detached from the shoots, washed and cleared in 10% (w/v) KOH at 90 °C. Roots are then soaked in 5% (w/v) HCl for 10 min at room temperature and stained with 0.1% (w/v) aniline blue or trypan blue at 90 °C for 30 min.
   - The stained roots are spread to a Petri dish with 1 cm grid lines, and the presence and absence of colonization are counted using the intersect gridline method (McGonigle et al., 1990). More than 100 intersections per sample are to be counted in three replication samples.
   - Percentage of colonization is calculated as follows:

$$Colonization[\%] = \frac{(No.\ colonized\ intersections)}{(No.\ total\ intersections)} \times 100 \tag{7.2}$$

Mandatory information on the product label:

- Colonization [%] in bioassay and used host plant
- Used carrier material
- Applicable and non-applicable plants
- Expiration date

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Chapter 8

Evaluation of composts and potting mixes and their ability to support arbuscular mycorrhizal fungi.

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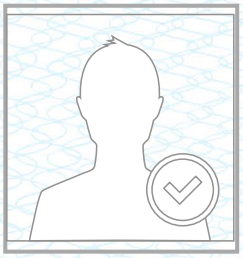

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Highlights

- Potting mixes and compost did not meet all national standards.
- Substrates were highly variable in terms of plant nutrient and metal concentrations.
- DNA-based analysis identified various plant pathogens present.
- Maize roots were well-colonized by arbuscular mycorrhizal fungi in most substrates.
- Continuous phenotyping gives valuable insights in combination with plant growth bioassays.

Abstract

The use of composts and potting mixes in food production systems is a promising way to counteract the effects of soil degradation and allows crop growth in soilless culture systems. Arbuscular mycorrhizal fungi (AMF) are a well-studied group of beneficial plant symbionts that have been shown to provide important ecosystem services. This study analysed the properties of nine commercial Australian potting mixes and composts and investigated whether they support colonization of maize plants with AMF in a plant growth bioassay. Analysis showed highly variable properties between the substrates, with some extreme values that limited plant growth. DNA-based analysis revealed the presence of various plant pathogens, which was linked to inhibited plant growth in one substrate. Some substrates did not meet national quality standards, such as for the concentrations of plant nutrients, heavy metals, or substrate maturity. Plant growth was mostly limited due to nitrogen immobilization, which required weekly fertilizer applications. Solid state ^{13}C nuclear magnetic resonance spectroscopy gave insight into the decomposition state of the substrates. Plant roots in most substrates were well colonized with AMF (>60% root length), regardless of most substrate properties. Root colonization was strongly affected in only one substrates, likely due to ammonium toxicity. Results of this study show that not all commercial substrates adhered to national quality standards. Potting mixes and composts can support high mycorrhizal root colonization when plant growth is otherwise not limited.

8.1 Introduction

New paradigms are required for accomplishing the goal of feeding an ever-growing global population whilst staying within our planetary boundaries (Steffen et al., 2015). One approach calls for the sustainable intensification of agriculture, which has been described as increased agricultural productivity with lesser impact on the environment (Rockström et al., 2017). These developments are key for the "half planet" movement to prevent the collapse of vital ecosystem services (Watson & Venter, 2017). To achieve

this goal, the use of soilless culture systems (SCS) should not be overlooked as a viable option (Muller et al., 2017). Especially in times of increasing land degradation (Gibbs & Salmon, 2015) and urbanization (Nations, 2019), the use of soilless plant substrates (e.g. potting mix) allows efficient food production where conventional agriculture is not possible. Such implementations are found in horticulture (Maher et al., 2008) and urban agriculture to avoid issues of soil compaction and contamination (Salomon et al., 2020). In both systems, the use of organic soil blends or soilless substrates is commonly practiced.

Another trend towards sustainable agriculture is the use of biological inoculants as an alternative to, or in concert with, agrochemicals. Research has identified a variety of plant growth-promoting bacteria and fungi that can reduce our dependency on agrochemicals (Parnell et al., 2016). These findings have significant economic and ecological implications, leading to a global market for biological inoculants that is expected to reach a value of $11.45 billion USD by 2026 (Consulting, 2018). Arbuscular mycorrhizal fungi (AMF) are among the most widely studied plant growth-promoting soil microbes. AMF associate with >80% of all plant species, including most crop plants (Smith and Read, 2008). They provide important plant and ecosystem services with significant potential for the sustainable intensification of agriculture (Gianinazzi et al., 2010). In addition to enhancing plant nutrient acquisition, especially phosphorus (P) and zinc (Zn) (Watts-Williams & Cavagnaro, 2014), the formation of arbuscular mycorrhiza (AM) can increase yield resilience through improved resistance against biotic and abiotic stresses (Rivero et al., 2018). AMF also improve soil aggregation which is associated with higher nutrient retention (Cavagnaro et al., 2015) and reduced greenhouse gas emissions from soil (Bender et al., 2014). As such, the management of AMF in agroecosystems, protected horticulture (De Pascale et al., 2020) and urban agriculture (Salomon et al., 2020), has been proposed as a viable option to increase sustainability in food production.

Although the use of biological inoculants in agriculture is increasing, there is a dearth of studies focusing on their use in soilless plant growth media such as potting mixes and composts. Previous research highlighted the sensitivity of AMF towards soil pH (Klichowska et al., 2019) and concentrations of nutrients, such as P (Liu et al., 2016), both of which can vary more widely in potting mixes and composts than natural soils (S. Clark & Cavigelli, 2005; Hashemimajd et al., 2004). Furthermore, these substrates undergo a period of heat treatment (Standard, 2012) which could establish microbial communities with suppressive effects on AMF (Svenningsen et al., 2018).

Given the potential benefits of AMF and a growing move towards to protected horticulture and urban agriculture (Dorais & Cull, 2017; Orsini et al., 2020), there is a need to assess their establishment in manufactured plant growth media. Therefore, we present results of a study in which we evaluated the quality of commercial potting mixes and composts and their ability to support AMF. We further aimed to identify limiting factors

of mycorrhizal root colonization in these substrates and whether they differ from the limitations posed by natural soils.

We conducted a greenhouse bioassay in which ten different substrates were inoculated with AMF and maize grown as a host plant. Plant growth was assessed *via* high throughput phenotyping and a destructive harvest at the end of the experiment. Root samples were stained and mycorrhizal root colonization quantified for the occurrence of AMF. Further analysis of the plant substrates provided additional insights into plant growth and mycorrhizal colonization. For this, all substrates were analysed for chemical, physical and biological properties. The dataset comprises information about plant-nutrient availability, the occurrence of plant pathogens, functional carbon groups and various indicators of substrate maturity.

8.2 Material and Methods

8.2.1 Plant substrates

This study included ten different substrates, of which one was a soil/sand mix, used as positive control. The remaining treatments were sourced from retail warehouses and landscape suppliers and are categorized as potting soils (n = 1), potting mixes (n = 3) and composts (n = 5). Hereafter, these are also referred to as a treatments or substrates interchangeably. Within this study, potting soils are defined as blends of natural soil and composted organic matter, whereas potting mixes and composts consist only of organic matter. Potting mixes are blends of organic materials that can be readily used for a wide range of container plants. Composts are organic materials that are rather used as soil amendment or conditioner. In order to identify limiting soil properties, plants were grown in undiluted compost to establish exceptional growing conditions. All product names have been de-identified for reasons of confidentiality, and all products were marketed as meeting relevant standards for composts or potting mixes.

The positive control treatment was a mix of natural soil and sand which has been used in previous experiments and supported high levels of mycorrhizal root colonization (Watts-Williams et al., 2019). It was mixed in a ratio of 1:9 of field soil to steamed fine sand. The field soil was collected in 2018 from the Waite Arboretum (coordinates: 34.9670°S, 138.6360°E) and has been classified as Urrbrae red-brown earth (Cavagnaro, 2016). Before being used in this experiment, the natural soil was sieved to <2 mm, twice-autoclaved at 121 °C for 60 min, and dried at 40 °C. Before autoclaving and mixing with sand, the natural soil contained: 10 mg kg^{-1} plant-available (Colwell) P, 28 mg kg^{-1} DTPA-extractable zinc (Zn), 22 mg kg^{-1} KCl-extractable NH_4^+ and 12 mg kg^{-1} NO_3^-.

Subsamples of all commercial substrates were taken to confirm the absence of naturally present AMF spores. For this, 30 mL of the substrate was taken in duplicates and

stirred in 300 mL water for 30 min. The suspension was wet-sieved through 250 μm and 53 μm sieves and the remaining substrate cleaned by centrifugation in a 50% sucrose gradient. The supernatant was again sieved to <53 μm and examined under a dissecting microscope at 50 × magnification (Brundrett et al., 1996). No naturally present AMF spores were found in any of the commercial substrates. Previous studies involving the same Arboretum field soil found 19 AMF spores g^{-1} soil. Double autoclaving proofed successful in inhibiting spore germination (Salomon et al., submitted).

8.2.2 Experiment set-up

Plastic pots were filled to a volume of 2.5 L and for each substrate, one pot was set aside for the determination of the gravimetric water holding capacity (WHC) after 2 days of free drainage (Vanderlinden & Giráldez, 2011). To all remaining pots, 30 g AMF inoculum was mixed into the top 5 cm. The AMF inoculum consisted of *Rhizophagus irregularis* WFVAM23 which was formerly described as *Glomus versiforme* (L.-L. Gao et al., 2001). The cultures were propagated in 2019 in closed pots on *Plantago lanceolata* as a host plant (Walker & Vestberg, 1994). Spores of AMF in the inoculum were counted after extraction from a sugar-gradient centrifugation with an average of 28 spores g^{-1} inoculum (Brundrett et al., 1996). After adding the inoculum to the pots (about 840 spores per pot), the pots were watered to their corresponding WHC. Seeds of the dwarf maize (*Zea mays*) cv. 'Gaspe' were surface sterilized in 5% NaOCl for 15 min and washed in de-ionized water (DI) for 45 min. This maize variety is a short-generation model which can grow from "seed to seed" in 60 days when grown under optimum conditions (McCaw et al., 2016). In January 2020, three seeds were planted into each pot and pre-grown on conventional greenhouse benches in the Plant Accelerator of the Australian Plant Phenomics Facility (APPF), prior to being transferred to the high throughput phenotyping system (see below).

