Tree Vigour in Apple Production:

Impact of Replant Disease and Mitigation Strategies

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Preface

This thesis was prepared within the framework of the BonaRes Initiative, project 'Overcoming Replant Disease by an Integrated Approach' (ORDIAmur) (FKZ: 031B0025D). The cumulative thesis is submitted to the University of Kassel, Faculty of Organic Agricultural Sciences (FB 11), Department of Soil Biology and Plant Nutrition, to fulfil the requirements for the degree 'Doktor/Doktorin der Agrarwissenschaften' (Dr. agr.) – kumulative Dissertation am Fachbereich Ökologische Agrarwissenschaften der Universität Kassel'.

The following publications are included in the here presented cumulative thesis:

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Summary

Cultivating apples on sites previously used for cultivation of apples leads to suppression of vegetative performance of apple trees and losses in fruit yields. This replant effect is attributed to an apple related form of soil fertility loss named 'soil fatigue' and 'Apple Replant Disease' (ARD). Replant disease strongly affects profitability of apple production in nurseries specialised in fruit trees, as well as on fruit orchards. Recent studies focus on causally linked agents of the replant disease and quantification of its impact on apple tree. Handling replant disease, however, is challenged by the lack of knowledge of causal agents of the soil-borne disease and interaction between replant soil and tree vigour.

The main objective of this study was to contribute to an agro-ecological estimation and mitigation of replant impact by soil management. To this end, three field studies were performed in apple fruit production on replant soil and in direct vicinity to no-replant soil. In-field studies were accompanied by pot trials for estimating effects of soil treatment by two management strategies.

Tree vigour is represented by trunk cross-sectional area (CSA). The CSA is highly correlated to above-ground tree area representing tree vigour. Apple trees are sensitive to replant soil by suppressed tree vigour. By quantitative PCR-analysis (qPCR) an effect of replant on soil fungal abundances are observed. The *Alternaria* group (Ag) (class: *Dothideomycetes*, order: *Pleosporales*, family: *Pleosporacea*) is identified as a replant-sensitive soil parameter responding to replant with abundances. Replant effect on soil structure was examined by dry- and wet-sieving procedure, as well as ultrasonication showing decreased aggregation level and stabilities of soil aggregates in replant soil in comparison to no-replant soil. By correlation analysis an interrelation between replant-related suppression of tree vigour and soil degradation processes were detected. An interrelation between apple tree vigour, soil fungal population and soil structure is only detectable at specific time intervals during vegetation period (flower bud, maturation).

For estimation of replant impact on orchard performance a methodological approach to quantify replant effects based on an indication was established. Replant effects were indicated by the algorithm $Q = \ln(Ag)/CSA$. By this algorithm replant impact can be indicated at the level of the single tree, independent of soil variant (no-replant/replant). Site-specific analyses have shown that replant effect on tree vigour does not occur continuously, but in different stages of suppression. On this basis, five clusters of tree vigour suppression (Q) were defined: vital (0%), escalating (- 38%), strong (- 53%), very strong (- 62%) and critical (- 74%). By means of clusters, the replant impact was assessed in economic terms. Classification of tree vigour has shown that replant impact

strongly differed among trees on replant soil, even between trees in direct vicinity. Random tests of tree vigour suppression required at least eighteen trees in row for solid results. The weighted frequency of clusters in the field allowed replant impact to be quantified at field level. Applied to case study, the calculated tree vigour was - 46% compared to the potential tree vigour on no-replant soil in the same field.

For mitigation of replant impact on orchard performance two biological soil management strategies were tested: (1) 'Müncheberger Dammkultur' (MDK) and (2) inoculation with arbuscular mycorrhizal fungi and bacterial strains (AMFbac). An initial treatment of soil at time of planting, as well as first-time treatment and intermittent treatment at later stages in established orchard were tested. Either biological management strategy fully mitigated replant effects within one vegetation period. A repeated application at later stages increased tree growth rate up to the level of growth rate on (non-treated) no-replant soil. The effect of AMFbac treatment showed no constant results. If replanting of apple is unavoidable, either biological soil management could be applied to stabilise productivity of the orchard. A repeated treatment of soil may become necessary.

In this study, ecological aspects of the replant disease were identified, particularly individual tree reaction on replant soil and replant-sensitive time intervals during growing season, suggesting for replant impact on soil structure. Continuous research should comparatively analyse regional soil climate conditions, to identify replant-sensitive production regions, that require an intensive biological management of replant soil.

Zusammenfassung

Der wiederholte Anbau von Apfel auf demselben Standort, kann zu einer gehemmten vegetativen Entwicklung von Apfelbäumen, sowie Einbußen im Fruchtertrag führen. Zurückgeführt wird dieser Nachbaueffekt auf eine spezifische Form des Verlustes der Bodenfruchtbarkeit, bezeichnet als Bodenmüdigkeit (engl.) ‚Apple Replant Disease'. Die Nachbaukrankheit kann sich nachteilig auf die Rentabilität der Apfelproduktion in Baumschulen, wie auch im Obstbau auswirken. Es gibt Ansätze zur Bestimmung der Ursachen und des Schweregrades, jedoch wird der Umgang mit der Nachbaukrankheit durch ein fehlendes Wissen über die explizit kausalen Faktoren im Boden und die Interaktion zwischen Nachbauboden und Apfelbaum beeinträchtigt.

Zielstellung dieser Arbeit ist es einen Beitrag zur agrarökologischen Bewertung und zur Minderung von Nachbaueffekten durch Bodenmanagement zu leisten. Dieses Ziel wurde anhand von drei Studien, durchgeführt in der laufenden Fruchtproduktion auf Nachbaustandorten und im direkten Vergleich zu benachbarten Nicht-Nachbaustandorten bearbeitet. Die Feldstudien wurden mit flankierenden Topfversuchen zur Untersuchung der Applikationswirkung von zwei Bodenmanagementstrategien ergänzt.

Die unterschiedliche Baumvitalität wird durch die Stammquerschnittsfläche (CSA) repräsentiert. Auf den Fruchtproduktionsstandorten wurde gezeigt, dass der CSA mit der Baumkronenfläche eng korreliert und damit die Baumvitalität gut abbildet. Apfelbäume reagieren sensitiv auf Nachbau durch eine gehemmte Vitalität, also einen geringeren CSA. Mittels quantitativer PCR-Analysen (qPCR) wurde zudem ein Einfluss der Nachbaukrankheit auf boden-pilzliche Abundanzen nachgewiesen. Auf genetischer Gruppenebene konnte die *Alternaria*-Gruppe (Ag) (Klasse: *Dothideomycetes*, Ordnung: *Pleosporales*, Familie: *Pleosporacea*) als Nachbau-sensitiv gefunden werden, verbunden mit signifikant erhöhten Abundanzen unter Nachbaubedingungen. Darüber hinaus wurden Auswirkungen des Nachbaus auf die Bodenstruktur untersucht. Dabei zeigten Trocken- und Nasssiebung, sowie Ultraschalldispergierung einen abnehmenden Bodenaggregationsgrad, sowie verringerte Aggregatstabilitäten in Apfel-Nachbauboden auf. Mittels Korrelationsanalysen wurde ein Zusammenhang zwischen der durch Nachbau bedingten Hemmung der Baumvitalität und boden-internen Degradationsprozessen nachgewiesen. Dieser Zusammenhang ist jedoch nur in spezifischen Zeiträumen innerhalb der Vegetationsperiode (Entwicklung der Blütenknospen, Fruchtreife) nachweisbar.

Zur Bestimmung des Grades der Bodenmüdigkeit/Nachbaukrankheit auf die Leistungsfähigkeit von Fruchtproduktionsstandorten wurde ein methodischer Ansatz zur Quantifizierung von

Nachbaueffekten auf Basis der Indikation der Baumvitalität entwickelt. Diese Indikation erfolgt auf Grundlage des Algorithmus $Q = \ln(Ag)/CSA$. Dieser Algorithmus ermöglicht eine Einzelbaum-spezifische Indikation von Nachbaueffekten, und kann auch unabhängig von der Nachbaustruktur (Nicht-Nachbau/Nachbau) angewandt werden. Anhand des Indikationswertes Q wurde jedem Baum ein Schadenscluster zugewiesen. Die Standortanalysen haben gezeigt, dass der Schaden sich nicht kontinuierlich, sondern schubweise zunehmend darstellt, so dass insgesamt fünf Schadenscluster (Q) für die Versuchsstandorte separierbar waren: vital (0%), eskalierend (- 38%), stark (- 53%), sehr stark (- 62%), und kritisch (- 74%). Auf Grundlage der Schadenscluster kann der Nachbaueffekt ökonomisch bewertet werden. Bei der Einordnung der Vitalität der Schadenscluster hat sich weiterhin gezeigt, dass der Ausprägungsgrad der Nachbaukrankheit Einzelbaum-spezifisch unterschiedlich stark ausgeprägt ist, auch in direkter Nachbarschaft. Diese Ausprägungsvariabilität erfordert eine minimale Stichprobe von 18 Bäumen in Nachbarschaft. Anhand der prozentualen Häufigkeit der Schadenscluster kann ein mittlerer Nachbaueffekt auf Feldebene ausgewiesen werden. Dieser entsprach für die Versuchsstandorte einer Reduktion der vegetativen Entwicklung der Apfelbäume von rund -46%.

