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E-CHAPTER FROM THIS BOOK

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Assessing the benefits of temperate cropland agroforestry for promoting soil biological health

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1 Introduction

Soil is one of the most valuable finite natural resources available to humankind. Soils are not only essential for production of food but also for air and water quality, climate mitigation, biodiversity, food security, and consequently human health. Yet, due to climate change and unsustainable management practices, the integrity of soils is at stake across the globe (FAO 2015). Soil 'health' is the most recent attempt to describe the vitality of soil. Soil health emerged from the concept of soil quality, which has been evolving ever since the importance of soil was recognized beyond soil fertility (i.e. suitability of soil to produce food, feed, timber, and fiber) in the 1990s (Bünemann et al. 2018). Since then, in addition to its agricultural potential, soils have been recognized as crucial for maintaining the functioning of terrestrial ecosystems by regulating soil-derived ecosystem functions and services at various scales (e.g. nutrient cycling, climate regulation, biodiversity, and the maintenance of air and water quality). Moreover, the biological component of soils has been acknowledged in addition to physical and chemical soil properties (Pankhurst et al. 1997). In contrast to the concept of soil quality, the scope of soil health extends beyond

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the anthropocentric notion to the broader sustainability aims that include planetary health (Pankhurst et al. 1997; Lehmann et al. 2020). More than two decades ago, soil health has been defined as 'the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant, animal and human health' (Doran and Zeiss 2000). However, the term 'soil health' is often used synonymously to 'soil quality' as the conceptualization and operationalization of soil as a multi-functional system continues to be a matter of an ongoing debate (Bünemann et al. 2018).

A decade ago, the essential role of soil in provision of ecosystem services that are crucial for human health and existence was emphasized by the term 'soil security' (McBratney et al. 2014). Furthermore, analogous to food and water security, soil security has been proposed to be equivalent to other human rights (Koch et al. 2013). As soil security covers the need for access to soil-derived ecosystem services, which strongly depends on soil health, soil health is in a broader sense an important pillar of soil security and therefore a part of a political sphere. On account of recognition of the importance of soil health, research efforts have been undertaken to develop approaches to quantify and monitor soil health. Within this context, identification of suitable indicators of soil health is essential and an ongoing process (Bünemann et al. 2018; Rinot et al. 2019).

Indicators of soil health can be classified into physical, chemical, and biological indicators (Bünemann et al. 2018), although some indicators cannot be assigned exclusively to just one of the three categories (Lehmann et al. 2020). Even though the importance of soil biota for soil health has been recognized over 25 years ago (Pankhurst et al. 1995, 1997), biological indicators usually account for less than 20% of indicators in soil health assessments (Lehmann et al. 2020). Inadequate inclusion of biological indicators can be attributed to several constraints, such as (1) long history of soil fertility assessments that have been predominantly based on chemical and physical soil indicators, (2) complexity of soil biota and biological processes, (3) highly contextual-dependent interpretation of biological indicators, and (4) ongoing elaboration of effective and practical methodological approaches. The most commonly included soil biological indicators are microbial biomass and activity, earthworm abundance, and mineralization rates (Lehmann et al. 2020). However, numerous other biological indicators with strong emphasis on soil microorganisms are being initiated for inclusion in assessment of soil health (see Bünemann et al. 2018; Lehmann et al. 2020; Fierer et al. 2021).

Soil biota are crucial drivers of numerous soil-derived ecosystem services (Kibblewhite et al. 2008; Wall et al. 2012; Schulte et al. 2014) and healthy soils are often associated with extremely complex communities of soil organisms, which range from microscopic microorganisms to soil megafauna. The large

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diversity in shape and size of soil organisms is also mirrored by their enormous taxonomic and genetic diversity. A recent study estimated that soil is the most biodiverse habitat on our planet as it is home to approximately 59% of all species (Anthony et al. 2023). The high biodiversity of soil biota is also reflected by their community assemblage and the provision of functions across spatial and temporal scales as well as in dependence of environmental conditions and management (Jeffery and Verheijen 2020). The selection of suitable biological indicators and quantitative assessment of their contribution to soil health is thus highly context-specific. Therefore, it has been proposed that research on soil health should rather revolve around identifying optimal management practices for individual farming systems under local environmental conditions rather than on finding a universal set of soil health indicators (Jeffery and Verheijen 2020; Fierer et al. 2021).

In addition to the preservation and restoration of soil health, there are multiple ecological, economical, and socio-cultural expectations placed on agricultural systems. For example, agricultural systems should not just be resistant and resilient to climate change but should also be able to mitigate climate change. They should improve yield stability, be accepted by society, and preserve biodiversity. Overall, agricultural systems are expected to deliver multiple functions simultaneously. In this context, agroforestry systems are proposed as promising multifunctional land-use systems that have the potential to meet the high requirements above while improving soil health, especially its biological components (Marsden et al. 2020; Beule et al. 2022b). In this chapter, we present recent advances in the field of soil biological health in temperate cropland agroforestry systems with a focus on alley cropping and shelterbelt systems.

2 The promotion of soil biological health through agroforestry – from microorganisms to earthworms

Soil organisms have various lifestyles and assemble in complex communities across space and time. Compared to agricultural systems without trees, agroforestry systems introduce additional complexity in space and constancy. Through the incorporation of woody perennials, agroforestry systems introduce spatial heterogeneity as pointed out for soil properties such as soil organic carbon (Bambrick et al. 2010) as well as soil microbial (Beule and Karlovsky 2021a) and faunal communities (Cardinael et al. 2019). Although less studied, the tree component of agroforestry systems also introduces temporal heterogeneity due to differences in phenology and longevity (Stamps and Linit 1997). This spatio-temporal heterogeneity within agroforestry systems is expected to introduce new habitats for soil organisms.