8.2.3 Plant growth bioassay and plant analysis

Fourteen days after adding the seeds to the substrate, seedlings were reduced to one seedling of homogenous size and the pots were moved onto the tracks of the automatic phenotyping system. Plants were imaged (Al-Tamimi et al., 2016) and watered with reverse osmosis water (RO) to their corresponding WHC daily. The average greenhouse temperature ranged from 31 °C during the day to 22 °C during the night with a mean photosynthetically active radiation (PAR) during mid-day of 900 μmol m^{-2} s^{-1}. Preliminary experiments identified depressed plant growth with signs of nitrogen (N) deficiency; thus, the decision was made to apply 10 mg NH_4NO_3-N weekly to all treatments. After 41 days on the phenotyping systems (56 days after seeding), plants had reached the reproductive phase (R1 – R3) and were destructively harvested. For this, plants were carefully removed from the pots and the soil washed from the roots with water. About 300 mg of fresh roots were taken and stored in 50% ethanol for the quantification of mycorrhizal root colonization. Fresh weights of roots and shoots were recorded, as well as

dry weights after 60 °C for 48 h. Shoot nutrient concentrations were determined as follows: Shoots were homogenized and a subsample of approximately 250 mg was digested in 2 mL of HNO_3 and 0.5 mL of H_2O_2. The digestate was further diluted and analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES, PerkinElmer Avio 200) for the elements boron (B), calcium (Ca), copper (Cu), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulphur (S) and zinc (Zn). NIST 1515 (apple leaves) was used as the certified reference material with recovery percentages between 79% and 108%. The remaining shoot samples were analysed for N concentration through Dumas combustion by the Australian Precision Ag Laboratory (APAL). The preserved roots were washed free of ethanol using de-ionized (DI) water and cleared in 10% KOH (w/v) at room temperature for 5 days. Roots were washed again and then stained in 5% ink in vinegar solution at 60 °C for 15 min (Vierheilig et al., 1998). The roots were then destained in acidified water for one day and mycorrhizal root colonization was estimated using the grid-intersect method (McGonigle et al., 1990).

8.2.4 Plant substrate analysis

At planting, subsamples from each substrate were collected for further analysis, as follows. The first subsample was used for the measurement of bulk density following the Australian Standard for potting mixes (Standard, 2003). The second subsample was used for the measurement of the gravimetric water content after drying at 105 °C for 24 h. The third subsample was used for the colorimetric determination of NH_4^+ and NO_3^- after extraction in 2 M KCl (Cavagnaro et al., 2006). The fourth subsample was sent to an external laboratory for the DNA-based quantification of soil-borne pathogens (PREDICTA® Research, SARDI Australia) (Ophel-Keller et al., 2008). The fifth subsample was incubated under anaerobic conditions for 14 days at 37 °C for the measurement of potentially mineralizable nitrogen (PMN), as described in (Drinkwater et al., 1997). The sixth subsample was dried at 40 °C and used for the measurement of pH, electrical conductivity (EC), plant-available (Colwell) P, water-extractable P, total C:N, carbon dioxide (CO_2) respiration, nitrogen drawdown index (NDI), metals, and analysis of carbon chemistry by solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

The pH and EC of each substrate was measured in a 1:5 water extract using a TPS WP-81 pH, TDS, Temperature & Conductivity Meter (EnviroEquip Biolab, Australia). Colwell P was extracted in a 0.5 M sodium-bicarbonate solution for 16 h and determined colorimetrically using the Murphy and Riley colour reagent (Murphy & Riley, 1986). Water-extractable P was measured similarly in a 1:5 water extract which was shaken for 90 min at 10 rpm (Standard, 2012). Total C:N analysis was performed using the combustion method by the Australian Precision Ag Laboratory (APAL). Basal respiration was measured as CO_2 flux using an infrared gas analyser (IRGA, Model 6262, Li-Cor, Lincoln, NE, USA). For this, 10 g of dried substrate was re-wetted to its corresponding WHC and incubated in 250 mL bottles. Gas samples were taken twice a day for 3 days and the CO_2 flux

calculated from the corresponding linear regression ($R^2 > 0.9$). Nitrogen drawdown index (NDI) was analysed as described by Handreck (1992). Bulk density, mineral nitrogen, plant-available (Colwell and water) P, PMN, NDI and CO_2 respiration were analysed as duplicates.

Metal concentrations were determined as follows: dried substrate was ground and sieved to 200 μm. Approximately 300 mg were digested in 3 mL *aqua regia* and 0.5 mL H_2O_2. The digestate was diluted and analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES, PerkinElmer Avio 200) for the elements arsenic (As), B, cadmium (Cd), Cr, Cu, Mn, nickel (Ni), P, lead (Pb), selenium (Se) and Zn. The reference soil Standard Stream Sediment (STSD) 3 was used with recovery rates between 58% and 91%. Internal soil standards ACU1 and ACU4 were used with recovery percentages between 91% and 144%. All treatments were analysed in triplicate.

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectra were acquired using a Bruker 200 Avance spectrometer. Fine ground substrate was packed into 7 mm cylindrical zirconia rotors with Kel-F end caps and spun at 5 kHz. Between 2000 and 10,000 scans were performed per sample. The empty rotor background signal was removed from the spectra before integrating. Functional carbon groups were identified based on their chemical shifts limits: 0 – 45 ppm (Alkyl), 45 – 60 ppm (N-Alkyl/Methoxyl), 60 – 95 (O-Alkyl), 95 – 110 (Di-O-Alkyl), 110 – 145 (Aryl), 145 – 165 (O-Aryl), 165 – 190 (Amide/Carboxyl), 190 – 215 (Ketone) (Baldock & Smernik, 2002).

8.2.5 Experimental design and statistical analysis (plant growth bioassay)

This single factor experiment involved ten different mycorrhiza-inoculated plant substrates, each replicated 6 times (n = 60). Pots were assigned a location in the greenhouse using a near-A optimal row-column design, obtained using the R packages "od" (Butler et al., 2020) and randomized using the package "dae" (Brien, 2020b) Treatment 5 was excluded from the analysis because no plants survived in this substrate. In treatment 4, one plant showed stunted growth and was also excluded. The final dataset for the plant growth bioassay comprised n = 53 plants across 9 substrate treatments.

Plant imaging was carried out daily from 14 – 53 days after planting (DAP). Due to technical problems, some images were unavailable on DAP 16, 17 and 20 and therefore excluded. From the remaining images, the Projected Shoot Area (PSA) of the plant was obtained using RGB cameras. The imaging data was prepared using the SET method described by Brien et al., 2020, employing the R package growthPheno (Brien, 2020a) for the computation. Imaging traits were calculated based on the smoothed Projected Shoot Area (sPSA). Shoot and root dry weight as well as mycorrhizal root colonization were used as harvest traits.

Penotypic estimated marginal means were produced for each trait using the R packages ASReml-R (Butler et al., 2020) and asremlPlus (Brien, 2020a) to fit a linear mixed model. The model included terms for treatment effects and spatial effects as well as three alternative variance models with different variance classes: (i) low - medium – high variance; (ii) normal – high variance; (iii) normal variance. Residual maximum likelihood ratio tests (REMLRT) with $\alpha = 0.05$ were performed to compare successive variance models for each trait, accepting the model with the most classes that was significant. The chosen model was checked for underlying assumptions. Based on diagnostic plots, the two biomass traits shoot dry weight and root dry weight were logarithmically transformed, with all other traits being left untransformed. For each trait, a Wald F-test with $\alpha = 0.05$ was conducted for difference between treatments. Estimated marginal means (Searle et al., 1980) and 95% confidence intervals were obtained for each treatments for all traits. In addition, p-values for all pairwise comparisons of the Soils have been calculated for each trait to allow the determination of the significance of the differences between the Soils. These p-values are equivalent to using Least Significant Differences (LSD) for pairs of treatments to determine significance at a nominated value of α.

8.3 Results

8.3.1 Substrate analysis

The control substrate showed the lowest concentrations of plant-available nutrients and EC (see Table 8.1). However, its bulk density was much higher than for the commercial substrates, which all ranged between 0.3 and 0.6 g cm^{-3}. Some substrates had very high concentrations of certain plant nutrients, such as Substrate 4 with over 1800 mg kg^{-1} NH_4^+ or Substrate 5 with over 190 mg kg^{-1} NO_3^-. The C:N ratio was highly variable between substrates and ranged from 9.7 to 26.3. The average concentration of plant-available (Colwell) P across all commercial substrates was 488 mg kg^{-1}, whereas Substrate 10 had the highest concentration (1200 mg kg^{-1}). Concentrations of water-extractable P correlated well with concentrations of Colwell P ($R^2 = 0.73$). Some substrates showed high availability of PMN (3, 5, 10), whereas others were more limited. Stable NDIs (close to 1) were only found in Substrate 1 and 8, the latter containing a slow release fertilizer. Substrate 4, a compost, had the highest concentration of NH_4^+ and had a NDI of 1.7. Respiration was much higher in the commercial substrates than in the control, with up to 141.5 mg CO_2-C kg^{-1} h^{-1} in Substrate 3. DNA-based analysis found pathogens in the control soil and in all four composts (Substrates 3 – 6). The most common pathogen was the microsclerotia-producing *Macrophomina phaseolina*, which can result in stem or root rot. Metal and nutrient analysis showed some strongly varying results between substrates, such as in the case of arsenic (As), which ranged from 0.4 to 13.9 mg kg^{-1}, however, still below the upper limit of the Australian Standard for composts. Most substrates exceeded the upper limits for nickel (Ni), and one substrate for lead (Pb) (see Table 8.2. Analysis of the composts by solid state ^{13}C NMR revealed that the most abundant chemical

shift regions were found in the O-Alkyl, Aryl and Alkyl groups. Overall, results were quite variable and with little consistence between the substrates. Stronger correlations were found between the Substrate 3 to 5, which had higher concentrations of Alkyl and Amide/Carboxyl groups, and lower concentrations of O-Alkyl and Di-O-Alkyl than the other substrates (see Suppl. Figure 8.3 A). Principal component analysis (PCA) supports the strong shift of compost substrate towards the Alkyl and Amide/Carboxyl group (see Suppl. Figure 8.3 B).

8.3.2 Plant growth and mycorrhizal colonization

Plants growing in Substrates 1, 9 and 10 resulted in the highest shoot and root dry matter. Substrate 4 resulted in depressed plant growth and no plants survived in Substrate 5 (see Figure 8.1 A and B). Shoot N analysis revealed high variability between the substrates, ranging from 0.9% to 2.7% (see Table 8.3). Similar variability is found in most metal concentrations, whereas the lowest concentrations were detected for B, Cu, Mn and Zn. Arbuscular mycorrhizal colonization was around 60% root length colonized for most substrates. Significant differences were found for Substrates 7, which was significantly lower than Substrates 1, 2, 3 and 8. Biggest differences were found in Substrate 4, which was significantly lower colonized than all other substrates (see Figure 8.1 C).