Zur Minderung von Nachbaueffekten auf die Leistung von Fruchtproduktionsstandorten wurden zwei biologisch-wirksame Bodenmanagementstrategien: (1) 'Müncheberger Dammkultur' (MDK), und (2) eine kombinierte Inokulation diverser arbuskuläre Mykorrhiza- und Bakterienstämme (AMFbac), geprüft. Getestet wurde die initiale Applikation zum Zeitpunkt der Pflanzung, sowie im bestehenden Bestand eine einmalige oder zweimalige Anwendung der MDK. Beide biologisch-wirksamen Managementstrategien haben die Nachbaueffekte innerhalb einer Vegetationsperiode vollständig aufgehoben, und bei wiederholter Anwendung im Bestand die Wachstumsrate auf Nicht-Nachbaulevel gesteigert. AMFbac lieferte jedoch keine konstanten Ergebnisse über verschiedene Versuche. Stehen für eine Neuanlage zur Apfelfruchtproduktion nur Nachbaustandorte zur Verfügung, so sollte zur Sicherung der Produktivität die Anwendung einer oder beider biologisch-wirksamen Bodenmanagementstrategien erfolgen. Eine erneute Anwendung innerhalb der Bestandslaufzeit kann notwendig werden.

In dieser Studie wurden ökologische Aspekte der Nachbaukrankheit identifiziert, insbesondere die stark ausgeprägte Einzelbaumreaktion und das Vorkommen Nachbau-sensitiver Phasen während der Vegetationsperiode mit ersten Ansätzen zu wahrscheinlich nachhaltig veränderten Bodenaggregatstrukturen. Weiterführende Arbeiten sollten die regional-spezifischen Bodenwitterungsbedingungen vergleichend analysieren, um besonders sensitive Anbauregionen auszuweisen, die ein intensiveres biologisch-wirksames Bodenmanagement erfordern.

Abbreviations

Ag	*Alternaria* group
AMF	Arbuscular mycorrhizal fungi
AMFbac	Microbial consortium of a variety of AMF species and bacterial strains
ARD	Apple Replant Disease
bac	Bacterial strains
CSA	Trunk cross-sectional area
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
ITS	Total fungal DNA
MDK	Müncheberger Dammkultur
MWD	Mean weight diameter
nr	No-replant soil
r	Replant soil
PCR	Polymerase chain reaction
Q	Clusters of tree vigour suppression
qPCR	Real-time quantitative PCR
WRB	World Reference Base for Soil Resources
WS	Water-stable aggregates

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1. Introduction

1.1 Apple Production

According to the Food and Agriculture Organization of the United Nations commercial apple orchards in at least ninety countries produce about 87.3 million metric tons of apples (*Malus domestica* Borkh.) harvested across a total area of about 4.7 million ha (2019) per year (FAOSTAT, 2021). The world's largest producer, accounting for almost half of the global output, i.e. around 41 million metric tons, is China. The European Union follows in second place with about 11.5 million metric tons (Shahbandeh, 2021). Within the European Union, Germany ranks on third place with about one million metric tons produced on about 68% (approx. 33.905 ha) of fruit tree orchards (Ahrens, 2022). In addition, fruit rootstocks are cultivated on about 837 ha nursery areas (Statistisches Bundesamt (Destatis), 2021a). Commercial apple orchards are located in every German federal state (Garming et al., 2017). The most relevant apple orchards are located along the Lower Elbe (Lower-Saxony, Hamburg, Schleswig-Holstein), in the Lake Constance region (Baden-Württemberg) and in Saxony-Anhalt (Statistisches Bundesamt (Destatis), 2021b).

An increasing intensification of apple production in specific geographical areas has led to highly specialised production regime, with replanting of apple trees on the same fields in continuous succession. A survey by expert interviews suggests that a proportion of about 50 to 75% of German apple orchards are already replanted. Within the traditional fruit production location lower Elbe, the percentage of replanted orchards is even higher, up to 75 to 90% (Fig. 1.1) (Cavael et al., 2018).

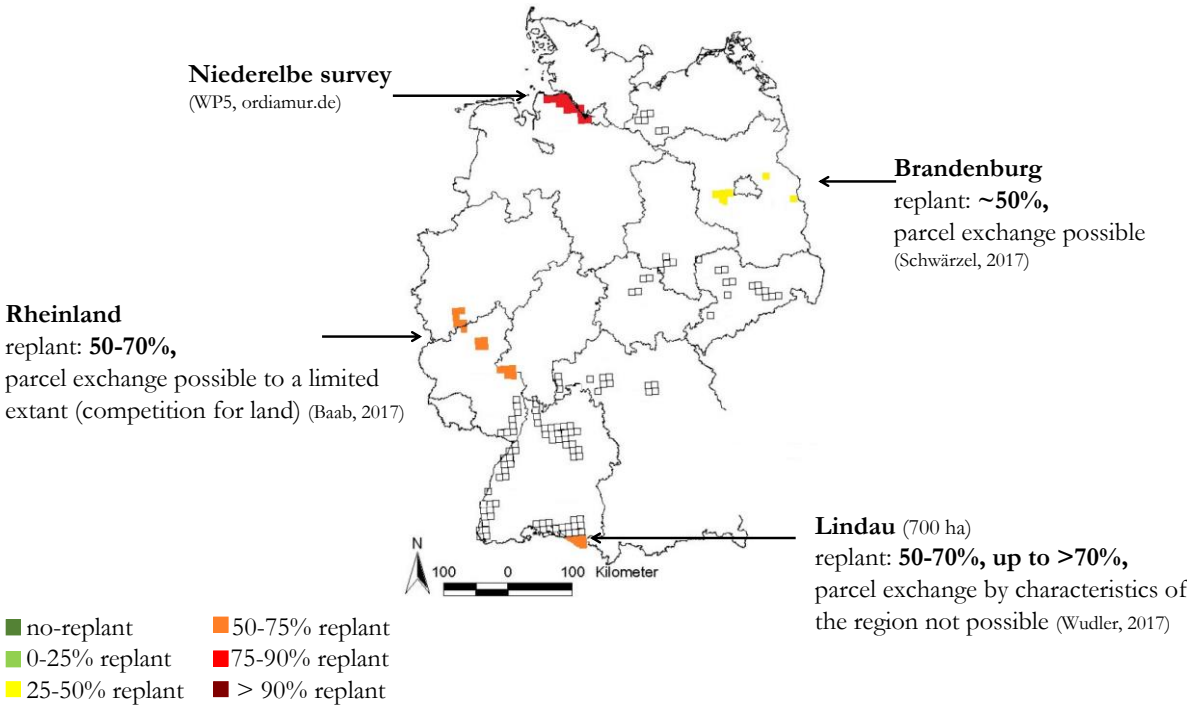

Fig. 1.1 Proportion of replanted orchards. ARD-severity reflected by fruit growers and fruticultural experts in apple-growing regions (Germany) (Map: Chmielewski, 2009; edited) (Cavael et al., 2018).

1.2 Tree vigour on replant sites

After replanting nurseries or fruit plantations within production-sites previously used for cultivation of the same crop, nursery owners and fruit growers are often faced with a general suppression up to complete failure of plant vigour. The suppression of plant vigour of nursery and fruit crops of the Rosaceae family is termed replant disease, and specifically 'Apple Replant Disease' (ARD) in terms of apple tree cultivation. The suppression of apple tree vigour on replant sites has been reported from fruit growing areas worldwide (Mai, 1981). ARD is a significant problem for nurseries specialised in raising fruit trees, affecting their production of rootstocks as well as the health of the finished trees, after the fruit-bearing variety is grafted. The apple fruit production system is perennial, therefore failure in managing replant disease will restrict productivity from several years to the whole production cycle of an orchard (15+ years).

Most obvious, replanted orchards show an uneven growth of apple trees. Suppression of tree vigour has been found individually pronounced in apple trees, resulting in a heterogeneous distribution of more or less replant symptomatic trees over plantation (Simon et al., 2020; Tilston et al., 2018). Symptoms of replant disease include reduction in root biomass by up to 50%, alterations in root structure, impairment of root hair formation, as well as cellular alterations in the outer tissue layers (blackening, necrosis, black inclusions, discoloration in the apoplast) along with

functional disturbance of root system by reduction of metabolic activity in feeder roots (Caruso et al., 1989; Grunewaldt-Stöcker et al., 2019; Yim et al., 2013). Root morphology and root physiology are closely associated with aboveground tree vigour (Reekie & Bazzaz, 2005; Waines & Ehdaie, 2007; Yang et al., 2008). Aboveground, replant symptomatic trees show stunting, shortened branch internodes, smaller and lighter green leaves as well as rosette growth (Atucha et al., 2014; Caruso et al., 1989; Emmett et al., 2014; Mazzola, 1998; Mazzola & Manici, 2012; Yim et al., 2013).

