

Apple Replant Disease: Causes and Mitigation Strategies

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Abstract

After replanting apple (*Malus domestica* Borkh.) on the same site severe growth suppressions, and a decline in yield and fruit quality are observed in all apple producing areas worldwide. The causes of this complex phenomenon, called apple replant disease (ARD), are only poorly understood up to now which is in part due to inconsistencies in terms and methodologies. Therefore we suggest the following definition for ARD: ARD describes a harmfully disturbed physiological and morphological reaction of apple plants to soils that faced alterations in their (micro-) biome due to the previous apple cultures. The underlying interactions likely have multiple causes that extend beyond common analytical tools in microbial ecology. They are influenced by soil properties, faunal vectors, and trophic cascades, with genotype-specific effects on plant secondary metabolism, particularly phytoalexin biosynthesis. Yet, emerging tools allow to unravel the soil and rhizosphere (micro-) biome, to characterize alterations of habitat quality, and to decipher the plant reactions. Thereby, deep insights into the reactions taking place at the root rhizosphere interface will be gained. Counteractions are suggested, taking into account that culture management should emphasize on improving soil

microbial and faunal diversity as well as habitat quality rather than focus on soil disinfection.

Introduction

Apple replant disease (ARD) affects plant propagation in nurseries as well as apple production worldwide by strongly reducing plant growth as well as fruit yield and quality (Mazzola and Manici, 2012; Manici et al., 2013). The disease occurs after repeated replanting of apple at the same site. On ARD soils, over the lifetime of an apple orchard, a 50 % reduced profitability has been estimated due to later and less fruit bearing of the affected trees (Mazzola, 1998; Van Schoor et al., 2009). Problems by ARD are increasing recently, mainly due to the concentration of tree nurseries to certain regions, such as the Pinneberg region in Germany or Pistoia in Italy, as well as due to the concentration of apple orchards in the respective fruit production areas. In addition, to achieve higher planting densities, the use of dwarf rootstocks results in a shorter life span of these orchards and in more frequent replanting (St. Laurent et al., 2010; Volk et al., 2015). A rapid improvement of this situation is unlikely, as installation costs increase (frost protection, irrigation) and areas for crop rotation become increasingly scarce due to alternative usage for industry, energy plants, or other purposes.

Various definitions of the term "replant disease" or related phrases such as "replant problem", "soil sickness" or "soil fatigue" exist (e.g. Klaus, 1939; Hoestra, 1968; Utkhede, 2006). According to Utkhede (2006) "replant problems" include both, abiotic and biotic factors which suppress plant growth, whereas "replant disease" comprises only all biotic factors. "Soil sickness" is used in cases, where the causes of the reduced growth are unknown or uncertain (Savory, 1966), thus excluding nematode damage (Spethmann and Otto, 2003). Here, we suggest the following definition of ARD: *ARD describes a harmfully disturbed physiological and morphological reaction of apple plants to soils that faced alterations in their (micro-) biome due to previous apple cultures.*

ARD is characterized by the specificity to the species *Malus domestica* (although cross reactions with other Rosaceae have been observed) and a persistence for decades (Savory, 1966). ARD is reversible after transplanting into virgin or healthy soil. Replant disease has also been reported for other plants, and especially members of the Rosaceae, such as cherry, peach, strawberry,

rowan, and rose, are prone to it. In this review, we summarize current knowledge on the causes of ARD and critically evaluate current lines of research to develop mitigation strategies.

Etiology and causes of ARD

The species specificity implies that ARD has its origin in the apple plant-soil interface: Based on root exudates (Börner, 1959; Wittenmayer and Szabó, 2000; Hofmann et al., 2009) or/and by decomposition products of dead apple plant material changes in the biomes of the rhizosphere and of the soil are induced. A recent study addressed the composition of root deposits, thus analyzing the important root soil interface, of different apple rootstocks for the first time (Leisso et al., 2017). The rhizodeposits were found to be highly dynamic and influenced by growth conditions, rootstock genotype and bacterial communities of the rhizosphere. Also intraspecific allelopathy cannot be excluded in this context, but the persistence of ARD over decades would suggest that the involved toxic or deleterious substances are extraordinarily stable and bioactive. It is well accepted that soils may store or cycle certain molecules such as carbohydrates, lipids or proteins for years to decades (e.g. Wiesenberger et al., 2004; Derrien et al., 2006; Amelung et al., 2008). However, this has not been proven for root exudates, as usually these molecules are quickly transformed by rhizomicrobial respiration before being adsorbed or bound to soil minerals, which would make them inert against microbial degradation (e.g. Oades, 1988; Kuzyakov and Larionova, 2005; von Lützow et al., 2006; Kögel-Knabner and Amelung, 2014). If they do get bound, then it is questionable whether such compounds really maintain their bioavailability. From experiments with pollutants like polycyclic aromatic hydrocarbons, pesticides, or antibiotics, we know that with increasing contact time, newly added compounds become sequestered, thus rapidly losing their desorbability, bioavailability, and potential effectiveness on soil microbes (e.g. Hatzinger and Alexander, 1995; Lueking et al., 2000; Ciglasch et al., 2008; Rosendahl et al., 2012). It therefore appears truly unlikely that toxic compounds can explain ARD persistence for years. However, they may still trigger initial shifts in microbiome composition.