TABLE 8.1: *Overview of soil analysis results. WHC = water holding capacity, EC = electrical conductivity, PMN = potential mineralizable nitrogen, NDI = nitrogen drawdown index, Colwell P = Plant-available (Colwell) P, Water P = Water extractable P, Respiration = Basal respiration, NA = no pathogens detected via PREDICTA® Research., kDNA = number of reads detected*

Treatment	Classification	Bulk density [g cm^{-3}]	WHC [%]	pH (1:5)	EC [μS cm^{-1}]	Total C [%]	Total N [%]	C:N	PMN [mg kg^{-1}]	NDI
1	Control	1.5	12.3	7.3	295	3.7	0.3	11.4	-2.4	0.9
2	Potting soil	0.4	154.5	7.2	2726	22.3	1.1	20.4	21	0.2
3	Compost	0.4	134.1	7.2	5830	22.5	1.9	11.9	351.5	0.1
4	Compost	0.5	99.6	7.8	7420	18.2	1.8	9.9	-173.9	1.7
5	Compost	0.6	71.2	7.7	6590	18.1	1.9	9.7	149.2	0.5
6	Compost	0.4	145.6	7.3	2455	24.3	1.2	19.9	13.9	0.6
7	Potting mix	0.4	125.9	7.2	1697	23.1	1	23.4	0.4	0.3
8	Potting mix	0.3	171.1	6.9	2815	26.7	1.2	21.5	4.7	0.9
9	Potting mix	0.3	162.8	6.6	2509	15.8	0.6	26.3	-965.6	0.2
10	Potting mix	0.3	143	6.9	5500	22.1	1	22	515.6	0.1

Treatment	Colwell P [mg kg^{-1}]	Water P [mg kg^{-1}]	Respiration [mg CO_2-C kg^{-1} h^{-1}]	Detected pathogens
1	24	4.1	0.9	*Pythium clade I [1.0 pg DNA g^{-1}]*
2	277.9	14.5	44.3	*NA*
3	377.4	19.3	141.5	*Macrophomina phaseolina [2.3 kDNA g^{-1}]*
4	777	29	49.5	*Sclerotinia sclerotiorum [2.3 kDNA g^{-1}]*
5	733.1	27.2	35.9	*Pratylenchus thornei [0.1 nematodes g^{-1}]*
6	417.9	15.2	36.8	*Macrophomina phaseolina [0.05 kDNA g^{-1}]*
7	285.7	36.2	56.3	*Macrophomina phaseolina [0.09 kDNA g^{-1}]*
8	159.3	13.9	35.9	*NA*
9	170.8	4.8	26.7	*NA*
10	1198.2	64.3	71	*NA*

TABLE 8.2: *Nutrient and metal concentrations in substrates. Results displayed as means ± standard error (n = 3). BDL: Below detection limit. Upper limit AS = Unrestricted use limits for contaminants according to the Australian Standards for Compost (AS 4454-2012), Upper limit NEPM = National Environment Protection Measures (NEPM): Health investigation levels for residential areas with garden/accessible soil, NA = no upper limit defined.*

Treatment	As [mg kg^{-1}]	B [mg kg^{-1}]	Cd [mg kg^{-1}]	Cu [mg kg^{-1}]	Fe [mg kg^{-1}]	Mn [mg kg^{-1}]
1	0.4 ± 0.2	15.9 ± 0.9	BDL	2.6 ± 0.1	4280 ± 89.1	46.4 ± 1.2
2	BDL	35.5 ± 1.3	BDL	31.9 ± 0.6	7708 ± 35.0	125.0 ± 1.5
3	0.6 ± 0.3	40.1 ± 0.8	0.1 ± 0.03	55.6 ± 1.4	7688 ± 879.5	126.7 ± 1.5
4	2.6 ± 0.7	58.3 ± 0.5	BDL	52.8 ± 1.8	8232 ± 725	213.1 ± 3.1
5	3.5 ± 0.6	63.6 ± 1.1	0.7 ± 0.3	91.6 ± 2.3	10417 ± 912	201.4 ± 3.5
6	BDL	41.0 ± 3.8	BDL	23.1 ± 2.0	8053 ± 567	121.2 ± 10.6
7	BDL	21.9 ± 0.5	BDL	24.4 ± 0.6	9226 ± 62.2	157.1 ± 1.2
8	BDL	18.6 ± 0.1	BDL	16.7 ± 0.1	9105 ± 83.8	105.9 ± 0.5
9	13.9 ± 1.8	17.0 ± 0.1	BDL	78.0 ± 3.4	7631 ± 40.9	99.0 ± 0.8
10	9.8 ± 3.6	19.8 ± 1.3	0.3 ± 0.2	70.0 ± 2.4	6899 ± 184	239.0 ± 5.9
Upper limits AS / NEPM	20 / 100	100 / 5000	NA / 20	150 / 7000	NA / NA	NA / 3000
Treatment	Ni [mg kg^{-1}]	P [mg kg^{-1}]	Pb [mg kg^{-1}]	Se [mg kg^{-1}]	Zn [mg kg^{-1}]	
1	147.6 ± 6.8	63.6 ± 2.1	3.7 ± 0.1	BDL	8.6 ± 0.3	
2	97.2 ± 1.7	1540.3 ± 20	19.1 ± 0.2	BDL	114.0 ± 2.0	
3	12.1 ± 0.8	2319.6 ± 51.5	39.1 ± 1.2	BDL	181.6 ± 6.4	
4	66.3 ± 0.8	3216.13 ± 34	25.1 ± 1.4	BDL	223.0 ± 2.6	
5	124.1 ± 8.9	3586.9 ± 31.2	30.9 ± 0.2	BDL	245.1 ± 4.3	
6	69.8 ± 6.8	1223.2 ± 107	49.3 ± 22.0	BDL	77.4 ± 5.1	
7	174.1 ± 1.6	1171.5 ± 5.6	11.9 ± 0.3	BDL	65.3 ± 0.9	
8	84.4 ± 1.6	704.3 ± 11.7	0.9 ± 0.05	BDL	30.9 ± 0.5	
9	270.6 ± 1.2	600.9 ± 4.3	1287.7 ± 115.5	BDL	143.0 ± 2.3	
10	14.7 ± 1.7	2650.4 ± 51.0	31.9 ± 0.7	BDL	184.8 ± 5.7	
Upper limits AS / NEPM	60 / 400	NA / NA	150 / 300	5 / 200	300 / 8000	

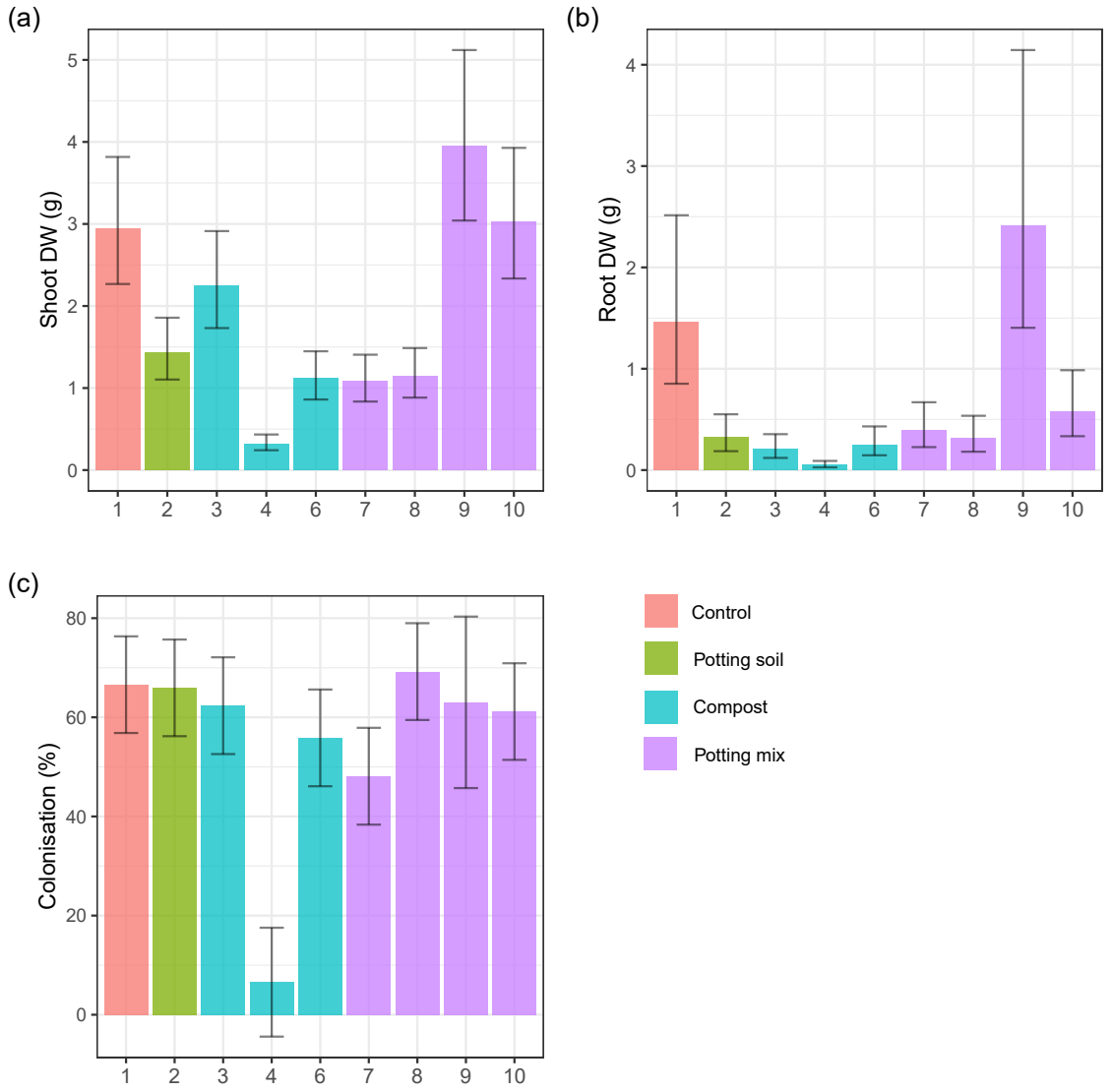

FIGURE 8.1: *Estimated marginal means (EMMs) for (a) Shoot Dry Weight (SDW), (b) Root Dry Weight (RDW) and (c) Mycorrhizal Colonisation of roots (%). Error bars correspond to 95% confidence intervals. No plant growth in Substrate 5 (omitted).*

The first imaging for phenotyping was performed at 14 days after planting (DAP). At this time, plants with high a high projected shoot area or growth rates also resulted in higher dry weights at the end of the experiment (see Figure 8.2). Only plants in Substrate 3 started with a relatively small projected shoot area and growth rate but performed better towards the end of the experiment. Plants with high growth rates at the beginning of the bioassay also showed an earlier peak in their shoot area than plants with lower growth rates (see Suppl. Figure 8.4).