The general suppression of tree vigour on replant sites is observed shortly after replanting within two weeks to three months and can ultimately lead to tree death within the first growing season (Grunewaldt-Stöcker et al., 2019; Mazzola & Manici, 2012). Due to the critical importance of tree growth in the years establishing an orchard, any vigour suppressive effect is adversely felt (Van Schoor et al., 2009). Thus, vegetative and generative performance of apple replant sites have been found suppressed by up to 50%, to reduced fruit size and weight up to 10%, to discoloration of fruit skin and altered aroma, as well as delay of fruit bearing on trees by 2-3 years (Liu et al., 2014; Mao & Wang, 2019; Mazzola, 1998; Mazzola & Manici, 2012). Resulting loss of profitability, by as much as 50% throughout the production cycle of an orchard, may render replant sites unprofitable for cultivation (Peterson & Hinman, 1994; Geldart, 1994).

1.3 Estimation of tree vigour

Tree vigour is defined as the intensity of vegetative growth (Nesme et al., 2005), and is related to the reproductive potential of trees (Lepsis & Blanke, 2006). Targeting the morphology of fruit tree, tree vigour is measured e.g. by scion-rooting, annual shoot length, sum of fruiting branch sectional area or trunk cross-sectional area (CSA). The CSA is an often-used measure to evaluate tree vigour, and modelling fruit yield, by fruit growers and fruticultural experts such as technical advisers in field (Lepsis & Blanke, 2006; Nesme et al., 2005). The estimation and control of tree vigour is of importance in orchard management with regard to soil and specific cultivation measures, such as soil fertilisation, pruning and pest management.

The detection of replant impact on tree vigour is severely impeded by the general nature of replant symptoms on trees. In field, there is often a lack of opportunity to compare trees on sites with identical cultivation management differing only in terms of soil (no-replant/replant). Therefore, an estimation of tree vigour under replant conditions is often performed using (greenhouse) bio-tests, generally performed prior to planting in orchards or on nursery sites. Replant-related suppression of tree vigour is estimated by growth-response using replant-soil, treated replant soil (e.g. thermal treatment, chemical fumigation) and no-replant soil (Yim et al., 2013). Bio-tests enable the

detection of several stages of severity of replant in soil by gradual suppression of tree vigour (Tewoldemedhin et al., 2011a). However, conducting bio-tests under controlled conditions generates high uncertainty regarding the prediction of tree vigour in field, and thus the results are less valid for field conditions (Gilles, 1974; Merwin et al., 2001).

1.4 Tree vigour linked to soil biotic and abiotic properties on replant sites

Tree vigour is the result of combined effects of biotic and abiotic factors that interact with tree ontogeny (Visser et al., 2016). Under replant conditions, imbalances in soil biotic and abiotic conditions have been observed that ultimately may result in suppression of tree vigour. Soil biotic conditions, specifically shifts in the soil microbiota, are assumed as a primary cause of (apple) replant disease.

By meta-analysis of replant soil microbial sequencing studies, Nicola et al. (2018) showed, that healthy soils contain distinct and more diverse microbial communities than replant soils. Among microbial genera, potentially pathogenic fungi, including *Cylindrocarpon* spp., *Rhizoctonia* spp. and oomycetes, e.g. *Phytophthora* spp., *Pythium* spp., (Manici et al., 2003; Tewoldemedhin et al., 2011a,b; Van Schoor et al., 2009), and bacteria with biocontrol or plant growth promoting potential, including *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp. (Jiang et al., 2017; Mahnkopp-Dirks et al., 2021; Mazzola et al., 2002), have been related to tree vigour suppression on replant soils. However, it is still controversial if shifts in dominance of several microorganisms, or perhaps shifts in the microbiome community structure of the bulk soil and the rhizosphere are causally linked to replant disease. In general, if reported microorganisms cause replant disease on their own or in combination is still controversially discussed, furthermore their relative importance varies between geographically distantly located replant sites (Nicola et al., 2018), indicating site-specific effects on the microbiome. Functional consequences of reported shifts in the soil microbiome in replant soil, affecting tree vigour, are not well understood (Radl et al., 2019). In addition, tree vigour suppression on replant soil is also related to soil-borne organisms, including nematodes, recently reviewed by Winkelmann et al. (2019).

The symptomatic effect of replant on tree vigour is linked to structural soil conditions. Replant sensitivity of apples differs by soil type and soil texture. Several studies state greater replant-related tree vigour suppression in light sandy soils than in heavy clay or loamy soils (Mahnkopp et al., 2018; Szczygiel & Zepp, 1998; Tewoldemedhin et al., 2011a). Tewoldemedhin et al. (2011a), for example, classify effects of replant disease on tree vigour by growth response on non-treated versus treated replant soil: low – on soils of clay and loamy texture, moderate – on soils of loamy texture and

severe – on soils of sandy texture, whereas Sheng et al. (2020) report a contrary effect of soil texture on tree vigour. At the example of replanting peach (*Prunus persica*) plant performance is shown to be linked to aggregation of replant soil (Lǔ et al., 2019; Zhang et al., 2015). Replant soil is found less aggregated, along with decreased stability of aggregate fractions. Soil structure related physico-chemical soil properties, including bulk density, poor soil structure, water logging, plus related soil chemical properties, particularly soil pH (Jonkers et al., 1980; Utkhede, 2006) are also linked to tree vigour on replant sites. As well, (micro-) nutrient imbalances in soil, due to perennial apple (monoculture) cultivation, are discussed to be linked to suppression of tree vigour (Forge et al., 2016; Simon et al., 2020; Von Glisczynski et al., 2016). However, reports about connections between soil abiotic properties and replant-related tree vigour suppression varies between study sites (e.g. (Utkhede, 2006; Willett et al., 2018). For example, Willett et al. (2018) report symptoms of replant disease less frequently in soils with low pH of 4-4.5 than with elevated pH, whereas Simon et al. (2020) report an unknown effect of pH on the growth of apple trees. The current understanding in research is, that certain abiotic factors may influence soil biotic factors, and thus influence the severity of replant disease on tree vigour (Mazzola & Manici, 2012; Winkelmann et al., 2019).

Most studies indicating soil biotic and abiotic properties linked to replant disease focus on non-treated replant soil versus treated replant soil using thermal treatment or chemical fumigation, and/ or no-replant soil. However, suppression of tree vigour is individually pronounced between apple trees within replanted production-sites (Simon et al., 2020; Tilston et al., 2018). In field study, Tilston et al. (2018) associate replant-symptomatic and non-symptomatic trees within shifts in the density of several fungi, e.g. class *Dothideomycetes*, order *Pleosporales*, suggesting for a local heterogeneously distribution of replant disease supporting agents. Physical and chemical soil properties are major reasons for the variation in soil microbial communities. The general heterogeneity of soil structure supports a high diversity of microhabitats with different physico-chemical gradients and discontinuous environmental conditions (Ditterich, 2016; Torsvik & Øvreås, 2002), even when the overall environment of the soil is constant (Nunan, 2017).

1.5 Soil-management strategies to improve tree vigour on replant sites

Management strategies based on the principle of exclusion of replanting, including plot exchange and long-term crop rotation are reliable measures to prevent effects of soil-borne disease on plant performance (Winkelmann et al., 2019). However, plot exchange is less feasible to growers and nurseries specializing in fruit trees. The location of production sites in specialised fruit growing areas, and the general intensity of land use, render plot exchange as improbable on a larger scale.

Tree placement within the orchard (Wilton, 2002) or swapping soil, as well, may not be a feasible tactic in many situations, e.g. due to field topography (Leinfelder & Merwin, 2006). In addition, high investments in orchard infrastructure, e.g. construction systems or technology for irrigation, lead to exclusion-based measures economically unattractive. From a grower's perspective the challenge is in the maintenance of economically viable production within given site.

Pre-plant soil fumigation by chemicals, e.g. methyl bromide, metam sodium or chloropicrin, is a prevailing method for soilborne pest control (Noling, 2008), it is, however, no longer an alternative measure to prevent replant disease. Chemical fumigants have a broad spectrum of activity on pathogenic microorganisms as well as non-pathogenic microorganisms alike (Noling, 2008). Environmental and safety concerns led to an international legislation prohibiting, or severely restrict in these ecologically harmful fumigants (Ajwa et al., 2010). Among other chemicals, the application of fungicides, e.g. difenoconazole or metalaxyl, has enhanced growth of apple trees on replant soils of different locations (Mazzola, 1998). The use of chemical fungicides is also disputable because of a small specificity of compounds and the need of continuous application (Winkelmann et al., 2019).

Pre-plant steam sterilisation or pasteurisation with aerated steam are environmentally safe and effective measures (Rechcigl & Rechcigl, 2018; Waschkies et al., 1993). Nevertheless, steaming is energy- and time-consuming and thus, more expensive than using chemicals (Winkelmann et al., 2019). Therefore, steaming is accepted for potted cultures, but less practical for field use (Allan & Chalton, 1981).

Alternative management options to agrochemicals focus on cultivating and biological measures, targeting on improvement of plant performance due to improving plant tolerance (Lü & Wu, 2018) or modulating the soil microbiome (Winkelmann et al., 2019).