Shifts in bacterial and fungal communities in soil as a trigger for ARD development

Previous studies allow the conclusion that ARD is assumed to be a disease-complex (Figure 1) which is influenced by the soil type and the climate of the

respective site. The fact that soil disinfection leads to the restored regular plant growth clearly points to biotic causal factors (e.g. Mai and Abawi, 1981; Mazzola, 1998; Yim et al., 2013; Spath et al., 2015). In earlier studies on ARD soil microorganisms, various genera differing in dominance between sites were suggested to be involved in the disease complex. These are the oomycetes *Pythium* and *Phytophthora*, the fungi *Cylindrocarpon* and *Rhizoctonia* as well as actinomycetes and other bacterial genera like *Bacillus* and *Pseudomonas* (Jaffee et al., 1982; Otto et al., 1994; Mazzola, 1998; Utkhed, 2006; Tewoldemedhin et al., 2011). Also evidence for an involvement of root endophytic fungi (e.g. *Cylindrocarpon*-like fungi) in apple plant growth reduction on ARD affected soils was presented (Manici et al., 2013, 2018). More recently, numerous studies on microbial community analyses

of ARD soils were published, strongly fostered by new sequencing technologies (Rumberger et al., 2007; Tewoldemedhin et al., 2011; Yim et al., 2013, 2015, 2016; Sun et al., 2014; Caputo et al., 2015; Franke-Whittle et al., 2015; Mazzola et al., 2015; Hewavitharana and Mazzola, 2016; Nicola et al., 2017). These total DNA based analyses confirmed previous data on changes in the microbial community composition in replant soils. Obviously, the soil-inherent microbial diversity, the plant species, and the plant growth stage influence the microbiome in the rhizosphere of apple plants. Differences are observed in the bacterial and fungal community composition in ARD affected and in healthy soils from the same site (e.g. Franke-Whittle et al., 2015). However, up to now it is unclear, whether the missing or additional microorganisms as well as shifts in abundances caused ARD or