TABLE 8.3: *Nutrient concentrations in maize shoots. Results as means ± standard error (n = 6). NA: No results available due to limited plant material. BDL: Below detection limit. Adequate: recommended levels based on Reuter and Robinson, 2020 and whole shoot analysis.*

Treatment	N [%]	B [g kg^{-1}]	Ca [g kg^{-1}]	Cu [g kg^{-1}]	Fe [g kg^{-1}]	K [g kg^{-1}]
1	1.4 ± 0.1	0.03 ± 0	3.5 ± 0.3	BDL	0.07 ± 0.02	19.8 ± 1.2
2	2.2 ± 0.1	0.01 ± 0	1.6 ± 0.2	0.01 ± 0	0.12 ± 0.03	29.6 ± 0.5
3	2.7 ± 0.1	0.04 ± 0.01	1.6 ± 0.1	0.01 ± 0	0.10 ± 0.01	38.2 ± 1.5
4	*NA*	0.1 ± 0.01	2.7 ± 0.7	0.01 ± 0	0.47 ± 0.26	65.3 ± 2.2
5	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
6	1.8 ± 0.1	0.02 ± 0	1.4 ± 0.01	BDL	0.08 ± 0.01	30.5 ± 0.9
7	1.4 ± 0.1	0.01 ± 0	1.3 ± 0.1	BDL	0.12 ± 0.05	32.8 ± 1.2
8	1.8 ± 0.03	0.02 ± 0	2.4 ± 0.1	BDL	0.09 ± 0.02	26.0 ± 0.7
9	0.9 ± 0.1	0.03 ± 0	1.7 ± 0.1	BDL	0.05 ± 0.01	27.8 ± 0.8
10	1.7 ± 0.1	0.02 ± 0	1.1 ± 0.1	0.01 ± 0	0.05 ± 0.01	56.0 ± 3.8
Adequate	3.5 – 5	0.007 – 0.025	9 – 16	0.007 – 0.002	0.05 – 0.3	30 - 50
Treatment	Mg [g kg^{-1}]	Mn [g kg^{-1}]	P [g kg^{-1}]	S [g kg^{-1}]	Zn [g kg^{-1}]	
1	2.2 ± 0.3	0.05 ± 0	5.5 ± 0.2	1.3 ± 0	0.03 ± 0	
2	1.7 ± 0.1	0.01 ± 0	5.5 ± 0.1	2.9 ± 0.1	0.08 ± 0	
3	1.9 ± 0.1	0.01 ± 0	4.0 ± 0.2	3.0 ± 0.1	0.08 ± 0.01	
4	2.3 ± 0.2	0.05 ± 0	4.2 ± 0.2	3.2 ± 0.2	0.06 ± 0	
5	*NA*	*NA*	*NA*	*NA*	*NA*	
6	1.5 ± 0.1	BDL	4.6 ± 0.1	2.5 ± 0	0.03 ± 0	
7	1.9 ± 0.1	0.02 ± 0	5.6 ± 0.3	2.5 ± 0.1	0.05 ± 0	
8	1.7 ± 0.04	BDL	2.6 ± 0.1	2.9 ± 0.1	0.04 ± 0	
9	1.7 ± 0.1	0.07 ± 0	2.2 ± 0.3	2.4 ± 0.1	0.03 ± 0.01	
10	1.7 ± 0.2	0.03 ± 0	6.9 ± 0.2	2.3 ± 0.2	0.1 ± 0	
Adequate	3 - 8	0.05 – 0.16	4 – 8	2 – 3	0.02 – 0.05	

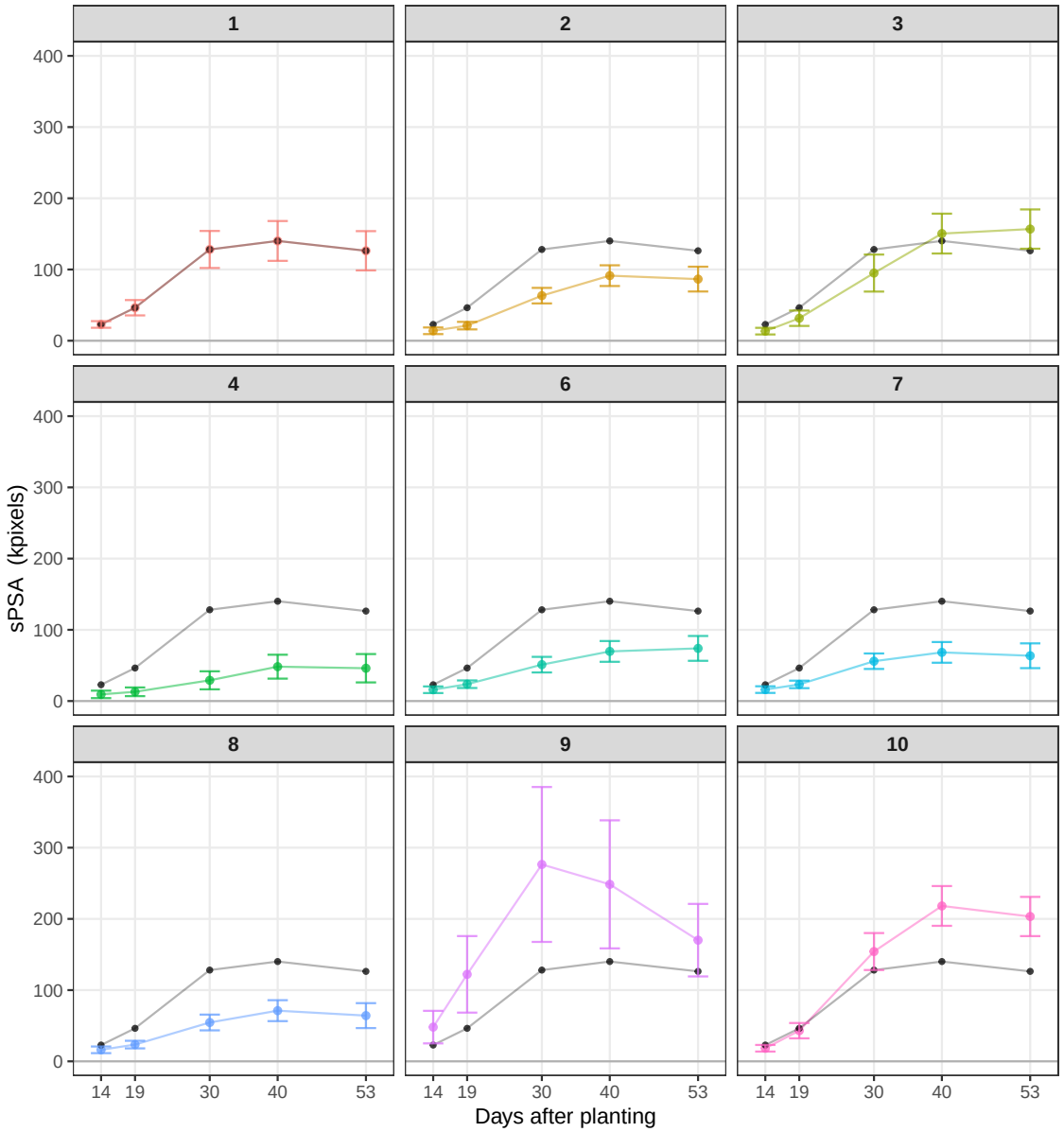

FIGURE 8.2: *Estimated marginal means (EMMs) over the imaging period for smoothed projected shoot area (sPSA) at days after planting (DAP) 14, 19, 30, 40 and 53. Error bars correspond to 95% confidence intervals. To aid visual comparison of Soil treatments, the Control results are indicated by grey curves without error bars. No plant growth in Substrate 5 (omitted).*

8.4 Discussion

8.4.1 Plant substrate analysis

Besides the control treatment, all types of substrate in this study are commonly used in horticulture or urban agriculture. In theory, potting soils are used for garden beds (Standard, 2018), whereas potting mixes are more suitable for container plants and raised beds due to their lower bulk density (Standard, 2003). However, there were no difference in bulk densities between the potting soils and potting mixes in this study. Composts are commonly used as soil amendments, such as for organic fertilization (Standard, 2003).

This practice has shown to improve the chemical, physical and microbial properties of soils (Bonanomi et al., 2007; Diacono & Montemurro, 2010). As such, composts are important tools to restore degraded soils and to close the nutrient cycle towards sustainable food production (Halloran et al., 2014).

Physico-chemical analysis showed that the bulk density, WHC and pH of all commercial substrates can be considered sufficient for most plants. As such, they provided lower bulk densities and higher WHC than the control treatment and most natural soils, which are around 1.6 g cm^{-3} and 30% WHC for agricultural sandy-loam soils (Rasool et al., 2008). The pH of all substrates ranged from 6.9 to 7.8; this is higher than the pH of around 5.5 that is often desired in horticulture to improve nutrient availability in the substrates (Barrett et al., 2016). Nevertheless, most crop plants can grow within the pH ranges measured in this study. The EC values of the commercial substrates were high, especially in most composts. Such high levels are typical for composts due to the presence of soluble salts. These could consist of ions which are essential plant minerals, such as K^+ or Ca^{2+} or be indicative of high concentrations of Na^+, which could hinder the water uptake of plants (Reuter & Robinson, 2020). However, Na^+ was not measured in this study. The recommended EC (water 1:5) for maize seedlings is below 1000 μS cm^{-1} and depressed plant growth is observed above this threshold (Maas et al., 1983). High EC values are one reason to use composts only as soil amendments, rather than as a sole plant growth substrate. However, Substrates 6 (compost) had lower EC values than the potting mixes. This could be explained by different composting processes, source materials, and/or the addition of bulking agents (Gondek et al., 2020).