Selecting replant disease tolerant rootstock genotypes is recognised as a promising measure to prevent replant effects on orchard performance (Rumberger et al., 2007). For example, the Geneva lines G.11 and G.31 were found less susceptible than rootstocks of the Mailing line M9 (Auvil et al., 2011; Wang et al., 2019) most widely used in commercial high-density orchards (Boomkwekerij Morren V.O.F., 2021). Less replant disease prone rootstock genotypes and popular apple varieties may be genetically mismatching. Furthermore, breeding is a lengthy process involving long growth periods and the risk of effectively being overlaid by regional and temporal phenomena (Diehl et al., 2020; Sharma et al., 2020).

Intercropping with tagetes (Yim et al., 2017) or wheat (Gu & Mazzola, 2003; Mazzola et al., 2002; Mazzola & Gu, 2000) prior to planting, as well as incorporation of organic amendments, such as Brassicaceae seed meal formulations or Brassicaceae green manure (Hanschen & Winkelmann, 2020; Mazzola et al., 2001; Mazzola et al., 2007) and (local) composts (Franke-Whittle et al., 2019; Kelderer et al., 2016; Wilson et al., 2004) target on enhancing biocontrol properties of soil. In addition, organic substances exercise an influence on soil structure and supply nutrients (Lucas et al., 2014; Van Zwieten, 2018). The combination of these amendments is reported to significantly increase aboveground tree performance on replant soils (Mazzola & Gu, 2000; St. Laurent et al., 2008; Van Schoor et al., 2008). Effectiveness of these measures appears to be formula- and soil-, respectively site-specific.

The inoculation of potential bacterial antagonists *Bacillus subtilis* (Strain EBW-4) (Utkhede & Li, 1989; Utkhede & Smith, 1992), *Enterobacter agglomerans* (Strain B8) (Utkhede & Li, 1989) *Agrobacterium radiobacter* (Catská & Taube-Baab, 1994) are found to have potential for field control of the replant disease. The application of inocula lead to improved vegetative (trunk cross-sectional area, total shoot) growth of apples in replant soils and, in terms of *Bacillus subtilis* (Strain EBW-4), even to an increase in fruit yield, for several years after planting (Utkhede & Smith, 1992), suggesting that apples may recover its natural tree vigour on replanted plantations.

After inoculation of arbuscular mycorrhizal fungi (AMF) mitigation of the severity of replant disease on tree vigour on different fruits, e.g. apple and peach (*Prunus persica*) has been reported (Taube-Baab & Baltruschat, 1993; Lǔ, Zou & Wu, 2019). Apple growth suppression is mitigated after inoculation of e.g. *Glomus mosseae* (Catská & Taube-Baab, 1994; Gąstoł & Domagała-Świątkiewicz, 2015; Ridgway et al., 2008), *Glomus fasciculatum* (Catská & Taube-Baab, 1994; Mehta & Bharat, 2013) and *Acaulospora leavis* (Ridgway et al., 2008). AMF affect and regulate soil biotic factors that are may be causally linked to replant disease, e.g. soil and root microflora (Jamiołkowska et al., 2017), as well as abiotic factors modulating the impact of replant disease, e.g. soil physico-chemical conditions (Rillig & Mummey, 2006). Nevertheless, reports are often AMF strain- and site-specific.

As yet, singular control measures aiming on a direct impact have not resulted in a reliable and transferable management strategy for remediation in different locations and settings. Due to the complex nature of replant disease an integrated pest management by combining cultivating and biological (and chemical) measures are advocated (Granatstein & Mazzola, 2001; Sharma et al., 2020). For example, Utkhede and Smith (2000) reported a significant increase in tree growth (trunk-cross sectional area) and fruit yield on replant soil treated with *Bacillus subtilis* (Strain EBW-4),

respectively *Enterobacter agglomerans* (Strain B8) combined with *Glomus intraradices*, then observed by single inoculation of the potential antagonistic bacteria. Gąstoł and Domagała-Świątkiewicz (2015) presented best productivity of replanted apple treated with a microbial consortium of bacterial strains and a variety of AMF species.

Most interventions to control replant disease, respectively mitigate the symptomatic effect of replant disease on tree vigour must be performed prior to or at planting (Bradshaw, 2016). For growers replant effects on tree vigour becomes only obvious after new root arise. Thus, growers are faced with financial challenges before knowing about the severity of replant disease and consequently its economic impact at respective site.

1.6 Definition of the problem

The replant disease on apple is a challenge to fruit growers and nurseries specialised in fruit trees, who are in general strongly tied to production locations – a problem hardly to be solved. Highly specialised and fund-intensive cultivation leads to replanting apples on the same production-sites for several times. Producers are often not aware of problems and consequences of the replant disease. Reasons include the lack of opportunity to compare tree vigour on no-replant and replant sites, lack of diagnostic and indicative methods, as well as the absence of (on-field) tools to indicate and estimate the extent and implication and thus, economic losses by replant-related tree vigour suppression.

Tree vigour suppression due to replant disease is detected as a significant variable in assessing the economic viability of orchards when replanting. Still, as long as the degree of growth and fruit yield suppression after replanting is not estimable, neither the need, nor the effect of soil management strategies, nor the risk of financial losses can be determined. From a producer's point of view, therefore, it is highly important to determine accurately indicative and quantitative parameter, as well as measures, that predict the urgency of soil management strategies on replant sites.

1.7 Research objective

The main objective is to provide a practical handling to estimate and mitigate losses of orchard performance impacted by apple replant disease, even though the causal agents of the disease itself are unknown.

For this purpose, this work has two main approaches. The first approach targets the quantification of tree vigour suppression caused by replant disease by plant- and soil-parameters. Aim of the quantification is to estimate replant impact on orchard performance. The questions addressed are:

(a) are there interlinkages between tree vigour, soil-fungal population, and soil structure under replant conditions, and (b) can tree vigour suppression caused by replant disease be estimated by assessing soil-plant interaction? The second approach targets on the mitigation of replant impact on orchard performance. Aim of the mitigation is to improve tree vigour under replant conditions. The question addressed is: (c) does an inoculum of AMF species and bacterial strains (AMFbac treatment) and the 'Müncheberger Dammkultur' (MDK treatment) effectuate tree vigour on replant sites?

1.8 Organisation of Research

The study is subdivided into an introduction (**Chapter 1**), a research sections addressing the estimation of replant impact on orchard performance and addressing the mitigation of replant impact on tree vigour (**Chapter 2**), discussion (**Chapter 3**) and conclusion (**Chapter 4**).

The research section comprises three Chapters 2.1 to 2.3. Chapters 2.1 and 2.2, analysing and estimating the site-specific impact of replant on tree vigour. To that, tree vigour is related to replant-sensitive soil parameters. In **Chapter 2.1** plant- and soil-parameters associated with replant disease are derived and the impact of replant on soil and plant is quantified. By means of soil parameters replant-sensitive time intervals over growing season are detected. In **Chapter 2.2** an algorithm for indicating replant impact on tree vigour is established. Based on an indication and quantification of replant impact on single tree level, replant impact is ecologically and economically estimated. A quantitative estimation of replant impact is performed up to field level. The methodological approach presented in Chapter 2.2 is used for impact analyses in Chapter 2.3. **Chapter 2.3** addresses the mitigation of replant impact on tree vigour by biological soil-management. Two complex soil management strategies, (a) AMFbac treatment: a microbial consortium of a variety of AMF species and bacterial strains and (b) 'Müncheberger Dammkultur' (MDK treatment): organic fertilisation with a specific mulch composition, are both tested for their potential to improve tree vigour under replant conditions. By application of soil management strategies further details of tree reaction on replant soil are disclosed.

In **Chapter 3** the results are discussed with a focus on tree vigour, concluding with **Chapter 4**.

1.9 Study sites, data analysis and statistical analysis

Following the aspiration for relevance to everyday practice studies are performed on-farm in field tests, supported by pot trials, and assisted by fruticultural experts and fruit growers specialised in commercial fruit growing industry.

Study sites

Field tests are performed on three study sites (orchard A, B and C). Orchard A and B are located in one soil-climate region in the lowland area located in north-eastern Germany (Brandenburg). In this area apple is cultivated on sandy brown, dry and diluvial Eutric Retisols (Geoabruptic, Arenic, Aric) and Geoabruptic Luvisols (Arenic, Aric, Cutanic) (according to World Reference Base for Soil Resources, WRB) (LBGR, 2021). For both orchards previous use is well documented.

Orchard A is located within the test-station for fruit cultivation 'Obstbauversuchsstation Müncheberg (OBVS)' (Müncheberg, longitude: 52.520496, latitude: 14.127071) specialising in the conservation of cultivars. On orchard A two test-sites A1 and A2, each spanning replant and no-replant soil in direct vicinity and cultivated by identical site-management and using 'Oeschberg cultivation', only differing by soil (no-replant/replant), were selected for analysis. Test-site A1 (no-replant and replant site) was set up in 2009. Apples of four different top-varieties are cultivated on understock M9. Test-site A2 (no-replant and replant site) was set up in 2010. As a characteristic for conservation and test-station various top-varieties of apple are cultivated on understock M9.