2.1 Promotion of soil microbial communities and processes

Soil microorganisms comprise prokaryotic and eukaryotic organisms such as bacteria, fungi, and archaea. Microbial communities make substantial contributions to soil health, thus, their community characteristics (e.g. biomass, diversity, and community composition) and activity are important indicators in soil health assessments.

Among soil microbial indicators, quantification of the microbial biomass is frequently included in soil health assessments (Lehmann et al. 2020). There are various methodological approaches to quantify the biomass of soil microorganisms ranging from the traditional fumigation–extraction method to molecular techniques that target marker genes of specific microbial groups (Jörgensen et al. 2024). Although each method comes with its own advantages and limitations, across methods, a growing body of literature revealed that trees in temperate agroforestry systems promote the biomass of soil microorganisms (Unger et al. 2013; Beuschel et al. 2019; Beule et al. 2020; Guillot et al. 2021). While some studies showed that microbial populations are exclusively promoted under the trees (Beuschel et al. 2019), others were able to show a gradual decrease with increasing distance from the trees (Luo et al. 2022). These discrepancies are likely related to differences in tree age across sites (Clivot et al. 2020; Beule and Karlovsky 2021b) as well as sampling time and frequency (i.e. single or repeated sampling) within sites (*cf*. Beuschel et al. 2019; Luo et al. 2022). Additionally, studies using molecular techniques (Beule et al. 2019a, 2020, 2022a) or phospholipid fatty acid (PLFA) analysis (Guillot et al. 2021) revealed that spatial gradients from the trees are present for some but not all microbial groups, which may serve as an additional explanation for the absence/presence of spatial gradients across studies.

In addition to the biomass of soil microorganisms, the importance of including their diversity in soil health assessments has been pointed out over two decades ago (Nielsen et al. 2002). Back then, the authors pointed out the urge for methodological development of tools to assess microbial diversity. Since then, methods that investigate microbial diversity developed rapidly, culminating in the development of high-throughput sequencing technologies. As of writing, amplicon-sequencing approaches of marker genes to assess soil microbial diversity are the most commonly used (Schöler et al. 2017).

Microbial diversity is commonly assessed using indices of alpha-, beta-, and gamma-diversity, whereas alpha diversity describes the diversity within a given system (e.g. Shannon diversity and species richness), beta diversity measures the diversity among systems (e.g. community similarity), and gamma diversity measures the diversity at large spatial scales (e.g. diversity at landscape level). To date, only a few studies have used amplicon sequencing to analyze soil microbial diversity in temperate agroforestry systems.

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Studies that used amplicon sequencing to investigate microbial alpha-diversity in temperate agroforestry systems showed variable responses of soil bacteria and fungi. While soil bacterial diversity remained largely unchanged in response to the poplar trees in alley cropping systems in Germany (Beule and Karlovsky 2021a), soil fungal richness in the same systems increased with decreasing distance to the trees (Beule et al. 2021). A recent study that investigated the response of the soil fungal community to the establishment of an alley cropping system over one-and-a-half years was able to show that species richness decreased under the trees, whereas Shannon diversity remained unaffected (Beule and Karlovsky 2021b). In contrast, in Canadian windbreak systems, the Shannon diversity of bacteria increased with increasing distance to the trees, whereas the Shannon diversity of fungi showed no consistent response to distance (Rivest et al. 2020). Another study investigated tree-distance and tree-species effects on soil fungal communities in a temperate alley cropping system and found no effects of tree-distance and tree-species on fungal diversity (Vaupel et al. 2023). Therefore, responses of soil microbial alpha-diversity to agroforestry practice remain inconsistent and the reason for the observed discrepancies among studies remains yet unknown. We speculate that these discrepancies are due to differences in the plant species composition and management of the studied agroforestry systems among studies and require further investigation, especially across time given that all studies above determined alpha diversity only at a time point.

In contrast, studies that investigated variations in microbial community composition within alley cropping systems (i.e. beta-diversity) using amplicon sequencing consistently showed that soil bacterial (Beule and Karlovsky 2021a) and fungal communities (Beule et al. 2021; Vaupel et al. 2023) under trees are compositionally different from those in neighboring crops. Effects on community composition were reported as early as 6 months after the establishment of the agroforestry system (Beule and Karlovsky 2021b), which is unsurprising considering the complex interactions in the plant–soil–microbe system. These results confirm earlier studies that used PLFA analysis and were able to show that temperate agroforestry can alter microbial community composition (Lacombe et al. 2009; Unger et al. 2013).