Concentrations of mineral N (NO_3^- and NH_4^+), PMN and total N were high for most commercial substrates, consistently exceeding critical or recommended concentrations for maize, which are around 6 mg kg^{-1} for mineral N (Peng et al., 2013), 32 mg kg^{-1} for PMN (J. D. Clark et al., 2019) and 0.15% total N (W. Gao et al., 2015). Yet, all plants were deficient in N, even though an additional 10 mg of NH_4NO_3-N was applied weekly. These results suggest that the N-dynamics of the commercial substrates are dominated by (microbial) N-immobilisation, which was also confirmed by the NDI analysis. Most substrates had unstable NDIs with high N-drawdown, whereas only the control and Substrate 8 had values close to 1. For the control, this can be explained by the high concentration of sand, which contribute little to N immobilisation. Substrate 8 contained a slow release fertilizer which might have counteracted possible N-drawdown during the incubation of the NDI analysis. Most other substrates showed high levels of N-drawdown, as indicated by low NDI values (Handreck, 1992). The NDI of Substrate 4 was positive, most probably due to the very high concentrations of NH_4^+ and its subsequent nitrification during the incubation. However, this possible advantage in terms of N-supply to plants was diminished through depressed growth, most probably due to NH_4^+-toxicity (Reuter & Robinson, 2020). N-immobilisation, as found in this study is generally increased in soils with labile carbon pools and high microbial activity, due to the uptake of mineral N

by microorganisms (Geisseler et al., 2010). All potting mixes and composts in this study had presumably high microbial activity, as indicated by the basal CO_2 respiration which was approximately 50 times higher in the commercial substrates than in the soil/sand control; on average, all commercial substrates released 50 mg CO_2-C kg^{-1} h^{-1}, whereas the control soil only released 0.9 mg CO_2-C kg^{-1} h^{-1}. Another important aspect regarding N availability is the C:N ratio. Ratios above 15 are more prone to N-immobilization, whereas greater N-mineralization (S. Clark & Cavigelli, 2005) is observed at lower ratios (Qian & Schoenau, 2002). In this study, the C:N ratio was not strongly correlated with shoot N (R^2 = 0.30). This suggest that other factors, such as the decomposability of the various carbon pools play a more important role than the C:N ratio in the N competition between microorganisms and plants (Månsson et al., 2009). Together, these data highlight the need to incorporate only matured C sources to avoid issues of N-immobilization (Jedidi et al., 1995). Where this is not possible, the addition of slow-release or quick-release fertilizer can counteract microbial N immobilization (Lazicki et al., 2020).

Different C pools are captured by the ^{13}C NMR analysis which, overall, show rather variable spectra between the substrates. This is likely a representation of different organic materials with varying stages of decomposition between the substrates. However, one interrelationship is found between the substrates 3 to 5 within the Alkyl and Amide/Carboxyl functional groups. These groups are indicative for lipids and proteins where N is most likely contributed by microorganisms, rather than plant material (Caricasole et al., 2011). Conversely, the O-Alkyl and Di-O-Alkyl groups in these substrates are lower compared to the other substrates. These two groups are commonly derived from polysaccharides, such as cellulose or hemicellulose (Amir et al., 2010; Chen et al., 1989). Taken together, these results suggest that Substrates 3 to 5 are either predominantly derived from organic non-plant materials, such as animal manures, or, if they are derived from plant material, they have undergone a high degree of decomposition where polysaccharides have transformed into microbial biomass. This is further supported by the low C:N ratio of these substrates (C:N = 10 – 12). This observation also correlates with the fact that highest shoot N concentrations were found in Substrate 3, indicating lower N immobilization in the substrate. However, no shoot N concentrations are available for Substrates 4 and 5 due to high plant mortality (see below).

Plant-available (Colwell) P was abundant in all commercial substrates and well above the critical concentration for most horticultural crops. The critical concentrations of plant available (Colwell) P in soil for maize was reported at 32 mg kg^{-1} (Moody et al., 2013). Hence, shoot concentrations of P were adequate for most substrates, with the exception of Substrates 8 and 9. These substrates also had the lowest amounts of water-extractable P. Generally, plant-available (water and Colwell) P and total P were poor predictors of shoot P (Colwell P: R^2 = 0.28, water-extractable P: R^2 = 0.38, total P: R^2 = 0.16). Shoot micronutrient concentration were mostly adequate or above, with the exception of concentrations of Ca and Mg which were below the recommended values for all substrates.

One likely explanation is a different nutrient uptake due to a varietal difference of the dwarf cultivar Gaspe, compared to the reference varieties (Iken et al., 2002). The highest correlation between shoot micronutrient concentration and soil micronutrient concentration was found in Zn (R^2 = 0.49), whereas all other micronutrients in shoots were poorly correlated (R^2 < 0.1). Concentrations of Ni exceeded the upper limit of the Australian Standard for Compost in all substrates. However, these results should be interpreted in light of the high metal binding capacity of Fe oxides and the geochemical ratio of elements with Fe (Hamon et al., 2004). Substrate 9 had Pb concentrations well above national health investigation levels. One possible source of contamination could be the use of painted timber which has been processed to wood chips and added as bulking agent (Rodrigues et al., 2020). For commercial substrates it is important to consider that, the exposure pathway of contaminants is not limited to dietary uptake of crops growing in such substrates, but also *via* dermal contact and dust inhalation after opening the bags and handling the substrate (Cramp et al., 2010).

All substrates were assessed for 22 common horticultural plant pathogens using a commercial DNA-based soil diagnostic test (PREDICTA® Research). Macrophomina phaseolina, which can cause stem or root rot, was found in three substrates. The other detected pathogens were *Pythium clade* (root rot), *Sclerotinia sclerotiorum* (white mold) and the root-lesion nematode *Pratylenchus thornei*. Although detected in the substrates at various concentrations, it remains unclear whether, or at which concentration and environment, these pathogens cause disease. The pathogen detection only indicates the presence of their DNA, whereas the development of a plant disease involves multifactorial inheritance (Francl, 2001). However, one can assume that unsuccessful plant growth in Substrate 5 was due to the presence of the nematode Pratylenchus thornei, against which maize plants have been described to have only medium resistance (Thomsen & Hart, 2018). The detected plant pathogens *Macrophomina sp.* and *Sclerotinia sp.* develop sclerotia as a survival mechanism. Even with proper phytosanitary practices, it might be difficult to eliminate such pathogens (Agrios, 2005). The Australian Standard requires different sanitation protocols for low-risk (plant material) or high-risk (manures) feedstock. For low risk material, three compost turns with internal temperatures of at least 55 °C for three days are required between each turn. For comparison, other regulations such as the German Ordinance on the Recovery of Bio-Waste require 55 °C for at least 2 weeks (of the Environment, 2013).

When comparing the results of the substrate analysis with the Australian Standard for Composts and Potting mixes (Standard, 2003, 2012), some criteria were not met. Potting mixes require NDIs above 0.2 (regular) or 0.7 (premium potting mix), which was not met by Substrate 10. The pH of all tested potting mixes was higher than the required pH range of 5.3 to 6.5. The concentration of NH_4^+ needs to be below 100 mg L^{-1}; this was not the case for Substrates 9 and 10. Various substrates exceeded upper limits for

metal concentrations as defined in the Australian Standard for composts. Contamination can be introduced due to the use of improper materials in the composting processes. These materials include non-organic waste products, such as plastic, sanitary products or special waste, such as road sweepings. Organic materials can also introduce metal contamination, for example due to the processing of timber which has been treated with paint or preservatives (Rodrigues et al., 2020). It is important to note that compost feedstock can be highly variable, thereby limit the producer's control over the end product (Reyes-Torres et al., 2018). Some composts also did not meet the required maturity indicators (Standard, 2012). Maturity describes the degree of organic matter decomposition and whether the turnover of organic material has sufficiently slowed down to provide stable plant growth conditions (Fourti, 2013). According to the Standard, 2012, compost maturity requires a NDI above 0.5, which was not met by Substrates 3 and 7. Respiration rates are required to stay below 91 CO_2-C kg^{-1} h^{-1}, which was not met by Substrate 3. Another visual indicator was the substrate shrinking in Substrate 3 during the greenhouse bioassay (Gruda, 2019). This, however, was a simple observation and not quantified.

8.4.2 Mycorrhizal colonization and plant growth

In addition to undertaking a detail analysis of the plant growth media (above), we also assessed their suitability for plant growth and arbuscular mycorrhizal colonization using a high throughput phenotyping experiment. For most substrates, there were no differences in mycorrhizal root colonization and only plants grown in Substrates 4 and 7 showed significantly lower colonization. However, plants in Substrate 7 were still well colonized with around 50% root length colonized. Based on the substrate analysis, there are no indications for certain chemical properties that would have decreased mycorrhizal root colonization. The reasons might be found within biological interactions between AMF and the microbial community (Svenningsen et al., 2018); this however, remains speculative. It is also unknown whether this relatively small, but statistically significant, difference in root colonization would result in different mycorrhizal functioning (Treseder, 2013). Compared to all other substrates, plants growing in Substrate 4 were much less colonized. This substrate contained about 1800 mg of NH_4^+-N, which, after rewetting to its WHC, could have caused substrate acidification due to the nitrification of NH_4^+ to NO_3^-, thereby supressing AMF development (Pan et al., 2020). However, the pH of the substrate was not measured during or after the bioassay, so this remains speculative. All other plants displayed similar mycorrhizal root colonization at around 50 - 60% root length colonized by arbuscules, vesicles or hyphae, which is typical for well-colonized maize plants (Tran et al., 2019; Wang et al., 2020; Watts-Williams et al., 2019). These results indicate that even strongly varying substrate properties, such as EC, plant-available P, total C or N had no discernible effect on the substrate's ability to support AMF. In practice, these results suggest that common potting mixes and composts which provide good plant growth could also be inoculated with AMF inoculum. The benefits of AMF in horticultural environments to further improve plant vigour and yields has been well studied (Rouphael et al., 2015). The introduction of beneficial microorganisms during

the composting process has been proposed to increase plant-available nutrients (Sánchez et al., 2017). Similarly, beneficial microorganisms, such as AMF, could be supplemented to the finished product (Fuchs, 2010).