Orchard B is an intensively managed commercial fruit orchard (Altlandsberg, longitude: 52.62623, latitude: 13.804264). On orchard B two test-sites B1 and B2 were selected for analysis. Test-site B1 is spanning replant and no-replant soil, test-site B2 is spanning treated replant soil. Both test-sites are in direct vicinity and uniformly cultivated with tall spindles from apple scions ROHO 3615 EVELINA® on apple understock M9 since 2009, and only differing by soil (no-replant/replant/treated replant). On test-site B2 the 'Müncheberger Dammkultur (MDK)' was applied by time of planting.

Orchard C is located in the region 'Kehingen' northwest of Hamburg (Balje, longitude: 53.828248, latitude: 9.135356). In this district apples are cultivated on tidal marshes (Fluvisol, according to WRB). Orchard C was cultivated for several years until grubbing-up trees in the end of 1980s. The former orchard was used as crop land. Orchard C was again set up in 2020 for analysis using understocks of type A2.

Data analysis

Data are analysed for no-replant soil, (untreated) replant soil and replant soil treated by AMFbac and MDK. For soil samples soil-microbial and soil structural parameters are analysed. Abundances of soil-fungal parameters, including total fungal DNA (ITS), *Alternaria* group (Ag) and *Fusarium* are analysed using quantitative PCR (qPCR).

Root colonisation by mycorrhizal fungi is counted by grid-line intersection method. Mean weight diameter (MWD) of aggregates and densities of (aggregate) sieve-size fractions are examined using dry-sieving procedure. Densities of aggregate-stability classes are examined using dispersive treatment by wet-sieving procedure and ultrasonication.

For apple trees phenotypical data including root morphology, trunk circumference and aboveground tree area are monitored. Data of aboveground tree area is analysed by standardised photography with a software-supported photography analyser (Program Krypten, Th. Bornhaupt, Herolab GmbH) which allows for calculation of aboveground tree area based on pixel density.

Statistical analysis

Plant- and soil parameters are examined via statistical analysis. The data for tree, respectively understock vigour parameters above ground (CSA, growth rate) and below ground (root morphology), soil fungal parameters (ITS, Ag, *Fusarium*, mycorrhizal fungi) and soil structure parameters (MWD, (aggregate) sieve-size fractions, aggregate-stability) are analysed using ANOVA (analysis of variance). Significant differences between no-replant soil, (untreated) replant soil and treated replant soil (AMFbac, MDK) are calculated by post-hoc test.

For correlations between tree vigour parameters (CSA, aboveground tree area), soil fungal parameters (ITS, Ag, ITS/Ag, mycorrhizal fungi) and soil structure parameters (MWD, (aggregate) sieve-size fractions, aggregate-stability classes) Spearman's rank coefficient (ϱs) is calculated. Significant correlations ($p < .05$) are calculated using non-linear regression analysis.

A k-means cluster analysis is performed to evaluate the suppressive effect on trees in replant soils by clusters with greatest possible distinction between the combined soil-fungal and plant growth parameters (as algorithm $Q = \ln(Ag)/CSA$).

2. Results

2.1 Correlations of soil fungi, soil structure and tree vigour on apple orchard with replant soil (Paper 1)

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Abstract:

The soil-borne apple replant disease (ARD) is caused by biotic agents and affected by abiotic properties. There is evidence for the interrelation of the soil fungal population and soil aggregate structure. The aim of this study conducted between March and October 2020 on an orchard in north-east Germany was to detect the correlations of soil fungal density, soil structure and tree vigour under replant conditions in a series of time intervals. By using the replant system as the subject matter of investigation, we found that replanting had an impact on the increase of soil fungal DNA, which correlated with a mass decrease of large macro-aggregates and an increase of small macro- and large micro-aggregates in the late summer. Increased proportions of water-stable aggregates (WS) with binding forces ≤ 50 J mL^{-1}, decreased proportions of WS > 100 J mL^{-1} and a decrease of the mean weight diameter of aggregates (MWD) emphasised a reduction of aggregate stability in replant soils. Correlation analyses highlighted interactions between replant-sensitive soil fungi (*Alternaria*-group), the loss of soil structure and suppressed tree vigour, which become obvious only at specific time intervals.

Keywords: aggregate stability, *Alternaria*-group, apple replant disease (ARD), growing season, soil aggregates, tree vigour

2.1.1 Introduction

Replant disease describes a phenomenon of disturbed physiological and morphological reactions of plants after replanting crop species at sites previously used for similar crop cultures (Winkelmann et al., 2019). Replant disease has been reported for several horticultural crops, including apples, peaches and cherries in nurseries and orchards all over the world (Rani, 2008; Verma & Sharma, 1999). On apple trees, symptoms of replant disease include damaged root systems; stunted growth above and below ground; and reduced fruit yields (Mazzola & Manici, 2012; Winkelmann et al., 2019). While the direct cause of the soil-borne replant disease has not been revealed, it has been attributed to a plethora of potential biotic and also abiotic factors. Biotic factors are generally believed to be the predominate causal agents of replant disorders, since replant soils treated with soil fumigation, soil pasteurisation and soil sterilisation have shown restored regular plant growth (Mai, 1978; Mazzola, 1998; Spath et al., 2015; Yim et al., 2013). Convergence has evolved around genera of oomycetes (*Pythium*, *Phytophthora*); actinomycetes or bacteria (*Bacillus*, *Pseudomonas*); and multiple fungal species (e.g., *Cylindrocarpon*-like fungi, *Rhizoctonia*, *Fusarium*) that appear to contribute to the complex disease (Franke-Whittle et al., 2015; Manici et al., 2013; Tewoldemedhin et al., 2011b,c). However, a definite relation between sequence data and replant disease in the microbiome of replant soils has not been shown yet (Nicola et al., 2018).

Abiotic factors, on the other hand, are understood as influences regulating the extent of the symptomatic effect of replant on tree vigour, rather than a primary cause (Mazzola & Manici, 2012; Winkelmann et al., 2019). Abiotic factors include water logging, soil pH and (micro)nutrient deficiencies (Fazio et al., 2012; Utkhede, 2006; Winkelmann et al., 2019). Replant-sensitivity of apple trees has been found to differ by soil type (Fazio et al., 2012; Mahnkopp et al., 2018) and soil texture (Sheng et al., 2020; Tewoldemedhin et al., 2011a). Tewoldemedhin et al. (2011a), for example, grouped the status of the apple replant disease (ARD) by growth response in non-treated versus pasteurised replant soil: low ARD-status on soils of clay and loamy texture; moderate ARD-status on soils of loamy texture; and severe ARD-status on soils of sandy texture. Replant-related suppression of apple tree growth performance has been found individually pronounced between apple understocks and trees, respectively (Simon et al., 2020; Tilston et al., 2018). The suppression results in an uneven growth by heterogeneous distribution of more or less replant, symptomatic apple plants across replanted apple orchards. The suppression of tree vigour in apple trees, and the consequential lack of a development of best-performing trees across the orchard, leads to decreased profitability of yields that can add up to 50% throughout the life cycle of replanted orchards (Cavael et al., 2020a; Van Schoor et al., 2009).

By meta-analysis, Nicola et al. (2018) showed that the soil microbial community significantly differs under replant conditions. However, shifted microbial communities show relatively small overlaps of microbial constituents between geographically distantly located replant sites, indicating site-specific replant effects on the soil microbiome (Nicola et al., 2018). In field studies, ARD-symptomatic and non-symptomatic trees have been associated with shifts in the density of several soil fungi, including the class *Dothideomycetes*, and more specifically in the order *Pleosporales* (Tilston et al., 2018). The genetically determined *Alternaria*-group (Ag) (order *Pleosporales*, family *Pleosporaceae*) has been identified as a replant-sensitive soil fungal population which responds to replanting by abundance (Cavael et al., 2020a). The proportion of Ag on the total soil fungal population was found to be 2% in replant soil; this was found to be 10-fold greater compared to no-replant soil. Such slight shifts in the Ag population can reflect larger shifts in the soil fungal community (beyond Ag) and thus be indicative for shifts in the distribution of sieve-size fractions and aggregate stabilities.

Most microbial studies indicating replant-related or even causal agent(s) of replant disorder have focussed on homogenized soil samples. Soil microbial interactions, however, occur in habitats much smaller than those generally captured in homogenized soil cores (Bach et al., 2018). Microbial community composition is strongly mediated by soil structure (Fox et al., 2018; Nunan, 2017). The general heterogeneity of the soil structure supports a high diversity of microhabitats with different physico-chemical gradients and discontinuous environmental conditions (Ditterich, 2016; Torsvik & Øvreås, 2002), even when the overall environment of the soil is constant (Nunan, 2017). Specific microbial taxa have habitat preferences that are linked to the morphological, chemical and physical properties of the interior and exterior interfaces of soil aggregates (Büks et al., 2016; Totsche et al., 2018). In general, the proportion of fungi within soil aggregates varies within aggregate size, as a greater proportion of fungi have been associated with macro-aggregates (> 250 μm), whereas bacteria were mainly associated with micro-aggregates (≤ 250 μm) (Bach et al., 2018; Kandeler et al., 2000). The microbial community was also found to vary within and among aggregate fractions of the same soil under different management and tillage practices (Kravchenko et al., 2014; Lŭ et al., 2019).