Several studies that investigated changes in community composition in response to agroforestry identified the members of the soil microbial communities that were associated with the compositional shifts. A recent study from a Mediterranean walnut–pea alley cropping system used PLFA analysis and found that the trees promote Gram-positive and Gram-negative bacteria as well as Actinobacteria (Guillot et al. 2021). The promotion of gram-negative bacteria and Actinobacteria agrees with the findings of other studies conducted in other temperate agroforestry systems (Unger et al. 2013; Beule et al. 2020). Another study that sequenced soil bacterial communities in three poplar-based alley

cropping systems in Germany found similar shifts in community composition in response to the trees across sites (Beule and Karlovsky 2021a). These shifts included, among other things, a greater relative abundance of members of the genera *Actinomycetospora*, *Bradyrhizobium*, and *Mesorhizobium* and lower relative abundance of *Pseudarthrobacter* spp. under the trees than in the crop rows and an adjacent open cropland. At the same study sites, the authors sequenced fungal communities and showed that *Basidiomycota* were strongly promoted by the trees (Beule and Karlovsky 2021a). Furthermore, the trees increased the community share of ectomycorrhizal fungi (EMF) such as *Cortinarius*, *Geopora*, *Inocybe, Laccaria*, and *Preussia* spp. (Beule et al. 2021; Vaupel et al. 2023). Likewise, studies investigating communities of arbuscular mycorrhizal fungi (AMF) found compositional differences between tree and crop rows of alley cropping systems (Chifflot et al. 2009; Battie-Laclau et al. 2019; van Tuinen et al. 2020) as well as between crop rows of alley cropping systems and open croplands (Bainard et al. 2012). We expect these compositional changes to translate into functional changes with consequences for soil and plant health. For example, changes in microbial community composition can affect the abundance of beneficial and pathogenic microorganisms in soil with consequences for agricultural production. Following the one health concept, soil microorganisms cannot just have indirect and direct impacts on plant health but also on the health of animals and humans (Banerjee and van der Heijden 2023). The importance of the soil microbiome for plant health has been recognized for decades and the ability of soils to suppress plant diseases in the presence of pathogens (soil suppressiveness) has become an important field of research. Given their importance, soil suppressiveness and plant health in temperate agroforestry systems are vastly understudied. Although a recent study conducted in an alley cropping system in Germany found the first evidence of enhanced soil suppressiveness through agroforestry (Vaupel et al. 2023), validation is yet lacking. The same holds true for measures of plant health. The first and yet only study on plant health and crop diseases in temperate alley cropping systems showed that crops cultivated in temperate alley cropping systems are at least as healthy as those cultivated in neighboring open croplands (Beule et al. 2019b). There is an urgent need for further studies on soil suppressiveness and plant health in temperate agroforestry systems.

Microorganisms further regulate biogeochemical cycles and a number of studies thus explored functional genes involved in the nitrogen cycle covering N_2 fixation, nitrification, and denitrification. The majority of studies showed that genes involved in denitrification are promoted through agroforestry (Beule et al. 2020, 2022a). A recent study demonstrated that denitrifier gene abundance may not be a reliable predictor of N_2O fluxes under field conditions (Luo et al. 2022). Despite functional gene abundance, numerous studies report changes in soil enzyme activities in response to agroforestry. Studies that determined

the activities of a range of common soil enzymes (e.g. β-glucosidase, N-acetyl-β-glucosaminidase, and dehydrogenase) in agroforestry systems generally reported higher activity under the trees as compared to the neighbouring crops (Mungai et al. 2005; Udawatta et al. 2008, 2009; Unger et al. 2013; Beuschel et al. 2019). These findings are congruent with studies that investigated substrate-induced respiration rates. For example, using a wide range of substrates, Mungai et al. (2005) and Beuschel et al. (2019) were able to show that substrate-induced respiration was increased through trees. Using a narrower range of substrates, Guillot et al. (2021) recently confirmed these findings. Furthermore, Mungai et al. (2005) and Beuschel et al. (2019) reported greater diversity of substrate utilization ability in the tree rows than in neighbouring rows. Since soil microorganisms are key drivers of biogeochemical cycles, we propose that composition and functional changes of the microbiome induced through agroforestry affect soil nutrient cycling in these systems. For example, Beule et al. (2022b) recently hypothesized that root-associated microorganisms support the 'safety-net'-role of tree roots for leached nutrients by enhancing nutrient uptake of tree roots and thereby contributing to nutrient pumping. Yet, the potential contributions of soil microorganisms to improved soil nutrient cycling in temperate agroforestry systems remain largely unexplored.

Overall, temperate agroforestry (1) promotes the population size of soil microorganisms, especially the population size of soil fungi and (2) alters the composition and functions of the soil microbiome, which may result in improved soil suppressiveness and altered soil nutrient cycling. For alpha diversity, however, variable effects are observed among studies with yet unknown underlying reasons. We suggest that future studies should aim to explore the contribution of the soil microbiome to soil functions in agroforestry systems.

2.2 Promotion of soil faunal communities

Faunal communities in soil are highly diverse and build complex food webs that highlight their interconnectedness. Since soil fauna is a key driver of several ecosystem functions, the global loss of soil biodiversity poses a serious threat to ecosystem functioning (Wagg et al. 2014). Soil faunal communities comprise a large variety of organisms that are considered indicators of soil health including earthworms (Paoletti 1999), nematodes (Neher 2001), and numerous arthropod groups (Menta and Remelli 2020). Among these, earthworms are a major component of soil faunal communities and are routinely used as biological indicators in soil health assessments (Lehmann et al. 2020). They contribute to numerous ecological functions (Blouin et al. 2013) such as enhanced water infiltration, improved nutrient availability, and suppression of phytopathogens. Because of these beneficial functions, the presence of earthworms has been shown to have a positive impact on crop yield (van Groenigen et al. 2014).

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Therefore, earthworms play a crucial role in global food production (Fonte et al. 2023).