Plant dry weights were highly variable between the substrates with the greatest biomass produced in Substrates 1, 9 and 10. Although there were no significant differences between these plants in terms of total dry matter, phenotyping data revealed that Substrate 9 provided optimal conditions for plant growth. Plants growing in this substrate achieved the highest leaf surface area at DAP 15 and the highest growth rates until DAP 30. Subsequently, the growth rate strongly decreased, probably from switching earlier than other treatments from the vegetative to the generative growth phase. Generally, plants with higher final dry weights showed an earlier decline in growth rates, indicating better nutrient supply and growth support. N-deficiency has been shown to delay vegetative and reproductive development of maize (Uhart & Andrade, 1995). Another factor with possible effects on plant growth is substrate phytotoxicity. Phytotoxicity in an organic growing medium refers to the inhibition of plant growth, either due to undesired properties, such as high EC, or transient compounds which can accumulate at certain stages of composting. Commonly found toxic compounds are short chain organic fatty acids, phenols or alcohols (Paradelo & Barral, 2011). Phenotyping data of plants growing in Substrate 3 showed a continuous increase in the absolute growth rate, which might be indicative of reducing phytotoxicity over time. Plant growth bioassays are a common method for determining phytotoxicity and are described in various standards for the determination of the effects of pollutants on soil flora (ISO 11269-2:2012). These tests capture continuous changes of the substrate, whereas germination tests only provide a snapshot of phytotoxicity (Cesaro et al., 2015). The results of this study show that continuous phenotyping of plants provides additional information that supplements the more commonly used plant dry weight, which is only measured at the end of the bioassay. In cases where the use of an automated high-throughput phenotyping system is not economical, a variety of affordable and flexible phenotyping platforms have been released over the last years (Araus & Kefauver, 2018).

8.5 Conclusion

Composts and potting mixes are commonly used to improve degraded soils or to grow food in soilless culture systems. AMF have been shown to improve plant growth and to provide essential ecosystem services, however, their development in plant substrates from organic origin is not well explored. This study showed that potting mixes and composts can be successfully inoculated with AMF. Strongly depressed mycorrhizal root colonization was only found in one substrate, probably due to NH_4^+ toxicity and substrate acidification. Although the effects of AMF were not evaluated within this study, a broad spectrum of scientific literature highlights the various benefits of AMF for plants

and their surrounding ecosystem. In this study, one common element between all commercial substrates was a high level of N immobilization which required additional N fertilization for adequate plant growth. Further analysis suggests that this N immobilization is dictated by varying proportions of the different carbon pools, whereas more plant N is available in substrates at a more advanced decomposition stage. Other adverse properties were discovered, such as the presence of plant pathogens or Pb concentrations above the national compost standard. For scenarios where composts and potting mixes are used as plant substrates, seed germination tests can be used to identify the presence of phytotoxic components. Organic or inorganic N fertilizers could be added to counteract N immobilization. For the scientific or regulatory evaluation of composts and potting mixes *via* plant growth bioassays, continuous phenotyping can be used to obtain refined insights into the effects of substrates on plant growth.

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8.7 Supplementary material

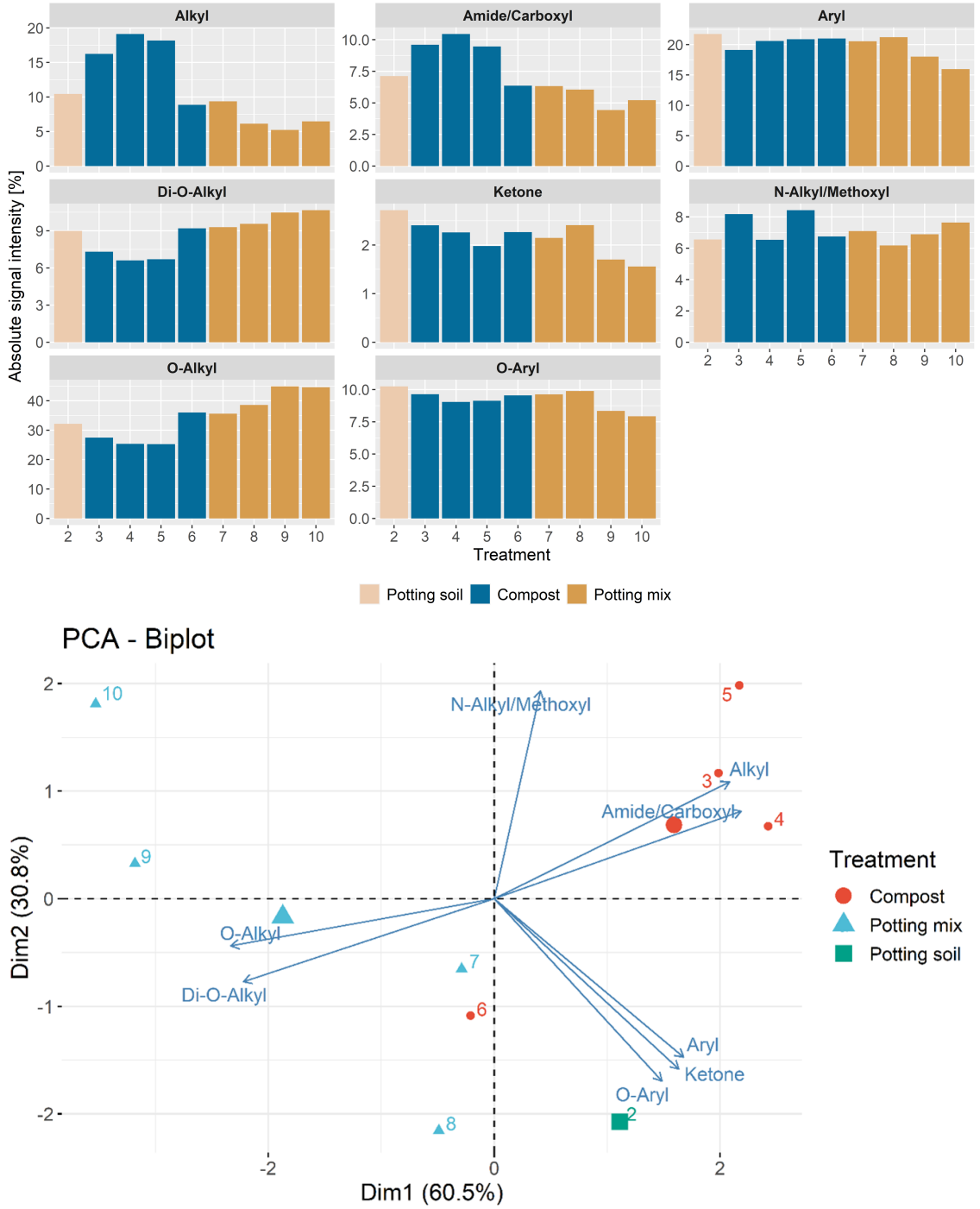

FIGURE 8.3: *A: Overview of ^{13}C solid state nuclear magnetic resonance (NMR) spectroscopy functional carbon groups between substrate Substrates 2 to 10. B: Principal component analysis (PCA) biplot of ^{13}C solid state nuclear magnetic resonance (NMR) spectroscopy based on functional carbon groups between Substrates 2 and 10.*

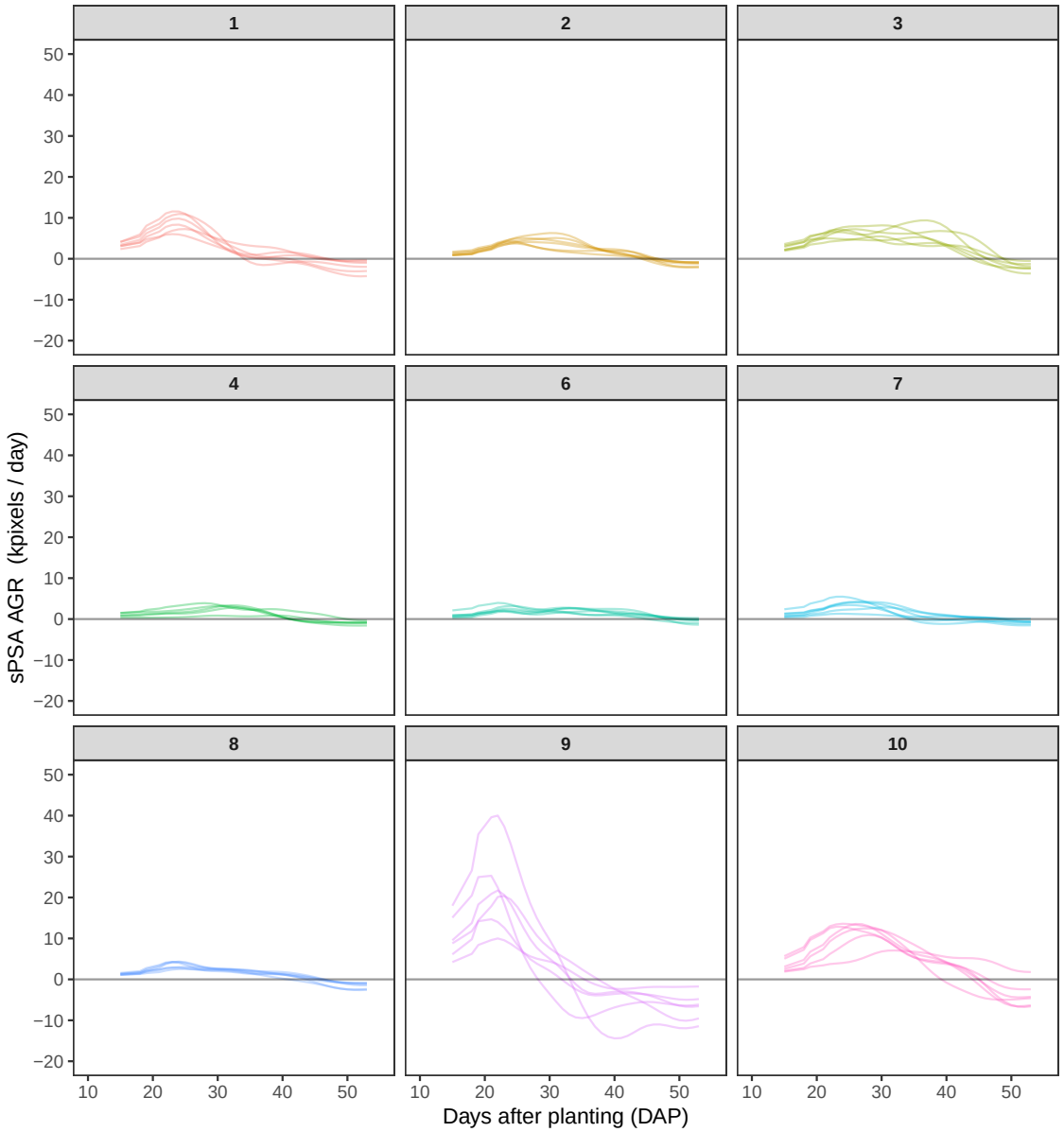

FIGURE 8.4: *Descriptive growth plots of individual plants, displayed as smoothed absolute growth rate (sAGR). One curve per plant. No plant growth in Substrate 5 (omitted).*