Soil microorganisms effect the formation and stabilisation of soil aggregates, and thereby significantly involve themselves in the processes of building soil structure (Büks & Kaupenjohann, 2016; Chotte, 2005; Lynch & Bragg, 1985). Microbes release excretions, including extracellular polymeric substances, which enmesh soil particles into aggregates. Similarly, soil particles can also be enmeshed into aggregates by fungal hyphae (Helliwell et al., 2014). Fungi have been found involved in the binding of larger particles, and are predominantly responsible for stabilization of

macro-aggregates due to their hyphae structure (Six et al., 2004; Tisdall & Oades, 1982; Totsche et al., 2018). The influence of fungi and bacteria on aggregate stabilization varies widely among species and depends considerably on the nature of the available substrates (Aspiras et al., 1971). In general, fungi are better correlated with aggregate stability and lead to stronger binding forces between soil particles than with bacteria (Abiven et al., 2009).

Soil physical structures and microbial community composition shift in short timescales (weeks) depending on environmental conditions, such as (soil-)climate and related soil ecosystem conditions, e.g., soil moisture. The extents of the shifts in soil abiotic and biotic properties differ depending on the crop and the management system of the cultivation (Bach & Hofmockel, 2016; Rumberger et al., 2007; Shishido et al., 2008).

Overall, this indicates a seasonal connection between the soil fungal population and the soil structure, particularly the size and mass distribution of aggregates. Our aim was to explore this possible correlation between the soil fungal population and the soil structure in a case study for apple replant disease. This was done by analysing the sizes and mass distributions of soil sieve-size fractions, and their physical stability, and contrasting the results with the replant effects on tree vigour. For this, we analysed and compared the soils of an apple orchard where apples were cultivated on initially planted and repeatedly planted soils in the direct vicinity and under identical cultivation management. The data were collected over four time intervals in one growing season from March to October in 2018.

2.1.2 Materials and Methods

2.1.2.1 Test Site and Sampling Design

The study was conducted on an intensively managed commercial fruit orchard, located east of Berlin in the district Märkisch Oderland (Altlandsberg, longitude: 52.62623, latitude: 13.804264) in Brandenburg, a state in north-eastern Germany. On this orchard, a variety of fruit trees, including different varieties of dessert apples, are cultivated on sandy brown, dry and warm diluvial Eutric Retisols (Geoabruptic, Arenic, Aric) and physico-chemically very similar Geoabruptic Luvisols (Arenic, Aric, Cutanic) (according to World Reference Base for Soil Resources, WRB) (LBGR, 2021). Within the orchard we selected two test fields, one with initial apple cultivation (no-replant, nr) and one with repeated apple cultivation (replant, r), both in the direct vicinity of one another and identically managed. Both cultivations were set up in 2009 with tall spindles from apple scions ROHO 3615 EVELINA® cultivated on understock of M.9.

On both test areas we periodically collected data on tree vigour, and sampled soil cores to analyse soil fungal populations, starting in March 2018 with the beginning of the growing season. For the analysis, we selected three tree rows in the replant area and one row on the no-replant area. Within each row we selected a consecutive number of 18 trees. The selected trees stood in parallel with a minimum of inter-row distance of 3.5 m, in order to reduce the influence of the inherent soil-related spatial variability in soil physiochemical properties, and thus, soil microbial structure (Tilston et al., 2018). Within each row we selected three trees for the analysis of soil. This selection of planting spots for further soil analysis was based on tree vigour, so that the three selected tree spots each represented the strongest, a medium and the lowest tree vigour of the respective 18 trees in line.

We started sampling just after a strong period of ground frost (WetterKontor GmbH, 2020) up to 50.0 cm soil depth, and ended in October with vegetation dormancy. Sampling started in week 10 (5 March); was repeated in week 16 (19 April), week 25 (20 June) and in week 31 (2 August); and was last performed in week 43 (22 October). The sequence of sampling aligned with the annual growing season of the apples and the cultivation plan of the farmer.

2.1.2.2 Measurement of Tree Vigour

The trunk cross-sectional area (CSA) is a practical and robust parameter for tree vigour (Cavael et al., 2020a). Therefore, trunk circumference was measured by standard folding ruler at 40.0 cm above soil surface and a millimetre tapeline. CSA was calculated using Equation (2.1):

$$CSA = \pi/4 \times (trunk\ circumference)^2 \quad (2.1)$$

2.1.2.3 Soil Sampling

Soil cores were sampled from the top 20.0 cm of the trees, 10.0 cm distance from the tree trunk with a Puerckhauer sampler. Three soil cores were collected from each sampling point in a distance of 15.0 cm at each sampling time. The topmost 2.0 cm of each soil core were removed. Soil cores were stored at 4° C.

2.1.2.4 Size Fractionation of Soil

Soil samples were fractionised to determine the mass distribution of soil fractions across size classes and to quantify soil fungal densities per size fraction. Soil fractionation was performed with dried soil material. As the process of slowly air-drying soils changes the microbial growth and activity (Roberson & Firestone, 1992; Zornoza et al., 2007), the soil samples in a moist-field state were

dried rapidly by 70° C in a pre-heated kiln for 2 h to minimise the effect of drying by a rapid reduction of soil moisture. Soil samples were spread out in a thin layer to ensure even drying of soil material. After half time of drying, soil samples were turned and the drying procedure was continued for an additional hour.

Dried soil material was sieved by dry-sieving procedure. Bach and Hofmockel (2014) suggest that dry-sieving is a useful alternative to wet-sieving to more closely capture shorter in situ measures of seasonal and intra-annual soil microbial activity. Soil microorganisms and associated activities have been found to be sensitive to (re-) wetting events, while dry-sieving prevents cross-contamination between fractions due to 'washing' (Blaud et al., 2017) and lysis of microbes. Furthermore, different spatial domains of microbial diversity can be distinguished by patterns in the adhesive forces (Blaud et al., 2017). Wet-sieving can either enhance or diminish these adhesive forces between aggregate particles, and thus alter measured communities.

Dried soil material > 6300 μm was removed using a hand-held flat sieve. Remaining soil material was separated into six sieve-size fractions: 2000-6300, 1000-2000, 500-1000, 250-500, 125-250 and ≤ 125 μm, corresponding to Wentworth's (1922) classification scheme of soil particle sizes, which allows for a higher resolution of soil parameters than would be observed by micro (≤ 250 μm), small (250-1000 μm), medium (1000-2000 μm) and large macro-aggregates (> 2000 μm) (Wentworth, 1922). The disaggregation of less stable soil structural units due to mechanical stress (Diaz-Zorita et al., 2005) was largely avoided by applying manual sieving, thereby imitating horizontal movements at a stroke of 20 min^{-1}. Flat sieves were filled with 5 mL soil material and rotated for 3 min. Each sieve-size fraction was separately fractionised to ensure the same time of cycling of soil material on each sieve mesh and weighed. Sieved soil material was locked in bags to avoid moistening during storage at 4° C. As a concession to the analysis of the microbial community, with this method we did not separate water-stable from water-labile aggregates and non-aggregated primary particles, but measured total aggregate masses. The mass distribution of sieve-size fractions in soil was calculated by using the weighed masses of the sieve-size fractions and normalising them with respect to the total soil material sieved.

The mean weight diameter (MWD) of aggregates was calculated according to Van Bavel (1950) by Equation (2.2):

$$\mathrm{MWD} = \sum_{i=1}^{n} x_i w_i \tag{2.2}$$

where x_i is the mean diameter of any sieve-size fraction, and w_i the weight proportion of this fraction. We used the MWD as a parameter of the soil aggregation level.

2.1.2.5 Fractionation of Aggregate-Stability Classes

2.1.2.5.1 Calibration of the Ultrasonication Device

The dispersion of soil samples was performed using an ultrasonic apparatus (Sonoplus 2070 Ultraschall-Homogenisator, BANDELIN electronics GmbH and Co. KG, Berlin, Germany) with a V 70 T sonotrode (Ø 13.0 mm). The power of the device was 70 W with an oscillation frequency of 20 kHz. The cavitational action of the sonotrode (J s^{-1}) was determined by measuring the heating rate of deionized water inside a Dewar vessel (North, 1976). The calibration was performed by subjecting five replicates of 180 g deionized water to successive ultrasonications of 1, 2, 3, 4 and 5 min with the respective measurement of temperature. The performance of the ultrasonic device was then calculated following Schmidt et al. (1999) (Schmidt et al., 1999). Graf-Rosenfellner et al. (2018) demonstrated that this way of calibrating the power output creates replicable results when applied with different sonication devices and procedural details (Graf-Rosenfellner et al., 2018).

2.1.2.5.2 Dispersive Treatment

The proportions of water-stable aggregates and their stability in the face of mechanical stress were determined by consecutive applications of 0, 50 and again 50 J mL^{-1}, respectively, for wet-sieving and weighing. Wet-sieving was performed following the operator's manual (Eijkelkamp Soil and Water, Giesbeek, the Netherlands; stroke length 1.3 cm, at 34 stroke min^{-1}), which is similar to existing methods (e.g., Kemper & Rosenau, 1986). Each 3.0 g of soil material was placed into a sieve. The mesh size was always the lower limit of the sieve-size fraction tested at the time (125, 250, 500 μm). The soil samples were remoistened with deionized water by capillary action and then submerged. The soil material was wet-sieved for 3 min. Material smaller than the mesh diameter passed the sieve and was caught in stainless steel cans.