Soil health indicators related to earthworms include population size, biomass, community composition, activity (e.g. number of casts on the soil surface), and functional parameters (e.g. water infiltration and aggregate formation). Methods suitable for the assessment of earthworm population size, biomass, and community composition include, among other things, hand sorting, chemical extraction (e.g. with formaldehyde, allyl isothiocyanate, or mustard), electrical octet method, Kempson extraction, or combinations of some of these methods. In contrast, the functions of earthworms are usually assessed indirectly by inferring them from the population parameters listed previously. Especially information on the abundance of ecological groups (anecic, endogeic, and epigeic as per Bouché 1972) is widely used to estimate earthworm functions. Recently, however, it has been argued that the use of ecological groups of earthworms can be misleading as ecological groups are not necessarily congruent with functional groups (Bottinelli and Capowiez 2021). Consequently, it has been proposed that future research should combine different methodologies and include advanced technologies such as stable isotopic techniques, X-ray tomography, and earthworm tagging in order to improve our understanding of the role earthworms in soil processes (Bartlett et al. 2010; Blouin et al. 2013). Additionally, the impact of earthworm community shifts on certain ecological functions can be investigated directly by, for example, measuring macroporosity and water infiltration rates (Capowiez et al. 2009). However, even though earthworms are among the most studied faunal groups in temperate cropland agroforestry systems, direct measurements of earthworm functions in these systems are yet missing.

A quarter of a century ago, Seiter et al. (1999) investigated bacterial and fungal biomass in an alley cropping system in the USA and made a noteworthy field observation on earthworm activity. Although the authors did not show their data, they observed that the majority of tree litter was pulled into earthworm burrows and that earthworm casts accumulated in close proximity to the trees (Seiter et al. 1999). Since then, numerous studies investigated earthworms in temperate cropland agroforestry systems and found predominantly positive effects (Price and Gordon 1998; Cardinael et al. 2019; D'Hervilly et al. 2020, 2021, 2022, 2024; Vaupel et al. 2023). For example, Cardinael et al. (2019) investigated earthworms in 13 alley cropping systems in France and were able to show greater population densities of earthworms in the tree rows of the agroforestry systems than in adjacent open croplands without trees. More than 20 years earlier, Price and Gordon (1998) found a similar promotion of earthworms in the poplar tree rows as compared to the crop rows of an alley cropping system in Canada. In the same study, however, the authors observed a decrease of earthworm population densities in the tree rows with ash trees

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as compared to the crop rows, suggesting an influence of tree species (Price and Gordon 1998). In 2023, Vaupel et al. published a study that investigated earthworms in an alley cropping system in Germany comprised of blocks of three different tree species (two different poplar clones and black locust). The authors found a strong promotion of earthworm population densities in the tree rows as compared to the crop rows and an adjacent open cropland, irrespective of the tree species. Furthermore, the authors were able to show a gradual decline of the population density of endogeic earthworms from the tree rows into the crop rows of the agroforestry system, which they attributed to the increased input of tree litter in close proximity to the trees (Vaupel et al. 2023). Additionally, they observed that anecic earthworms were strongly promoted in the tree rows, leading to a community shift towards anecics. In contrast to endogeic species, the positive impact of the tree rows on anecic earthworms did not extend into the crop rows, which was attributed to the presence of tillage in the crop rows (Vaupel et al. 2023). Similar results were obtained by D'Hervilly and co-authors (2020) who reported increased relative abundance of anecic earthworms within the earthworm communities in the tree rows as compared to the crop rows of two alley cropping systems in France. One year later, these results were confirmed in another alley cropping system in France, highlighting the positive impact of trees on this ecological group of earthworms (D'Hervilly et al. 2021). Overall, the existing literature reports predominantly positive effects of temperate cropland agroforestry systems on earthworms.

However, a number of knowledge gaps remain regarding the influence of tree row design and management on earthworm communities (e.g. tree age, tree species, tree harvesting cycles, and tree row orientation). Furthermore, the magnitude of spillover effects of earthworms from the trees into the neighboring crops is highly understudied. The quantification of spillover effects requires the investigation of migration patterns of earthworms from the trees into the crops and vice versa. In a recent study, D'Hervilly et al. (2024) aimed to quantify earthworm migration patterns in an alley cropping system in France by investigating earthworm fluxes from the tree rows into the crop rows and vice versa. The authors observed greater earthworm fluxes from the crop rows into the tree rows than from the tree rows into the crop rows. However, fluxes were largely based on the presence of a single endogeic earthworm species (*Allolobophora chlorotica*) and the authors suspect different migration patterns for other species that could not be successfully quantified using their traps (e.g. epigeic species). Finally, the authors found a strong temporal variation of earthworm densities within the agroforestry system between March and May, suggesting that migration patterns might differ throughout the year (D'Hervilly et al. 2024).

Similarly, D'Hervilly et al. (2022) observed seasonal variations of the spatial distribution of several soil faunal groups within a walnut-based alley cropping

agroforestry system in France. The authors found seasonal variations across spring, summer, and autumn for Chilopoda, adult Coleoptera, Isopoda, and Diplopoda. Furthermore, the authors classified their soil faunal individuals into soil-, litter-, animal-, and plant-feeding organisms. While population densities of soil-feeding organisms were greater in the tree rows than in the crop rows throughout all seasons, tree rows promoted litter-feeding organisms only during spring and summer (D'Hervilly et al. 2022). While animal-feeding organisms were promoted through the tree rows in spring, plant-feeding organisms showed lower population densities in the tree rows than in the crop rows during this season (D'Hervilly et al. 2022).