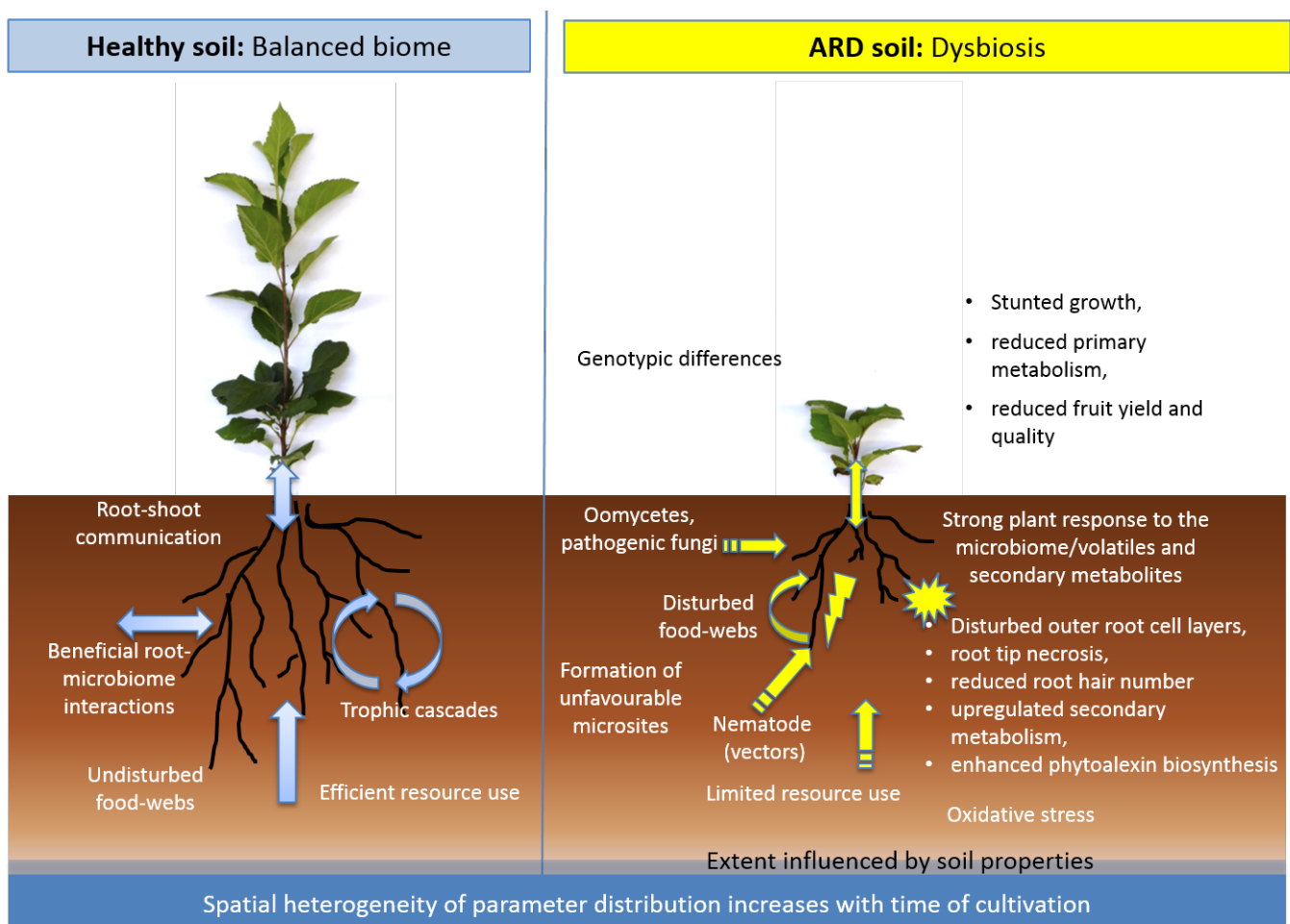


Figure 1. Apple replant disease (ARD) has multiple causes with a strong impact of dysbiosis regarding the microbiome and is influenced by soil properties, faunal vectors, and trophic cascades, with genotype-specific reactions.

occurred as a result of ARD. The analysis of amplicon sequencing data of microorganisms in ARD soil after treatment with heat, gamma irradiation, Basamid® or biofumigation revealed numerous bacterial and fungal populations with significantly increased abundance (responders) compared to that in untreated ARD soil. Although the 16S rRNA gene and ITS sequence data need a careful interpretation, notably, many of the responders belonged to taxa of which strains with plant beneficial traits or antagonistic activity were described, such as *Burkholderia* spp., *Arthrobacter* spp., *Podospira* spp. or *Penicillium* spp. (Franke-Whittle et al., 2015; Mazzola et al., 2015; Yim et al., 2016, 2017). However, the analysis of diversity revealed on the base of 16S rRNA gene fragments or ITS fragments amplified from DNA directly extracted from soil (total community DNA - TC-DNA) is limited in its resolution down to the genus or species level depending on the used primer systems. But, for many species which colonize the rhizosphere, it is well known that traits differ on a strain specific level and bacteria of the same species can act as phytopathogens or plant growth promoting bacteria. Even the same strain can change the gene expression pattern depending on its environment and thus develops different interaction patterns with plant roots. Thus, analyses of the functions and the expression pattern of genes of interest provided by the below-ground microbiome are needed to improve our mechanistic understanding on the role of microbes in ARD development.

In upcoming studies oomycetes need to be included in the analysis by molecular tools as the primers targeting fungal ITS do not amplify oomycetes and thus these data are still missing. Furthermore, qPCR systems need to be established to determine the changes in abundance of potential harmful or beneficial microorganisms in the apple rhizosphere microbiome in response to different soil management treatments.

Soil fauna affecting ARD

Besides nematodes (see below), important soil mesofauna groups are Collembolans and soil mites, which play an important role in soil food webs as decomposers, plant parasites, microbivores as well as predators (Hopkin, 1997; Wardle, 2006, 2013; Walter and Proctor, 2013). Both groups of organisms are neither discussed in the context of causing agents nor considered at all in the current ARD literature (Utkhede, 2006; Mazzola and Manici, 2012; Vukicevich et al., 2016). Nevertheless, there

is much evidence in the literature that soil mesofauna affects not only bacterial and fungal communities in the rhizosphere, i.e. by selective feeding on pathogenic or non-pathogenic microorganisms (Larley et al., 1994; Sabatini and Innocenti, 2000, 2001; Innocenti et al., 2009; Böllmann et al., 2010), but also promotes mycorrhizal fungi (Steinaker and Wilson, 2008; Kanters et al., 2015) and other beneficial microorganisms (Larley et al., 1994). Having in mind that Collembolans and soil mites are also strongly affected by land management (fertilizer, water, soil structure) (Schrader et al., 1997; Larsen et al., 2004; Innocenti et al., 2011; Roy et al., 2014) and pesticide use (Frampton, 2002; San Miguel et al., 2008; Chelinho et al., 2014), it would not be surprising that cascading trophic effects driven by consumers either directly or indirectly influence the etiology of ARD. So far, own results indicate decreased abundance of Collembola and soil mites, as well as shifts in Collembolan species composition in ARD compared to healthy soils (Meyhöfer et al., unpublished data).

Many studies suggested a role of root lesion nematodes in the ARD development (Hoestra and Oostenbrink, 1962; Dunn and Mai, 1972; Mai and Abawi, 1981; Jaffee et al., 1982) citing notably uneven distribution pattern of *Pratylenchus penetrans* in apple orchards (Mai and Abawi, 1978; Jaffee et al., 1982; Mai et al., 1994). Yim et al. (2013) inactivated nematodes in ARD affected soils by heat treatment and could prove that apple plants grew significantly better in heat-treated ARD soil compared to the untreated ARD soil, confirming the role of nematodes in ARD development. However, nematicide applications in affected orchards were inefficient to enhance apple growth (Hoestra and Oostenbrink, 1962; Covey Jr et al., 1979; Caruso et al., 1989; Mazzola, 1998). Furthermore, the low frequency of endoparasitic nematodes in roots did not give evidence for a contribution to growth reduction in apple in ARD affected soils (Manici et al., 2013). Nevertheless, root lesions induced by nematodes can cause synergistic damage to apple by acting in combination with some notable pathogenic fungi or oomycetes such as *Rhizoctonia*, *Phytophthora*, *Cylindrocarpus*, and *Pythium* (Mazzola, 1998). Furthermore, a high abundance of nematodes feeding on microbes can modify the microbial community by altering the relative abundance of populations (Djigal et al., 2004; Hai-Feng et al., 2014; Gebremikael et al., 2016) thus causing a significant reduction of microbes that induce plant growth promotion. Indirectly,