Immediately after the wet sieving procedure, the sieves, then containing only the water-stable aggregates, were placed into new cans, each filled with 70.0 g of deionized water. For subsequent ultrasonication, the sonotrode was submerged 1.5 cm into the sieves and 50 J mL^{-1} was applied. The sieves and cans were placed together into the wet-sieving apparatus and wet-sieving was repeated as described above. Ultrasonication and wet-sieving were then conducted once again. As a result, we gained disaggregated soil material of three distinct stability classes: the water-labile class (WL), the water-stable class with binding forces ≤ 50 J mL^{-1} (WS ≤ 50 J mL^{-1}) and the water-stable class with binding forces ≤ 100 J mL^{-1} (WS ≤ 100 J mL^{-1}). A fourth stability class, of water-stable aggregates with binding forces of > 100 J mL^{-1} (WS > 100 J mL^{-1}), comprised the soil sample that remained in the sieve and was not disaggregated by the procedure.

The disaggregated soil fragments were air-dried at 105° C until the weight remained unchanged by evaporation any further (> 17 h). After drying, the masses of the first three stability classes were weighed. The mass of the water-stable class with binding forces > 100 J mL^{-1} was calculated by subtracting the sum of the fragments from the original dry-weight of the sample. The procedure of disaggregation was repeated three times per sampling point and sieve-size fraction. Repeated measures were standardised for each 3 g of origin soil material.

2.1.2.6 Quantification of Soil Fungal Densities

Total DNA was extracted from 0.5 g soil according to the standard protocol of the NucleoSpin soil kit (Macherey-Nagel GmbH and Co. KG, Düren, Germany). The total amounts of purified DNA were assessed using a NanoDrop 1000 microvolume spectrophotometer following the NanoDrop ND-1000 standard protocol (Kisker Biotech GmbH and Co. KG, Steinfurt, Germany). Total fungal DNA was amplified using the highly conserved fungal rRNA gene primers ITS1F and ITS4 (Gardes & Bruns, 1993; White et al., 1990). The total fungal DNA in a sample was quantified by SYBR green fluorescence qPCR (QuantStudio 12 K flex, Applied Biosystems) using 5 µL of template DNA in a 20 µL reaction mix (qPCR HRM-mix, Solis BioDyne, Tartu, Estonia). The PCR thermal protocol consisted of an initial 15 min denaturation step at 95° C; 32 amplification cycles of 95° C for 30 s, 55° C for 30 s and 72° C for 60 s; and a final extension step of 72° C for 10 min.

For the quantification of the *Alternaria*-group, standard curves were generated based on dilution series of DNA from *Alternaria tenuissima* GH50t (efficiency > 0.91 and $R^2 > 0.998$) (culture collection of microorganisms of the working group "Fungal Interactions" at the Leibniz Centre of Agricultural Landscape Research Müncheberg). The primers and probes used for detection of Ag were as described by Grube et al. (2015), for the detection of all genetically defined species of Ag according to Lawrence et al. (2013) and Woudenberg et al. (2015). The PCR conditions were adapted to the qPCR mix (3mMMgCl2, Solis BioDyne, Tartu, Estonia (T. Müller et al., 2018)). Different strains of plant-associated fungal species were used as negative controls, as they were reference strains of *Verticillium* (CBS 130603, CBS 130339, CBS 130340, DSM 12230 and CBS 447.54), *Gibellulopsis* (CBS 747.83), *Trichoderma* spp. (St365) and *Fusarium* (Korn et al., 2011).

2.1.2.7 Statistical Analyses

The data for all soil fungal and soil structural parameters (MWD, mass distribution of sieve-size fractions, aggregate-stability classes) were analysed using ANOVA (analysis of variance) and significant differences between no-replant soil and replant soil were calculated by Games-Howell post-hoc test. $p < 0.05$ was accepted as significant.

As datasets of plant parameters and soil parameters did not follow a normal distribution, Spearman's rank correlation coefficient (ϱs) was calculated for correlations between plant and soil parameters. Significant correlations were accepted at $p < 0.05$. Subsequently, significant correlations were calculated using non-linear regression analysis. All statistics were conducted using IBM SPSS Statistics 22.

2.1.3 Results

2.1.3.1 Soil Fungal Densities in No-Replant Soil and Replant Soil

Densities of soil fungi exhibited seasonal dynamics and differed between no-replant soil and replant soil, with stronger effects by season, as demonstrated for Ag and total fungi (ITS) in Fig. 2.1. The Ag density in no-replant soil differed marginally between sampling dates from March to August, followed by a strong rise with a 70-fold increase until October. Starting from similar fungal densities on both planting areas after strong ground frosts in March, Ag density in replant soil continuously increased 21-fold following a logarithmic scale ($R^2 = 0.92$) to a maximum in October. The differing trends of Ag proportion among total fungi between no-replant soil and replant soil were found most pronounced in August ($p \leq 0.01$) and least in October (Fig. 2.2).

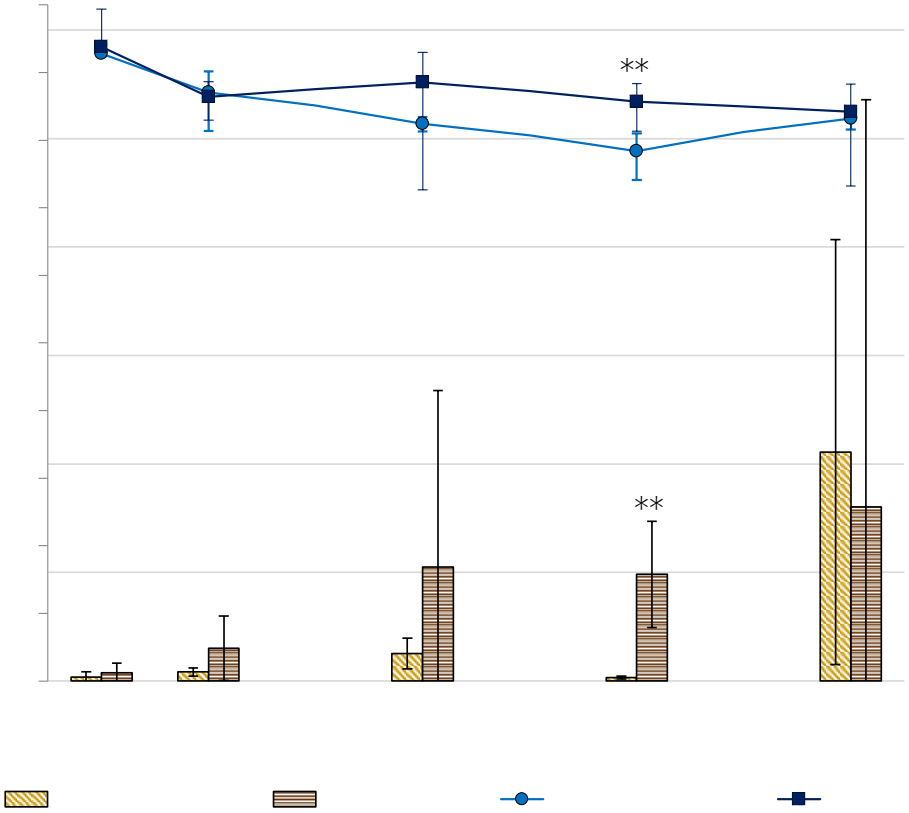

Fig. 2.1 Concentration of *Alternaria* group (Ag) (genome/g soil) and total fungal DNA (log (ITS)) in no-replant soil (nr) and replant soil (r). Significances calculated between nr and r soil. $\alpha = 0.05$, ** $p \leq 0.01$.

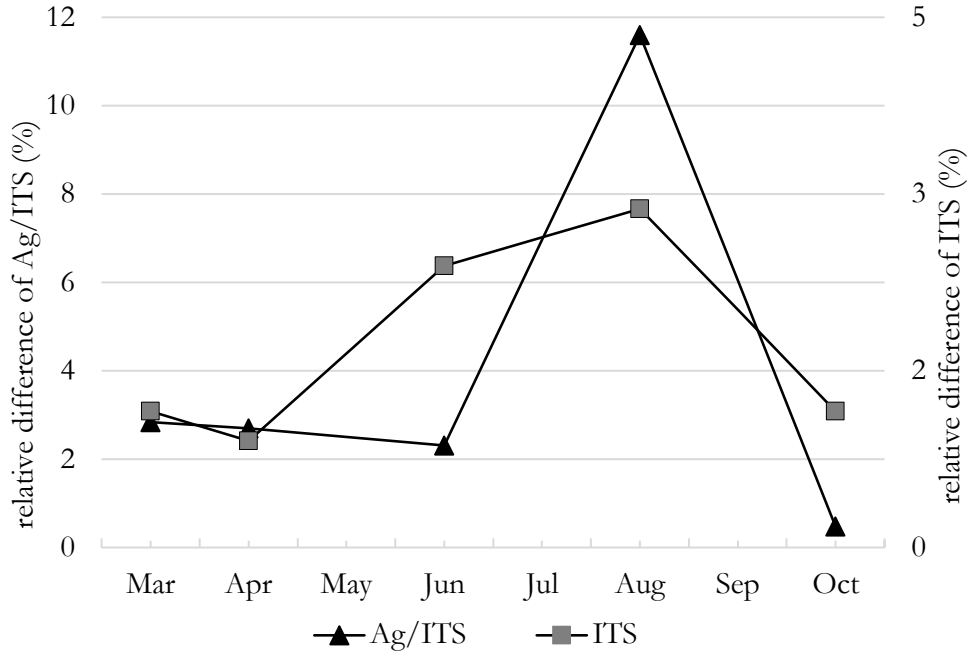

Fig. 2.2 Relative differences (%) of proportion of Ag out of total fungal DNA (Ag/ITS) and total fungal DNA (ITS) between no-replant soil and replant soil.