The effect of temperate agroforestry practice on soil invertebrates has been investigated by Park et al. (1994). The authors obtained invertebrates from soil cores (collected at 0–15 and 15–30 cm soil depth) using Tullgren funnels in a poplar-based alley cropping system in the United Kingdom. Their results showed greater invertebrate populations and biomass in the tree rows and at 1 m distance into the crop rows than at 3 and 6 m into the crop rows as well as in an adjacent open cropland (Park et al. 1994). Almost 30 years later, Szigeti et al. (2022) applied Tullgren funnels to investigate microarthropods at 0–10 cm soil depth in two shelterbelts and an adjacent open croplands in Hungary. The shelterbelts harboured greater population size and species richness of *Collembola* as compared to the neighbouring crops (Szigeti et al. 2022). Likewise, the population densities of Acarina were greater in the shelterbelts than in the crops (Szigeti et al. 2022). Similar results were obtained by Olejniczak (2007) who used the same methodological approach as Szigeti et al. (2022) in three shelterbelt systems of different ages (6, 7, and 170 years) in Poland. The authors found mostly decreasing population densities of Acarina with increasing distance from the trees and reported that these trends were depending on shelterbelt age and sampling time (Olejniczak 2007). In contrast to Acarina, the effect of shelterbelts on the population density of Collembola remains inconclusive and was strongly affected by sampling season (Olejniczak 2007).

In 1998, Griffiths et al. published a study in which they investigated the impacts of alley cropping agroforestry on slugs in the United Kingdom. By utilizing pipe, refuge, and pitfall traps, they found greater diversity and activity density of slugs in the tree rows as compared to the crop rows and open croplands. Additionally, the authors assessed damage to pea plants cultivated at their study site and reported increased plant damage and reduced counts of emerged pea seedlings in close proximity to the trees (Griffiths et al. 1998). In contrast, Boinot et al. (2019b) investigated overwintering invertebrates with emergence traps in seven alley cropping systems in France and found that population densities of economically relevant pests (*Aphidae*, *Elateridae*, and slugs) were lower in the tree rows than in the crop rows. At their study sites, the majority of investigated overwintering invertebrate groups was promoted

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by the tree rows. For example, they found greater numbers of Heteroptera, Hymenoptera, Lepidoptera, Neuroptera, Psocoptera, Blattoptera, Collembola, Isopoda, Acarina, Araneae, Opiliones, snails, and Pseudoscorpionida under the trees than in the crop rows. Coleoptera, Homoptera, and Myriapoda, however, showed the opposing trend (Boinot et al. 2019b). Furthermore, tree rows had a positive effect on the overwintering of beneficial invertebrates, such as *Coccinellidae*, *Chrysopidae*, and *Formicidae*, whereas numbers of overwintering *Carabidae*, *Staphylinidae*, and *Syrphidae* declined under the trees as compared to the crop rows (Boinot et al. 2019b). In the same year, Pardon et al. (2019) published a study in which they investigated ground-dwelling arthropods in six alley cropping agroforestry systems in Belgium using pitfall traps. They observed greater activity densities of woodlice and millipedes in the tree rows than in the crop rows as well as decreasing activity densities in the crop rows with increasing distance from the trees. For Carabids and rove beetles, however, the authors reported the opposing trend (Pardon et al. 2019). Another study from 2019 that used pitfall traps in a walnut-based alley cropping agroforestry system in France found similar results on the activity density of ground beetles (Martin-Chave et al. 2019b). In the same system, the authors conducted one of the few studies that directly investigated the functions of soil fauna by determining the predation of *Cydia pomonella* larvae (Martin-Chave et al. 2019a). Predation was mainly driven by ground beetles and Opiliones and was greater in the agroforestry system than in the reference system without trees. Predation of *Cydia pomonella* larvae, however, showed temporal variations between June and August (Martin-Chave et al. 2019a). In 2024, Matevski and colleagues installed pitfall and emergence traps at five paired alley cropping and open cropland systems in Germany and assessed spider abundance, taxonomic and functional diversity. The authors found that agroforestry systems generally promoted the abundance as well as taxonomic and functional diversity of the overall spider communities as compared to open croplands. Interestingly, they found that the promotion of forest specialists and eurytopic spiders in the agroforestry systems without negatively affecting open-habitat specialists. Furthermore, they found that spider abundance and taxonomic diversity in the crop rows peaked when crop pests arrived, which likely resulted in increased pest control potential through agroforestry (Matevski et al. 2024).

Overall, the existing literature on soil fauna in temperate cropland agroforestry systems reports predominantly positive effects on their population density and diversity. In a recent review article, Marsden et al. (2020) pointed out that so far only a few studies conducted direct measurements of functions of soil fauna in agroforestry systems and stressed that future studies should aim to include such measurements. We strongly agree with Marsden et al. (2020) and argue that direct measurements of functions are needed to understand the

advantages and disadvantages of agroforestry systems for essential ecosystem services driven by soil fauna.