nematodes might contribute to ARD by dissemination of microbes (Freckman, 1988; Wang and McSorley, 2005) or activation of specific microbial growth by the release of growth limiting nutrients (Wang and McSorley, 2005). Recent findings by Adam et al. (2014) and Elhady et al. (2017) confirm specific bacteria and fungi to be attached to infective stages of *Meloidogyne incognita* and *P. penetrans* in different soil types indicating an ecological role of the association. Four way interaction between fungi, oomycetes, bacteria and nematodes was supposed to increase the ARD severity when these organisms were present at the same time (Utkhede et al., 1992; Mazzola and Mullinix, 2005).

Soil properties affecting ARD

Soil properties might modulate the degree of ARD observed in plants, if not even causally affecting it (von Bronsart, 1949). In general, sandy/light soils have often been observed to be more prone to ARD than loamy soils (<http://www.leicesters.co.nz/specific-apple-replant-disease/>). Own observations additionally confirm that areas with high groundwater levels or extended periods of water logging are less conducive for ARD most likely due to interfering with the development of pathogenic aerobic communities.

Von Bronsart (1949) stated that physical soil conditions like compaction, loss of specific pore sizes, dryness in macropores or stagnant water conditions above a compacted plough pan, may hardly be seen as major cause of ARD, because the latter is plant-specific while most soil-related effects are not. Nevertheless, physical soil conditions may drive the survival and competitiveness of phytopathogenic or beneficial nematodes and also of microbial communities, i.e., they might affect the intensity and duration of ARD symptoms. And indeed, ARD has often been observed to occur heterogeneously at a given site, as do many soil properties (Bogena et al., 2010; Gebbers and Adamchuk, 2010; Herbst et al., 2012). Mazzola and Manici (2012) concluded that abiotic factors may exacerbate ARD but do not appear to function as the primary cause of the disease.

Among chemical soil properties influencing ARD severity, pH is certainly a master variable affecting nutrient availability, microbial diversity and microbial nutrient mobilization and immobilization. Some authors reported that in soils with low pH values (around 4-4.5) ARD problems are less pronounced (Jonkers et al., 1980; Utkhede, 2006), while others

reported that low pH values make soils prone to replant disease problems (Willet et al., 1994). Additionally, the role of pH on ARD severity is obviously genotype dependent (Fazio et al., 2012).

With variations in pH, nutrient availability changes, and there has been focus on micronutrient controls on ARD expression, mainly related to Zn, Fe, and Mo (von Bronsart, 1949; Fan et al., 2010; Fazio et al., 2012). To avoid additional nutrient limitations, apple replanting should consider respective fertilizer recommendations. These may include, for instance, the use of selected micronutrients such as zinc while possibly excluding or at least very carefully operating with others like boron (Tukey et al., 1984). Adding mineral fertilizer, e.g., with P, (Sewell et al., 1988), as well as managing ground cover by adding compost or even biochar, may induce increased biocontrol properties of soils including a reduction of nematodes causing root lesions. Fertilization may thus affect ARD severity in nutrient-limited soils (van Schoor et al., 2009), but frequently failed to replace fumigation for replanting in temperate climates (St. Laurent et al., 2008; Mazzola and Manici, 2012; Glisczynski et al., 2016; Peruzzi et al., 2017).

To future study the role of soil in modulating the degree of ARD in plants, it will be inevitable to map spatial ARD heterogeneity in different orchards and to correlate it with spatial patterns of soil properties that likely also include subsoil properties. The finding of causal interactions between soil properties and ARD, however, is often hampered by insecurity related to sampling rhizosphere soil. Rhizosphere soil is commonly obtained by a vigorous shaking of the uprooted root system. This enables neither to differentiate between affected and non-affected root areas, nor to define precisely the distance from the root surface. As in general less material is obtained from affected roots compared to healthy root material, a dilution of effects might occur using total root sampling approaches.

Plant reactions to ARD

The level of susceptibility differs significantly between individual apple genotypes. Fully resistant genotypes were never observed yet, but less susceptible and/or tolerant genotypes can be found for different species of the genus *Malus* (Isutsa and Merwin, 2000). Symptoms of ARD are expressed early after the first contact with ARD affected soil and include belowground a root browning and blackening, root tip necrosis, reduced number of root hairs and destroyed outer root cell layers

(Caruso et al., 1989; Yim et al., 2013, Grunewaldt-Stöcker, unpublished data, Figure 2).