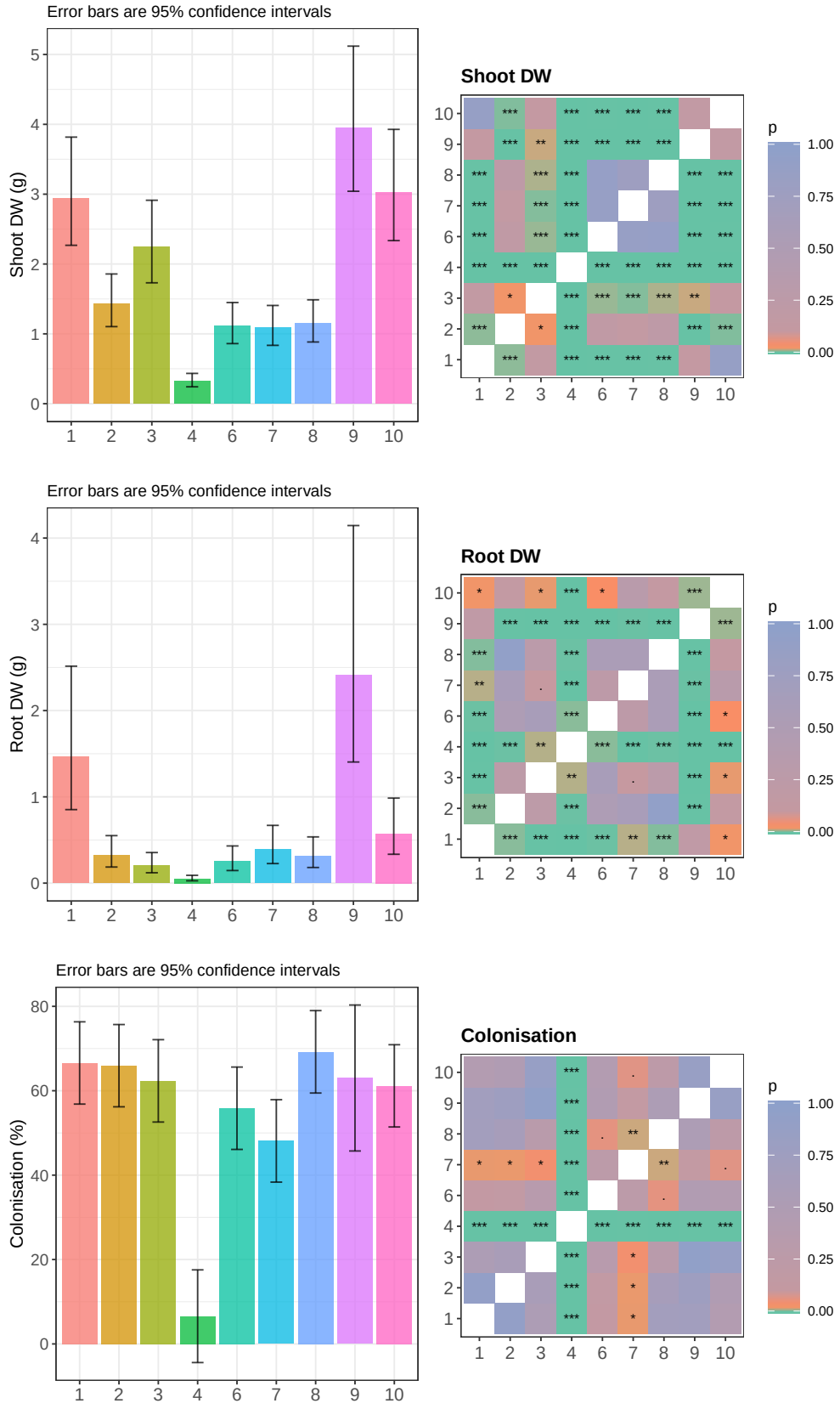

FIGURE 8.5: *Estimated marginal means (EMMs) for Shoot Dry Weight (SDW), Root Dry Weight (RDW) and Mycorrhizal Colonisation of roots (%). Error bars correspond to 95% confidence intervals. No plant growth in Substrate 5 (omitted). Heatmap indicating p-values and statistical significant differences between the Substrates.*

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Chapter 9

Discussion

9.1 Soil health in urban agriculture systems

Surveys on urban agriculture sites in Adelaide (Chapter 4 and 5) showed that soils contain substantial plant nutrients, with very high concentrations, especially of phosphorus (P). The median concentration of plant-available (Colwell) P in Chapter 4 was over five times the critical concentration for lettuce (*Lactuca sativa*). Plant-available P was mainly measured as Colwell P due to the method's common use in Australia and an abundant dataset of critical concentrations for important food crops. However, plant-available (Colwell) P might be biased in urban agriculture soils, because the extractant was designed for acidic agricultural soils (Colwell, 1963). Urban agriculture soils differ in various ways, including lower bulk density and higher carbon (C) concentration. When analyzing these soils by weight, this amounts to a greater volume of soil and potentially more plant-available P. High C concentration in soils might also interfere with the extraction of P, such as through adsorption (Yang et al., 2019). However, this is speculative, and the potential discrepancy between urban agriculture soils and different P solvents was not a focus in this thesis. One indication of accuracy between different plant-availability tests for P can be found in Chapter 8, where water-extractable P and Colwell P were strongly correlated in typical urban agriculture substrates (R^2 = 0.73). The correlation of Colwell P to the P concentration in shoot tissue was much lower for both tests (R^2 = 0.28 and 0.38). However, these unknowns in testing plant-available P do not deviate from the fact that general levels of P in urban agriculture soils are very high. This has also been confirmed through total P analysis of the soil (Chapter 4) and has a strong biological reason due to the intensive input of animal manures for organic fertilization. The reasoning behind measuring plant-available P is to provide improved fertilizer application guidelines, which are arguably of little importance in these cases. One might argue that the measurement of water-extractable P as an estimation of potential P runoff might be of higher importance (Kleinman et al., 2007).

P accumulation in soils can become an issue for plant growth and productivity once it reaches phytotoxic levels. At such concentrations, plant deficiencies might occur, mainly due to antagonistic interactions between Fe and Zn (Jones, 1998). For example, high P

concentration in soils leads to increased plant Zn requirements, which might then become the limiting factor in that system (Loneragan et al., 1979). Furthermore, high P soils pose an environmental risk due to potential runoff into waterways (Hart et al., 2004). Once P has accumulated in soil to phytotoxic levels, it is difficult to correct. One way to remove P from soils is through biomass production, where Fe and Zn are applied as a foliar application to facilitate plant uptake and thus overcome the limitation to growth (Zhang et al., 2012). Soils could also be exchanged or diluted with low-P soils, raising the question, whether excavated high-P soil needs to be treated as hazardous to avoid further environmental damage (such as to P sensitive native vegetation). Such options are labor-intensive, costly, and contradictory to the common urban agriculture principles of sustainable food production. Research on phytotoxic concentrations of P in soils is limited. In this thesis, the highest plant-available (Colwell) P found in a community garden bed was 1266 mg kg^{-1}, which corresponded to 6490 mg kg^{-1} of total P (Chapter 4). The corresponding community garden was created in 2003. Using a very simplified model for the annual increase of plant-available P, the garden bed in question accumulated 90.5 mg kg^{-1} of plant-available (Colwell) P per year. Given the lack of literature on P toxicity and its interactions with micronutrients, it is difficult to estimate certain threshold levels. However, when levels of plant-available (Colwell) P are already above the critical concentration of common crop plants by an order of magnitude, it is fair to assume that this trend needs to be corrected. One feasible solution includes to educate gardeners proactively about suitable composting materials which provide sustainable ratios of C:N:P.

When considering nitrogen (N) in soils, this thesis illustrates that urban agriculture soil contain high concentrations of total N and potential mineralizable nitrogen (PMN) (Chapter 4 and 5). Due to its highly dynamic cycle, the amount of plant-available or mineral N can be quite low at certain times of the year (Chapter 5). Research by Arrobas et al., 2017 showed that N concentrations in plant tissues were close to the lower critical value. Similarly, work in this thesis reports that low tissue N concentrations were discovered when maize plants were grown in common urban agriculture substrates (Chapter 8). In this case, the plants had even been fertilized with NH_4NO_3 to prevent severe symptoms of N deficiency. This suggests that N supply to plants in urban agriculture systems might be affected by environmental conditions which lead to leaching and reduced nitrification, but also through immobilization in the soil. Based on the organic farming principles that all urban agriculture sites adhere to, organic fertilizer would need to be applied multiple times throughout the season, depending on the plants' actual demand. This however raises concerns over food safety when organic fertilizers are applied too close to harvest times, especially for root vegetables. N availability is a similar concern in organic agriculture, which is addressed through crop rotations, intercropping, increased soil ecology and biofertilizers. Such measures could be similarly applied to urban agriculture. Issues of N immobilization can be mitigated by using only matured composts.

This thesis revealed an abundance of arbuscular mycorrhizal (AM) fungal spores in

urban agriculture soils (Chapter 4 and 5). When these soils were used in a growth bioassay, using tomato as a host plant, substantial mycorrhizal root colonization was observed in most treatments (Chapter 4). Percentage root colonization further increased after the addition of *Rhizophagus irregularis*. These results are surprising, since literature consensus describes an antagonistic effect of P on the colonization of arbuscular mycorrhizal fungi (AMF). This effect has been described *in vitro* (Hepper, 1983), in greenhouse studies (Nguyen et al., 2019; Watts-Williams & Cavagnaro, 2012), and in agricultural systems (Kahiluoto et al., 2001). Various reasons, or a combination thereof, could explain the phenomena that we see in urban agriculture soils. Research has long identified the importance of the soil microbial community for the establishment of AMF. One of the most common terms for a group of AMF-beneficial bacteria is "mycorrhiza helper bacteria (MHB)". These bacteria are taxonomically diverse and have been isolated from most natural habitats. They are closely associated with AM and show a high species specificity (Deveau & Labbé, 2017). Although MHB can always be found in the AM symbiosis, different strains can confer diverse functioning (Turrini et al., 2018). Soils with high concentrations of C, such as in urban agriculture systems, might provide a greater diversity of MHB that enable AMF to develop in high P environments. Research has identified changes in the soil microbial community in C-amended soils (Drenovsky et al., 2004). Although this study did not focus on MHB, it is plausible that mycorrhiza-beneficial bacteria could be found in urban agriculture soils. Another explanation is the natural selection of AMF species and strains with higher tolerance of high soil P concentrations. Functionally diverging AMF groups have been found within different ecosystems (Antunes et al., 2011). Some traditional farming practices such as tillage, inorganic soil fertilization or reduced crop rotations are associated with fewer dominant AMF species (Verbruggen & Toby Kiers, 2010). Similarly, research has focused on the impact of soil management practices on the abundance and diversity of AMF (Bowles et al., 2017). The most positive correlated activities are a high plant biodiversity, continuous active vegetation, and minimal soil disturbance. Organic farming is associated with higher AMF diversity and abundance than conventional farming (Verbruggen et al., 2010). Most of these principles are also found in urban agriculture systems. This thesis showed that most garden beds had year-round high plant biodiversity and that community gardens generally adhered to the same principles as those of organic farming (Chapter 4 and 5). The positive effects of organic farming for AMF development are often explained by lower nutrient concentrations in the soil. This is not the case in most urban agriculture systems but may be another compelling reason that the form of P is more important than its concentration. Research has identified that different sources, and availability, of P can impact arbuscular mycorrhizal activity differently (Reynolds et al., 2006; van Geel et al., 2016). Based on the results of this study, one might even deviate protective effects of AMF at extreme soil P conditions towards plant health and the uptake of micronutrients. This however is speculative, and warrants further investigation.