2.3 Mechanisms by which agroforestry promotes soil biological health

There are several different agricultural management practices that can promote soil health. For example, in a recent meta-analysis, Nunes et al. (2020) showed that reduced tillage intensity and crop residual removal and permanent plant cover enhance indicators of soil biological health. The authors further pointed out that biological indicators of soil health are most effectively promoted through perennial cropping systems with zero soil disturbance. The introduction of woody perennials into agricultural systems through the implementation of agroforestry creates areas with zero soil disturbance under the trees and many authors argue that this is a key factor for the promotion of soil life under the trees (Beuschel et al. 2019, Beule et al. 2020, D'Hervilly et al. 2021). Furthermore, the absence of soil disturbance under the trees leads to the emergence of a permanent herbaceous understory vegetation (Boinot et al. 2019a), which has been shown to be important in maintaining microbial communities (Battie-Laclau et al. 2019). Additionally, trees provide permanent plant cover and their aboveground tree leaf litter is not removed, serving as soil cover as well as a resource for soil organisms. In alley cropping systems, the amount of tree leaf litter generally decreases exponentially with increasing distance to the trees (Swieter et al. 2018, 2022), which may explain gradients of indicators of soil health from the trees into the neighbouring crops. In addition to tree litter quantity and its spatial distribution, the quality of the tree litter is yet another important driver of soil microbial activity in agroforestry systems (Clivot et al. 2020). The decomposition of fine root litter of trees is expected to further contribute to the maintenance of soil organisms, especially at deep soil depths (Beule et al. 2022a).

3 Measuring soil biological health in agroforestry systems

3.1 Spatial heterogeneity in agroforestry systems and sampling design

As reported by numerous studies, trees in temperate agroforestry systems introduce spatial heterogeneity of soil health indicators. The vast majority of studies that investigated spatial heterogeneity in these systems determined horizontal heterogeneity (e.g. spatial gradients from the trees into the neighboring crops at a defined soil depth). In alley cropping systems, sampling at multiple distances along gradients spanning from the tree rows into the

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crop rows is routinely done to capture spatial heterogeneity. Such a study design further allows calculating area-weighted averages in order to obtain aggregated values for the crop rows (Beule et al. 2019b; Schmidt et al. 2021) or the agroforestry system as a whole (Luo et al. 2022; Shao et al. 2023). This further enables comparisons of agroforestry systems with other land-use systems such as open fields (Veldkamp et al. 2023). The distances to trees, however, must be selected carefully as pointed out by Shao et al. (2023) who observed an overlap of fertilizer application in the middle of the crop rows due to the working width of the operating farmer.

Although soils are three-dimensional systems and their variation in physical and chemical composition can span up orders of magnitude – even along a single soil profile – most studies of soil organisms in temperate agroforestry systems do not account for soil depth. Since variations in soil properties with depth are among the main characteristics of soil (Mulla and McBratney 2001), it is unsurprising that soil microbial communities adapt to this and change in their community composition with depth (Fierer et al. 2003b). Furthermore, it is well established that soil microbial communities generally decrease in biomass and activity with soil depth, which is mainly attributed to resource limitations at deeper depths (Fierer et al. 2003a).

The introduction of trees into agricultural systems introduces both horizontal and vertical spatial variations in soil. For example, one of the most prominent benefits of agroforestry systems is the concept of the 'safety-net'-role of deep tree roots, which is based on differences in the distribution of crop and tree roots across soil depths that enables a complementary rather than competitive use of resources (Allen et al. 2004). While a large body of literature explored horizontal variations of soil biological health indicators in temperate agroforestry systems by collecting samples at a single soil depth, only very few studies explored both horizontal and vertical variations by additionally collecting samples at multiple soil depths.

In 2005, Mungai et al. investigated the activities of β-glucosidase and fluorescein diacetate (FDA) hydrolase at multiple depths (0–10, 10–20, and 20–30 cm) in two alley cropping systems in Missouri, USA. Although the differences in enzyme activity between the tree and the crop rows were mostly higher in the tree rows, they varied between sampling year, and soil depth. Ten years later, Udawatta et al. (2014) collected soil samples at 0–10, 10–20, and 20–30 cm soil depth at one of the two alley cropping systems of Mungai et al. (2005). The authors analyzed the activities of β-glucosidase, N-acetyl-β-glucosaminidase, FDA hydrolase, and dehydrogenase and found no differences between the tree row and the middle of the crop alley at any soil depth. In 2019, Beuschel and co-workers reported that patterns of spatial heterogeneity in microbial population size, enzyme actives, and substrate-induced respiration rates in three alley cropping systems were evident at

0–5 but not at 5–20 cm topsoil. The authors also accounted for horizontal heterogeneity and collected soil samples at multiple distances from the tree rows into the crop rows. For years following their soil sampling, Beule et al. (2022a) collected soil samples at two of the three study sites of Beuschel et al. (2019) in topsoil (0–15 and 15–30 cm) and subsoil (30–45 and 45–60 cm). They further collected soil samples at the same distances from the trees as Beuschel et al. (2019). Beule et al. (2022a) used real-time polymerase chain reaction to quantify taxonomical and functional groups of soil microorganisms and found that the microbial population size decreases with increasing soil depth and the promotion of microorganisms through trees in subsoil is stronger than in topsoil. Yet, the subsoil microbiome and its functions in temperate cropland agroforestry systems remain largely unexplored.