Aboveground plant parts show stunted or rosette growth (Caruso et al., 1989; Mazzola, 1998; Mazzola and Manici, 2012; Yim et al., 2013; Atucha et al., 2014; Emmett et al., 2014). These severe disorders result in a dramatically reduced plant biomass, fruit yield, as well as fruit size and flavor (Mazzola and Manici, 2012; Liu et al., 2014). The molecular and physiological reactions of apple plants to ARD soils resulting in these morphologically visible symptoms were only recently subject of first in-depth studies. The accumulation of phenolic compounds as antioxidants in roots and shoots under ARD, points to oxidative stress (reactive oxygen species) (Henfrey et al., 2015), and may be a consequence of plant damage from ARD induced plant secondary metabolites. Changes in patterns of phenolic compounds, like phloridzin and phloretin, benzoic acid and rutin (Börner, 1959; Hofmann et al., 2009; Yin et al., 2016, 2017; Leisso et al., 2017) could be the result or reason of ARD.

The exudation of these compounds may affect the complete soil microbiome or parts of it.

In addition, the abundance of antioxidative enzymes, such as peroxidases increased significantly four weeks after planting young apple rootstocks in ARD affected soil, whereas synthesis of these enzymes was lower in plants grown in gamma irradiated soil (Schmitz et al., unpublished results). Peroxidases oxidize hydrogen donors at the expense of peroxides. They are highly specific for hydrogen peroxide, but they accept a wide range of other hydrogen donors, including polyphenols. The higher activity of peroxidase could promote the oxidation of phenols into the antioxidative polyphenols in the roots (Ayyagari et al., 1996), which may lead to the visible browning and blackening symptoms as well as root tip necrosis.

After infection of roots of apple seedlings by *Pythium ultimum*, one of the potential causal agents of ARD, an upregulation of the expression of genes involved in the secondary metabolism occurred as well as differential expression of genes in plant

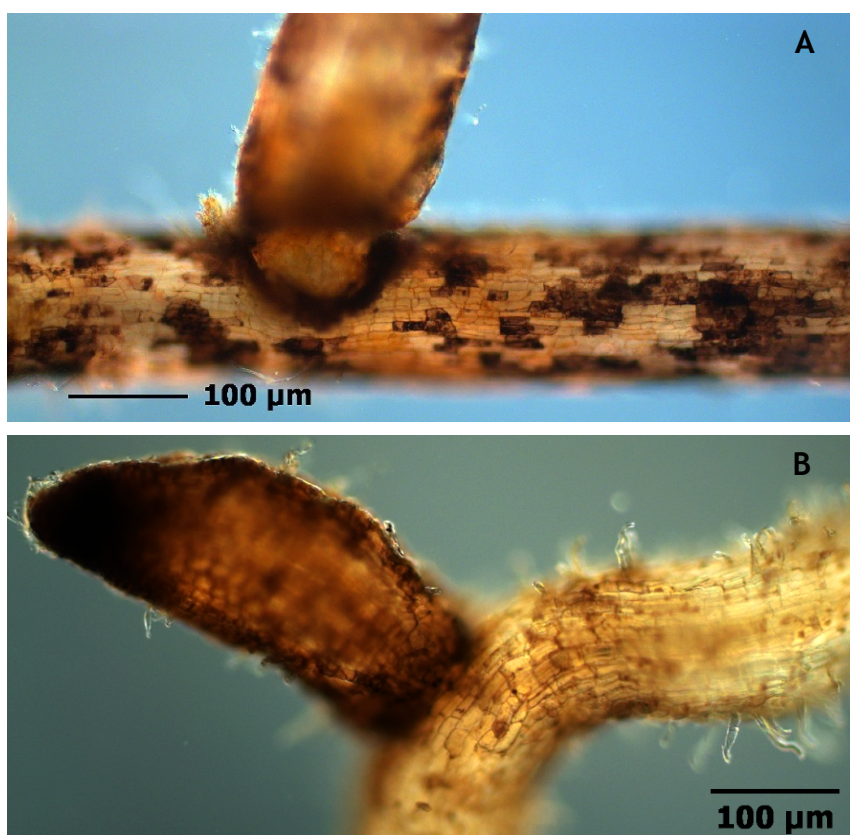


Figure 2. Cell necroses and blackening in the outer tissue layers of a branching fine root (A), and root tip necrosis (B) of apple rootstock *Malus domestica* M26, grown for two weeks in ARD affected soil.

hormone metabolism (Shin et al., 2014, 2016; Zhu et al., 2014). Comparative transcriptomic studies of roots of the sensitive rootstock M26 grown either in ARD or gamma irradiated ARD soils revealed several differences in the expression of genes involved in stress responses (Weiß et al., 2017a, b). Further, when grown in ARD soil the plants reacted with an upregulation of expression of genes of the secondary metabolism, especially concerning the phytoalexin biosynthesis. Also, the corresponding phytoalexin products, i.e. biphenyls and dibenzofurans were detected in relatively high concentrations (Weiß et al., 2017b). A more detailed understanding of the molecular interplay of apple plants and their microbiome in healthy and ARD affected soils is urgently needed to define causes and consequences of ARD for plants and microbes (Manici et al., 2017).