9.2 Commercial AMF inoculants

The focus on commercial AMF inoculants in this thesis revealed that most products did not contain viable propagules when tested in a plant growth bioassay. To interpret these results, the term 'viability' needs to be first defined. Within this thesis, inoculants were tested in a plant growth bioassay on tomato and leek as a host plant. Therefore, 'viability' in this context described the ability of AMF propagules to germinate in a plant substrate and to develop an arbuscular mycorrhizal symbiosis. This symbiosis was then measured as a percentage of arbuscular mycorrhizal root colonization. Theoretically, viability could also be measured through evaluating *in vitro* spore germination or spore staining for metabolic activity (Mayo et al., 1986; Walley & Germida, 1995). These methods provide faster results but with lesser value for applied scenarios. Besides root staining, biotechnological methods could be applied, which was also done in the North American part of this study. For this, colonized root pieces were collected and analyzed via amplicon-based sequencing to provide information on the AMF species assemblage. This is of special interest when AMF inoculum is added to natural soil, to observe potential shifts in the arbuscular mycorrhizal community. When used in sterilized soil, it would confirm the species' description of the added inoculum with its actual species taxonomy. Another infectivity assay is the most probable number (MPN) assay (Sieverding et al., 1991). Here, the inoculum or substrate is diluted by various orders of magnitude and applied to a host plant. The presence of AMF colonization is analyzed in root samples from each dilution treatments, and the MPN estimated through a mathematical model.

For this thesis, the most applied scenario was investigated by using a plant growth bioassay and adding the amount of inoculum as it is recommended by the manufacturer. Instead of the time intensive MPN assay, spores were extracted from the products and counted. Spore extraction revealed one reason for non-viable inoculants, which is a negligible number of AMF spores. For other products, ample spores were found, but low root colonization revealed insufficient germination rates. Why these spores did not germinate is speculative, but reasons could include prolonged storage periods or adverse conditions during formulation and packaging of the products. One could argue that the actual underlying issue is a lack of mandatory quality control mechanisms for microbial inoculants in most countries. In Chapter 7, we contend that a plant growth bioassay would be a sufficient means for AMF inoculant quality control, and that it could be implemented under a mandatory quality control scheme. Such a system has been implemented in Japan, where inoculum producers are required to provide percentage root colonization and declare the host plant identity (Chapter 7). From a consumer's perspective, the proposed quality control framework would increase product reliability and potentially increase the widespread adoption of AMF inoculants. From a producer's perspective, unreliable companies would be removed from the market, thereby reducing competition. This could also have economic benefits, which would then justify increasing costs of mandatory quality control. This, however, is speculative and has not been

investigated.

Whereas legal guidelines for quality control of AMF inoculants would help to provide fair conditions for the consumers, more work is necessary to improve the production of AMF inoculum for economic and quality benefits. To date, most inoculants are produced *in vivo* on living host plants, which is costly and raises phytosanitary issues (von Alten et al., 2002). Alternatives have been proposed, such as bioreactors, aquaponic or aeroponic systems (Ijdo et al., 2011). Latest work in this area showed that asymbiotic sporulation is possible under *in vitro* conditions (Sugiura et al., 2020). Using a combination of fatty acids and plant hormones, this system was further improved for the asymbiotic mass production of *Rhizophagus clarus* (Tanaka et al., 2020). These new insights into the metabolism of the arbuscular mycorrhizal symbiosis allows further exploration pathways, such as host plant breeding for increased lipid metabolism (Gargouri et al., 2021). These are important steps for future AMF inoculum production, which need to be complimented by other research questions. In personal communication with Stéphane Declerck, he termed the acronym "VIPS", which stands for viability, infectivity, purity and stability of inoculum. Following this principle, mass-produced spores need to retain their viability and infectivity for long enough to be used in applied scenarios. Purity describes the absence of phytopathogens, which is almost guaranteed when using axenic conditions. Finally, the inoculum requires functional stability, which will guarantee similar mycorrhizal growth responses (MGR) over multiple generations. In a study by Kokkoris and Hart, 2019, repeated *in vitro* sub-culturing of single species AMF in a dual culture system resulted in functional and morphological changes between 10 and 80 generations of subcultures. While the number of spores per culture increased over time, P uptake benefits to host plants were reduced. The theoretical explanation described changes in fungal strategy that favored spore and extraradical mycelia production, while the ability to form internal structures, such as arbuscules or vesicles, decreased. One possible attempt to overcome this issue is the ongoing selection of suitable AMF species *in situ* with limited cycles of *in vitro* sub-culturing (Abbott et al., 1992). Although not mentioned in this study, functional changes over multiple sub-cultures may also be attributed to changes within the associated MHB consortium. The questions regarding functionally different MHB between sub-cultured AMF species could be addressed through adding small agar discs of the initial culture to later generations, thereby re-introducing the original bacterial consortium.

9.3 Future research

Following the results of this thesis, various knowledge gaps unfolded, which could be addressed in future research. This is especially the case for topics related to urban agriculture which, compared to other food production systems, is still untapped land.

First, issues which stem from current urban agriculture practices need to be improved, such as imbalanced nutrient availabilities in soil. This could be addressed through composts that provide a balanced C:N:P stoichiometry. Various urban waste products with different nutrient profiles could be tested for their suitability as compost. Questions regarding compost hygiene and ability would need to be addressed when using urban waste products. Research could also be directed towards the development of a self-made, organic and sanitary N-fertilizer. Such fertilizers could be specifically applied when N availability is predicted to be low, such as after high precipitation and low temperatures. Liquid formulations of such a fertilizer would also allow its use in drip irrigation systems.

Second, increasing knowledge about soil health in urban agriculture systems could be further supplemented by maximizing caloric or economic efficiency of the crops grown. This could be achieved through evaluating various companion planting systems, crop rotations, and their respective yields. Systems analysis could provide economic results by considering all inputs and outputs. Inputs could be minimized through technical improvements, such as wicking beds and organic mulches for improved water use efficiency (Semananda et al., 2016).

Third, the occurrence of AMF in urban agriculture systems warrants further research into their biological role in high P soils. As a first step, hypotheses could be refined through mycorrhizal plant growth experiments under increasing P concentrations and with different forms of P. Analysis of shoot nutrient concentration and the quantification of AM root colonization provides additional information about mycorrhizal functioning at higher soil P concentrations.

Finally, the work in this thesis on commercial AMF inoculants revealed that more research and framework is needed to improve arbuscular mycorrhizal inoculants as commercial products. Recent advances in the mass production of axenic spores created an important foundation for achieving contaminant-free (pure) inoculum at competitive prices. Following the VIPS principle, more research needs to be directed towards understanding the viability, infectivity, and stability of this inoculum. To overcome issues of non-targeted selection and genetic shifts within axenically-produced spores over multiple generation, this production method could be combined with a fast and efficient selection process for new AMF strains from in situ environments. These new strains are then used to replace the previous culture after multiple generations of sub-culturing. Alternatively, adverse functional changes within *in vitro* produced AMF cultures might be avoided through changes in the nutrient medium composition after some generations, such as through providing high and low P conditions. The effect of MHB on functional changes within *in vitro* AMF cultures could be investigated through the addition of specific bacterial isolates. These isolates could be collected from endophytic bacteria that occur in axenic plant cultures (Abreu-Tarazi et al., 2010).

9.4 Contribution to the scientific body of knowledge

This thesis started very broadly with a survey on urban agriculture sites in Adelaide. We were able to confirm some previous knowledge of urban agriculture soils which stemmed from a very limited body of literature. This survey also provided novel insights into the occurrence of AMF in urban agriculture systems. However, one limiting factor was that it only provided a one-time overview of urban agriculture soil health. This was overcome through sampling three urban agriculture sites over the course of one year. This dynamic approach was supplemented by an amplicon-based metagenomic analysis which provided in-depth insights into the biological side of urban agriculture soils. As this thesis puts an emphasis on AMF, more research was undertaken on commercial AMF inoculants. This is also of relevance if biotechnological solutions, such as AMF, would need to be incorporated to urban agriculture. The need of this applied research is highlighted by the unsatisfying results of this study. To improve this situation, it was followed by an opinion paper which involved the cooperation and support of over a dozen AMF experts from all around the world. The circle of soil health in urban agriculture systems and AMF was closed through an in-depth study of commercial potting mixes and composts and whether they support the development of AMF. With most mycorrhizal research involving natural soils, this work filled another gap in the literature and provided clarity for using AMF inoculants in common urban agriculture soils. Our current understanding of soil health in urban agriculture and AMF as a biotechnological tool have been summarized and put in scientific context through two literature reviews.

Altogether, this thesis made significant contribution towards our understanding of urban agriculture soil health. It highlighted various pitfalls when using common urban waste products as soil amendment and provides solutions to overcome this situation. Special emphasis lies on the natural occurrence of AMF and whether the introduction of AMF inoculum in common urban agriculture substrates is a worthwhile endeavor. This thesis also showcased deficiencies of commercial AMF inoculants which, in the best case, serves as a wake-up call for industry and regulators. The outcome of this study supports the development of urban agriculture and the adoption of AMF towards a more sustainable food production.

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