3.2 Temporal heterogeneity in agroforestry systems

In addition to spatial variation, the examples previously discussed (cf. Mungai et al. 2005; Udawatta et al. 2014; Beuschel et al. 2019; Beule et al. 2022a) highlight the need to further account for temporal variation in soil health assessments in temperate cropland agroforestry systems. As of writing, only a few studies covered temporal dynamics of soil biological health indicators in agroforestry systems by using repeated sampling (Mungai et al. 2005; Beule and Karlovsky 2021b; Luo et al. 2022; D'Hervilly et al. 2022). Studies that investigated soil organisms across seasons reported strong temporal dynamics for communities of both soil micro- (Beule and Karlovsky 2021b) and macroorganisms (D'Hervilly et al. 2022). Temporal dynamics of soil organisms are strongly affected by agricultural management. For example, it is well established that crop rotation and its diversity are important drivers of soil communities (D'Acunto et al. 2016; Torppa and Taylor 2022). However, studies investigating soil organisms across an entire crop rotation within agroforestry systems are absent. Likewise, extensive studies on soil health in relation to tree age and/or tree harvesting cycles are absent. For example, in systems that use short-rotation trees, regular tree harvests will result in repeating temporal changes of, among other things, tree litter input (above- and belowground), root exudation, and shading with potential consequences for soil organisms. Yet, the effects of tree harvesting and tree age on soil life in agroforestry systems remain largely unexplored. We therefore encourage researchers to study soil health across time and under consideration of crop and tree rotation cycles in order to identify optimal combinations as well as management practices of trees and crops that maximize the soil health benefits of agroforestry systems.

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4 Future trends

4.1 Consideration of spatio-temporal heterogeneity

While a growing body of literature covers horizontal spatial heterogeneity within agroforestry systems (e.g. sampling at multiple distances from the trees into neighboring crops), only a few studies account for vertical heterogeneity (i.e. different soil depths). We thus encourage researchers to extend their study design to multiple soil depths and to consider subsoil. We further recommend accounting for temporal heterogeneity in agroforestry systems by sampling at multiple points in time under consideration of crop and tree rotation cycles. Such studies may be conducted by repeated sampling across multiple years or by using space-for-time substitution.

Finally, in order to evaluate the changes in soil health associated with the conversion of agricultural systems without trees to agroforestry, we would like to stress the importance of comparing agroforestry systems to land-use systems without trees. Although the importance of including non-agroforestry plots as a reference land use has been pointed out more than 25 years ago (Stamps and Linit 1998), several studies lack this comparison. A good example of a study that successfully implemented a reference land use at multiple study sites is the work of Veldkamp et al. (2023). The authors studied cropland and grassland alley cropping systems and compared those systems to neighboring open cropland and grassland systems without trees, respectively. Since the open cropland or grassland systems were managed identically to the corresponding crop or grass rows of the agroforestry systems, the authors were able to compare the land-use systems (i.e. agroforestry versus open cropland or grassland). The authors further accounted for horizontal spatial heterogeneity within their agroforestry systems. We argue that such study designs are of great value but are aware that they are difficult to realize.

4.2 Consideration of the full diversity of soil organisms

While soil bacterial and fungal communities are by far the most studied group of microorganisms in temperate agroforestry systems, other groups of microorganisms are highly understudied or yet ignored. For example, although soil archaea contribute to important soil functions in biogeochemical cycles (Offre et al. 2013), most prominently their contribution to the oxidation of ammonia in nitrifaction, this group of microorganisms has rarely been quantified in temperate agroforestry systems (Beule et al. 2019a, 2020; Vaupel et al. 2023) and has not yet been investigated using modern sequencing approaches in these systems. Therefore, their diversity and community composition in agroforestry systems remains unexplored. Likewise, soil protists are ubiquitous and contribute to soil nutrient cycling (Geisen et al. 2018; Oliverio et al. 2020)

but remain yet unexplored in temperate agroforestry systems. While there are a number of studies on earthworms in temperate agroforestry systems (Price and Gordon 1998; Cardinael et al. 2019; D'Hervilly et al. 2020, 2021, 2022, 2024; Vaupel et al. 2023) and few on Acarina, Araneae, and Collembola (Olejniczak 2007; Boinot et al. 2019b; Szigeti et al. 2022, Matevski et al. 2024), other soil faunal groups are highly understudied. Likewise, studies on burrowing mammals are scarce (Klaa et al. 2005). Finally, only a handful of studies performed direct measurements of the functions of soil fauna in temperate cropland agroforestry systems (Marsden et al. 2020). We thus argue that in addition to inferring potential functions from ecological count data, researchers should aim to directly assess the functions of soil organisms in agroforestry systems.

4.3 Consideration of the full diversity of temperate agroforestry systems

Over the last three decades, the literature investigating soil biological health indicators was largely focused on alley cropping systems with poplar (Park et al. 1994; Chifflot et al. 2009; Beaudette et al. 2010; Sun et al. 2018; Beuschel et al. 2019; Cardinael et al. 2019; Pardon et al. 2019; Beule et al. 2019a, 2020, 2021, 2022a; Beule and Karlovsky 2021a, 2021b; Luo et al. 2022; Shao et al. 2023, Matevski et al. 2024) or walnut trees (Griffiths et al. 1998; Cardinael et al. 2019; Guillot et al. 2019, 2021; Martin-Chave et al. 2019a, 2019b; Pardon et al. 2019; Gao et al. 2019; D'Hervilly et al. 2020, 2021, 2022, 2024; van Tuinen et al. 2020). Other tree species investigated cover, among other things, alder (Seiter et al. 1999), ash (Griffiths et al. 1998; Bainard et al. 2012), black locust (Nii-Annang et al. 2009; Sun et al. 2018; Szigeti et al. 2022; Vaupel et al. 2023, Matevski et al. 2024), maple (Bainard et al. 2012; Bardhan et al. 2013; Udawatta et al. 2014; Szigeti et al. 2022), oak (Olejniczak 2007; Udawatta et al. 2008, 2009; Weerasekara et al. 2016), pecan (Lee and Jose 2003; Mungai et al. 2005), and spruce (Bainard et al. 2012). Yet, there are only a few studies that investigated mixed tree species within the tree rows (Griffiths et al. 1998; Olejniczak 2007; Battie-Laclau et al. 2019; Cardinael et al. 2019; Pardon et al. 2019; Boinot et al. 2019b; D'Hervilly et al. 2020; Szigeti et al. 2022; Mettauer et al. 2023; Vaupel et al. 2025) or evaluated the effects of different tree species at the same study site (Price and Gordon 1998; Bainard et al. 2012; Vaupel et al. 2023). Furthermore, studies comparing the impacts of differences in soil (e.g. tillage vs. no-till), crop (e.g. major vs. minor crops), and farm management (e.g. organic vs. conventional farming) on soil biological health within agroforestry systems are either scarce or absent. Finally, as stated previously, most studies on soil biological health were conducted in alley cropping systems, while other types of cropland agroforestry systems dominant in the temperate zone, such as shelterbelts, remain largely unexplored. Likewise, except for a few studies