The role of spatial distribution of relevant parameters

There are several observations indicating that the ARD causing agent, whatever it is, lacks mobility. Hoestra (1968) already reported in 1968, that ARD affects the apple tree in the first years of planting, thereafter the roots grow into deeper soil layers less impaired by ARD. He showed in growth experiments with soil extracted from different depths that ARD was mainly observed in 0-15 and 15-30 cm soil depth. As also many soil properties and functions are heterogeneous under field conditions (Bogena et al., 2010; Gebbers and Adamchuk, 2010; Herbst et al., 2012), the patchiness of ARD related growth depression in the field as reported above, likewise hints in the same direction. Interestingly, ARD is induced more rapidly if the site is replanted frequently (nurseries) compared to sites permanently used for apple production. Frequent replanting is associated with more frequent mixing of soil due to uprooting and soil cultivation.

Restricted mobility of ARD causing agents, at least within the root system was also confirmed in a recent split-root experiment (Figure 3) of Lucas et al. (unpublished data). Apple plants grown in split-root systems with different combinations of ARD soil, sterilized ARD soil or control soil (same site but never planted with apple) gained no reduction in shoot growth if half of the root system had access to soil not affected by ARD. The spatial separation is obviously crucial as simple dilution of the ARD soil by sterilized or control soil did not lead to comparable results (Hoestra, 1968; Jaffee et al., 1982; van Schoor et al., 2009; Tewoldemedhin et al., 2011; Spath et al., 2015). The split-root



Figure 3. Root growth of *Malus domestica* M26 in a splitroot experiment. (Photo taken by Maik Lucas).

experiment of Lucas et al. (submitted) clearly showed that ARD is not systemic. Bacterial and fungal community composition in the rhizoplane and rhizosphere of the same plants differed significantly between the compartments containing ARD soil and those containing sterilized or control soil. However, some populations were only detected in the sterilized soil if the neighboring compartment contained ARD soil.

Further observations from our group (Zickenrott et al., unpublished data) indicate that apple plant roots avoid ARD soil patches, if given a choice. The mechanisms behind this are currently not known.

Assessment of mitigation strategies for ARD

Crop rotation is the first and oldest way to mitigate or circumvent ARD (Mazzola and Gu, 2001), but this is strongly limited or even not possible due to high investments in orchard infrastructure, for-instance in fences, hail nets, wells, pipes and more technology for irrigation. However, the main obstacle is the lack of areas for rotation in the production centers. Soil fumigation by chemicals is no longer possible in

many countries due to the phase-out of the ecologically harmful fumigants. Biofumigation (Brown et al., 1991) using the incorporation of Brassicaceae plants or seed meal has been suggested as a counteraction and has shown first promising results (Mazzola et al., 2001, 2007, 2015; Yim et al., 2016, 2017), but cannot fully restore plant growth in most cases. The authors could prove that the application of *Brassica napus* seed meal amendments resulted in an increased abundance of *Actinomycetes*, e.g. *Streptomyces*, and *Pseudomonas* in soil, bacterial groups being known for their high contribution to biocontrol of phytopathogens (Mazzola et al., 2007). The observed effects of applied *B. napus* seed meal were, however, variable and depended on the time of application, the concentration applied and the content of glucosinolates of the meal.

Further, several studies in the past have investigated the impact of fungicides like difenconazole or metalaxyl on the growth of apple trees in soils with replant disease symptoms (Mazzola, 1998). Although positive effects were obvious the issue of sustainability is questionable as a continuous application is needed. Because of the small specificity of the compounds other non-target populations like beneficial fungi might be affected with non-intended side effects.

Steam disinfection of soils is theoretically possible but too energy and time consuming and still fraught with technical problems, as demonstrated in current experiments in German nurseries. The costs of disinfecting soils with steam are 3-4 times higher than using chemicals (Nitt et al., 2015). Interestingly, the intercropping with *Tagetes*, conventionally used against nematodes, revealed increased growth of apple in two ARD soils, both in a bio-test as well as in field trials (Yim et al., 2017).

The idea to change more than the abundance of one microbial strain becomes more and more popular as it is well accepted that different microbial traits might contribute to overcome replant disease in soil, and that soil microbial diversity is strongly altered by replant disease (Sun et al., 2014; Berg et al., 2017). Already 25 years ago, Utkhede and Smith (1992) reported the promotion of apple tree growth and fruit production in a former ARD soil after inoculation with a strain of *Bacillus subtilis*, which showed biocontrol activities against various pathogens. The authors could prove that the inoculation procedure was more effective than a classical formalin fumigation, mainly, as it did not