(Amorim et al. 2023) soil health in silvopastoral (integration of woody perennials and livestock) and agrosilvopastoral systems (integration of woody perennials, crops, and livestock) remains highly understudied in the temperate zone.

Future research efforts should also focus on urban and peri-urban agroforestry given an ever-increasing urban population density and the need to increase food sovereignty and enhance ecosystem services provision in cities (Clark and Nicholas 2013). In a pioneering study on AMF and EMF colonization of 26 tree species in urban and rural environments in Canada, Bainard et al. (2011) found that urbanization reduces mycorrhizal colonization. The urban factors that led to the observed reduction in colonization, however, remained unclear. The anthropogenic stressors experienced by urban and agricultural soils have intersections but are not identical and urban soils are often strongly degraded through physical (e.g. sealing and compaction) and chemical stressors (e.g. contaminants). In this context, urban and peri-urban agroforestry practices may offer promising solutions to restore the soil health of urbanized soils. We acknowledge, however, that suitable study sites for temperate urban and peri-urban agroforestry may not be widely available. Furthermore, the high complexity of these systems creates large dissimilarities among systems, which may represent hurdles for certain scientific investigations. Despite these hurdles, urban and peri-urban agroforestry systems have tremendous potential to improve soil health as well as the overall sustainability of urbanized areas and thus require more investigations.

Overall, we strongly encourage researchers to consider the full diversity of temperate agroforestry systems as well as their management in future research.

5 Conclusion

Soil is a finite resource. The thin and fragile layer of soil that covers Earth's ice-free land surface sustains an incredibly large number of organisms, including us humans. Therefore, maintaining soil health must be among our top priorities as a species. Furthermore, soil health is recognized as one of 13 principles of agroecology, a concept that presents transition pathways to more sustainable food systems (Wezel et al. 2020). Consequently, the existential need to preserve soil health is mirrored by numerous political agendas and has become the goal of several multinational research funding initiatives. In this chapter, we show that temperate cropland agroforestry systems strongly promote the biological components of soil health. This includes, among other things, enhanced population densities and activities of soil microorganisms and fauna as well as increased diversity of soil fauna under cropland agroforestry as compared to open croplands without trees. Additionally, agroforestry alters the community composition of soil organisms, which is expected to result in altered functions of soil communities. Overall, the changes in soil communities

associated with agroforestry practices benefit soil biological health. In addition to the promotion of soil health, multifunctional agroforestry systems offer a variety of environmental benefits. We argue that the land-use change from open cropland to agroforestry provides substantial environmental benefits that outperform those of many sustainable agricultural management practices. However, cropland agroforestry does not exclude additional integration of sustainable management practices but allows their simultaneous inclusion in the system. Therefore, agroforestry can make major contributions to improving and maintaining soil health for future generations. Considering the annual global loss of fertile soil, restoration of degraded soils becomes ever more important and agroforestry offers promising solutions to rehabilitate degraded soils and restore soil health. Overall, agroforestry provides high multifunctionality and represents a key land-use system for the agroecological transition of our food systems. Consequently, we propose agroforestry as a priority land-use system that should receive adequate subsidies and political attention.

6 Where to look for further information

Standard introductions to the subject of improved soil health through agroforestry include:

- Barrios, E., Coe, R., Place, F., Sileshi, G. W., & Sinclair, F. (2023). Nurturing Soil Life through Agroforestry: The Roles of Trees in the Ecological Intensification of Agriculture. In Biological Approaches to Regenerative Soil Systems (pp. 265-278). CRC Press.
- Dollinger, J., & Jose, S. (2018). Agroforestry for soil health. Agroforestry Systems, 92, 213-219.
- Barrios, E., Sileshi, G. W., Shepherd, K., & Sinclair, F. (2012). Agroforestry and soil health: linking trees, soil biota and ecosystem services. In Soil ecology and ecosystem services (pp. 315-330). Oxford Academic.

Centres of expertise to reach out to for information resources include:

- The Center for International Forestry Research and World Agroforestry (CIFOR-ICRAF) (https://www.cifor-icraf.org/)
- The European Agroforestry Federation (EURAF) (https://www.euraf.net/)
- National Research Institute for Agriculture, Food and Environment (https://www.inrae.fr/en)
- USDA National Agroforestry Center (NAC) (https://www.fs.usda.gov/nac/)
- The authors' own consultancy, which have conducted numerous research projects related to soil biological health in temperate agroforestry systems

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