only increase shoot growth and the cross-sectional trunk area but resulted in higher yields, too. This concept of disease suppression via the inoculation of biocontrol microbes was further followed up, mainly as several authors could prove a high abundance of *Rhizoctonia* spp. from ARD soils (Mazzola, 1997). In 2007, the same author published data on the manipulation of rhizosphere bacterial communities to induce suppressive soils (Mazzola, 2007). Crop rotation, including wheat cultivation after apple growth, reduced the susceptibility of soils for ARD, and correspondingly an increase of fluorescent pseudomonads in the soil was observed (Mazzola et al., 2002). Therefore, it was suggested to use selected *Pseudomonas* strains of the species *P. fluorescens* or *P. putida* with biocontrol properties against *Rhizoctonia* for inoculation (Mazzola et al., 2002). These approaches seem to be promising, since the use of chemical substances can be avoided. However, it needs to be taken into account that microbe-based inoculation strategies need to consider on the one hand the potential risk of the inoculum for the environment. For example, *P. putida* has been recently classified into risk class II according to the German biosafety level, as several severe cases of infections of humans with *P. putida* have been reported (Carpenter et al., 2008). On the other hand, inoculation-based approaches often do not result in the expected outcome as the inoculated microbes did not establish well in soil and were outcompeted by the autochthonous microflora in the soil. Here developments using specific carrier materials for the inoculum have been proven to be successful, which give inocula a protected initial niche for performance (van Elsas and Heijnen, 1990). Furthermore, an improved understanding of the ecology of inoculants is required for more reliable and efficient use (Berg et al., 2017).

Numerous experiments, in which treatments with *Trichoderma harzianum* (Wrede, 2015), cyanamid, stone dust and fertilizers amended with organic compounds such as humus, alkaline substances and seaweed were tested, were not or not sufficiently effective. Also, by incorporating spent mushroom compost, a composted substrate from mushroom production, into ARD soil, an increase of microbial activity was achieved leading to a significant increase in shoot growth, an effect being comparable to that of pasteurization (Manici, 2015; Franke-Whittle et al., 2018). More research is necessary to support or reject the hypothesis that many of these compounds were ineffective in harming soil pathogens, because they might have

persisted in microhabitats different from those reached by the amendments.

The influence of important compounds exuded or released from roots ploughed into soil must be evaluated. In this respect, also carbon source-dependent effects of anaerobic soil disinfestation might be discussed (Hewavitharana and Mazzola, 2016).

Arbuscular mycorrhizal fungi (AMF) are essential endophytic players in the microbial network in the rhizosphere as well as in plant root systems. Besides the often-cited promotion of P acquisition, the mycorrhizal host plants have manifold advantages for their survival and productivity (Finlay, 2004; Smith and Read, 2008; Smith and Smith, 2012). Thus, a positive contribution of AMF to healthy apple growth and productivity is the normal case. The selection and application of AMF isolates for a recovery from ARD has been considered a possible strategy, but seems to be a rather difficult aim. Since long, this approach gained often less successful results in other instances of disease control, especially in field trials (Schönbeck et al., 1994; Linderman, 2000; Whipps, 2004). However, the AM symbiosis can lead to striking positive effects in plant productivity when damages of abiotic stress, e.g. drought (Pinior et al., 2005) or of infections by soil borne pathogens (Grunewaldt-Stöcker and von Alten, 2003; Whipps, 2004) and of nematodes (Calvet et al., 2001) were diminished. Regarding ARD, Čatská (1994) described a promising significant increase in productivity of apple plants (shoot and root biomass) due to *Glomus fasciculatum*, applied to ARD soil of two diverse soil types. Moreover, this mycorrhizal effect occurred together with an altered composition of the rhizosphere microbiome. Mehta and Bharat (2013) confirmed in tests with several AM fungi the specific success of a *Glomus fasciculatum* strain to overcome apple growth depression in ARD soil.

To apply selected effective AMF for an ARD therapy at a large scale in nurseries or field sites, the production of AMF inoculum, the formulation, shelf life and commercial supply are yet difficult (Azcón-Aguilar and Barea, 1997; Whipps, 2004). Nevertheless, the strategy to exploit naturally occurring or introduced AMF with a potential to alleviate abiotic stress and to control soil borne pathogens in combination with other biological agents or measurements against ARD seems attractive. AMF together with fine root endophytes (*Glomus tenue*, Orchard et al., 2017), are essential

in the microbiome network and need attention, promotion and protection by all cultural practices. The recently widened molecular methods (e.g. real-time PCR quantification of AMF, Alkan et al., 2004; Voříšková et al., 2017; DNA based sequencing for identification and diversity studies of AMF, Vasar et al., 2017) can help to determine fungal communities with positive effects on apple plants. Also, AMF isolates harboring mycoviruses (Ikeda et al., 2012) or endobacteria (Venice et al., 2017) are of interest with regard to their influence on the symbiotic performance of AMF in ARD soil as well as in biocontrol strategies.

Besides modulating the soil microbiome, several recent strategies include the improvement of plant tolerance towards replant disease. Breeding of less susceptible rootstocks seems feasible as tolerant genotypes are available in *Malus* germplasm (e.g. Isutsa and Merwin, 2000; St. Laurent et al., 2010; Robinson et al., 2012; Volk et al., 2013). In addition, an improved strategy for defense responses of plant roots by modulating cellular signals such as the oscillation of Ca^{2+} concentration, reactive oxygen species burst or protein kinase activity (Emmett et al., 2014) is under debate.

Finally, more work needs to be done to assess the socio-economic benefits of such approaches.

Conclusions

Despite increasing data on ARD, combined efforts of plant scientists, ecologists, microbiologists, soil scientists as well as socio-economists and growers are needed to fully understand and overcome ARD. The German consortium BonaRes ORDIAmur (Overcoming Replant Disease by an Integrated Approach; www.ordiamur.de) aims at finding indicators for infected soil, to restore its functional biodiversity, to identify and use genetic factors controlling ARD in apple and to optimize the composition of microbial communities to promote apple growth in ARD soil (Figure 4). Two important considerations for future research in ARD have to be taken into account: Firstly, a proper comparison to healthy or virgin soil is difficult, since even small spatial distances to sites where healthy soil is taken might involve very drastic changes in soil physical, chemical and biological properties. Moreover, the vegetation of the control site will also influence the (micro)biome of the soils. On the other hand, disinfected soil can neither be considered a proper control soil. Secondly, future studies should emphasize the soil sampling and distinguish bulk soil, rhizosphere and rhizoplane, as well as define

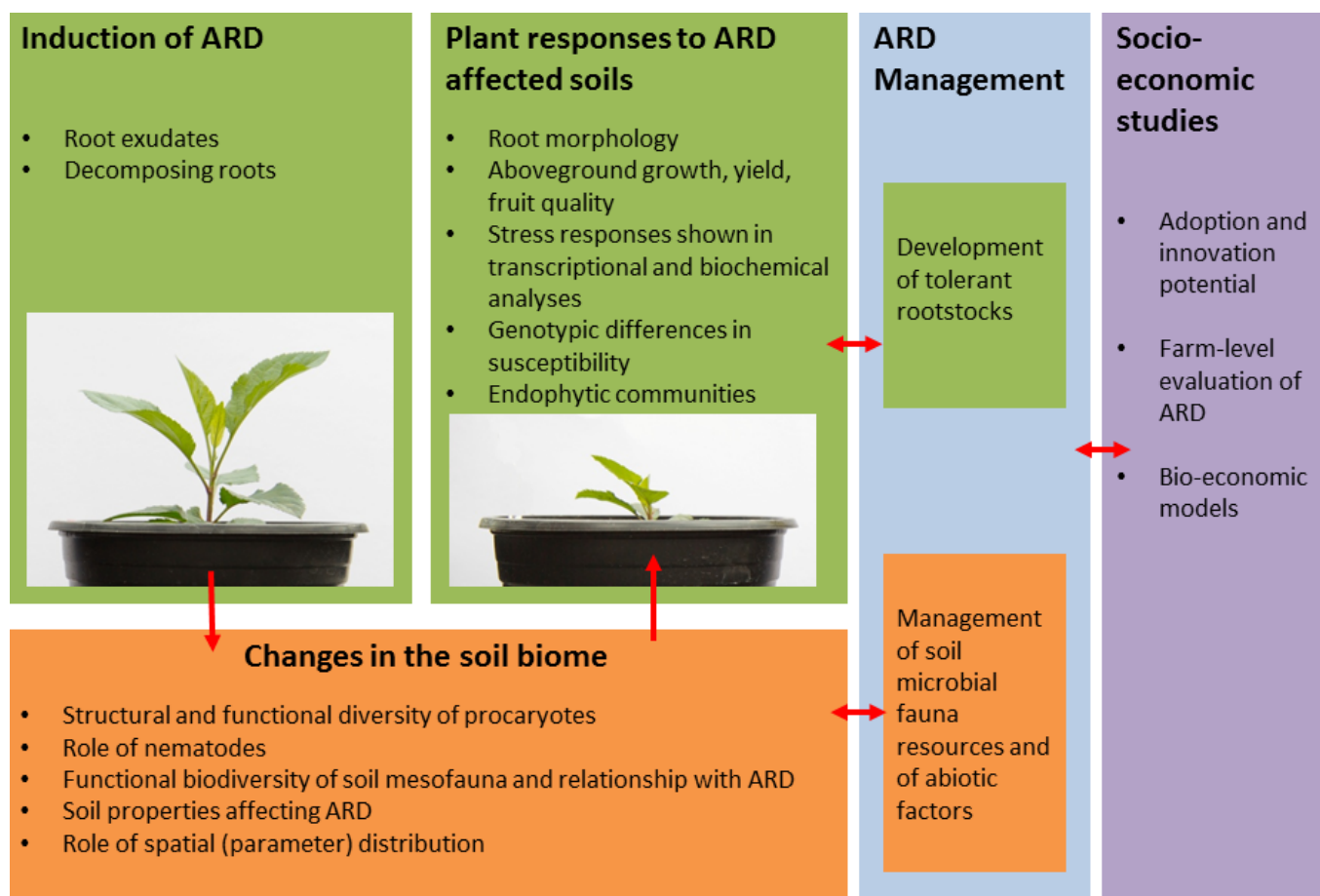


Figure 4. The understanding and management of apple replant disease (ARD) goes beyond disciplinary expertise - The ORDIAmur concept: Complex interactions of plant driven metabolites and soil-borne (micro)biome changes induce ARD. Future studies will have to focus on managing techniques including manipulation of plant attributes and soil microbial communities accompanied by socio-economic studies.

the root order and degree of damage. For both aspects, reproducible and internationally accepted definitions would be helpful.

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