In replant soil, the proportion of Ag among total fungal density increased disproportionally, as the increase in total fungal-density was not compensated due to increased Ag density. Total fungal density was at a maximum in March in both soils (no-replant/replant). Density of total fungi decreased by approximately 75% from March to October in soils, resulting in similar densities in no-replant soil and replant soil in October. In no-replant soil, total fungal density decreased at an exponential rate ($R^2 = 0.50$), followed by an increase from August to October. In contrast, the total fungal density in replant soil rapidly decreased from March to April (- 66%), followed by an increase up to June (+ 36%) and a further decrease from June to October (- 46%). Different dynamics of fungal densities between no-replant soil and replant soil resulted in increased total fungal density in June ($p > 0.05$) and significantly increased density in August ($p \leq 0.01$) under replant conditions.

2.1.3.2. Aggregation Level in No-Replant and Replant Soil

The soil aggregation level varied over time and between no-replant soil and replant soil (Fig. 2.3). Both soils had the highest MWD in March; however, the aggregates tended to differ by a lower MWD in replant soil ($p \leq 0.10$). During the first half of the growing season, the aggregation level of no-replant soil decreased to a minimum in June, and in turn, increased during second half of growing season. In replant soil the soil, aggregation level was at a minimum in April, and then remained at a constant level until August, which was followed by an increase up to October. In August, no-replant soil and replant soil differed significantly due to less soil aggregation of the replant soil.

Seasonal differences in MWD were mainly driven by the 2000-6300 µm fraction. A decrease in MWD in no-replant soil during the first half of the growing season was concomitant with an increase of soil in fractions ≤ 1000 µm, whereas it was concomitant with an increase in soil in fractions from 125 to 2000 µm in replant soil. In the second half of the growing season, an increase in MWD was driven by aggregation of the 125 to 1000 µm fractions in both soils. Throughout, the mass of the 2000-6300 µm fraction was lower, and masses of the 125 to 1000 µm fractions were higher in replant soil than in no-replant soil, with an exception in April (Tab. 2.1). Significant differences in the mass distribution of sieve-size fractions between soils (no-replant/replant) were observed in August and October. In August, sieve-size fraction 2000-6300 µm was significantly decreased by 36%, whereas fractions 125-250 µm (+ 29%), 250-500 µm (+ 43%) and 500-1000 µm (+ 54%) were significantly increased under replant conditions. In October, sieve-size fractions ≤ 125 µm (+ 12%), 500-1000 µm (+ 57%) and 1000-2000 µm (+ 17%) were significantly increased in replant soil compared to no-replant soil.

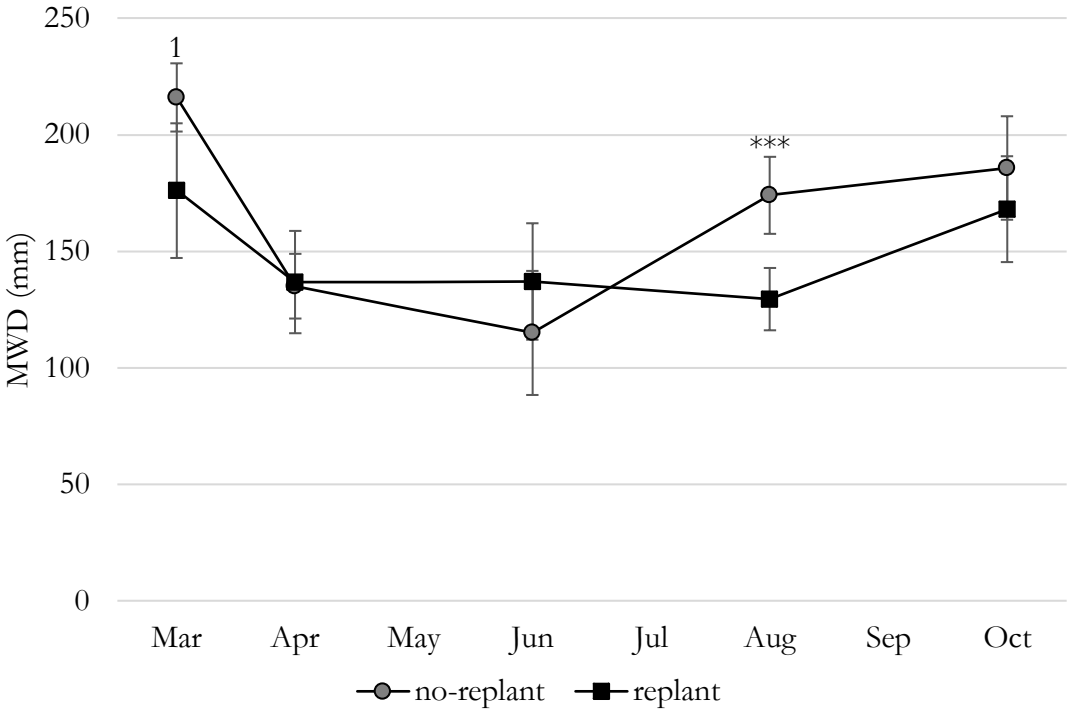

Fig. 2.3 Mean weight diameter (MWD) of no-replant soil (nr) and replant soil (r). Significances calculated between nr and r soil. $\alpha = 0.05$, *** $p \leq 0.001$, 1 $p \leq 0.10$.

Tab. 2.1 Mean mass values (g) of sieve-size fractions form ≤ 125 µm to 6300 µm in no-replant soil (nr) and replant soil (r).

Sieve-Fraction (µm)	Soil	March		April		June		August		October	
		MW	**SD**	**MW**	**SD**	**MW**	**SD**	**MW**	**SD**	**MW**	**SD**
2000-6300	nr	45.15	1.28	22.34	3.42	18.86	5.86	33.28	4.19	38.58	6.53
	r	35.93	7.14	22.39	4.82	22.92	5.96	21.24***	3.52	31.89	6.78
1000-2000	nr	11.46	4.88	13.64	1.45	10.96	2.04	13.86	1.02	8.34	3.14
	r	8.57	1.05	14.84	2.73	14.54	2.53	13.31	1.13	12.70*	2.88
500-1000	nr	6.56	2.76	15.44	1.70	12.09	0.85	9.47	2.05	6.81	2.08
	r	7.13	0.59	15.81	1.08	13.84	2.72	14.57***	1.64	11.06*	2.99
250-500	nr	7.98	7.24	15.85	0.46	14.97	0.88	10.57	1.93	6.81	0.71
	r	11.38	1.51	14.85	2.34	13.61	3.48	15.16***	1.34	10.90	2.18
125-250	nr	13.71	7.71	18.16	2.34	23.24	4.42	16.24	1.25	18.21	0.67
	r	17.88	3.35	17.54	3.23	18.97	3.20	20.89**	2.02	17.68	1.24
≤ 125	nr	15.14	7.83	14.56	1.98	19.88	3.83	16.59	2.78	18.83	2.90
	r	19.11	3.26	14.58	2.54	16.12	1.85	14.83	1.20	15.77*	1.73

Significances calculated between nr and r soil. $\alpha = 0.05$, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$.

2.1.3.3 Aggregate-Stability of No-Replant and Replant Soil

In line with the decreased aggregate size in replant soils observed in August, replant soils also showed a highly significant increase of less stable aggregates (WS ≤ 50 J mL^{-1}) with sizes < 500 µm, and a corresponding decrease of the more stable kind (WS > 100 J mL^{-1}), compared to no-replant soil (Tab. 2.2). With 74 to 97%, these two stability classes contain the bulk soil mass, whereas for WL and WS ≤ 100 J mL^{-1} neither have large masses (except for the coarse sand fraction within the 500-1000 µm fraction) nor show any significant effects.

Tab. 2.2 Mean concentration (mg) and proportion (%) of aggregate-stability classes in sieve-size fractions from 125 to 1000 µm in no-replant soil (nr) and replant soil (r) in August.

Aggregate-Stability Class	Soil	125-250 µm			250-500 µm			500-1000 µm		
		MW	SD	%	MW	SD	%	MW	SD	%
WL	nr	42	17	1	280	84	12	711	200	24
	r	58	14	2	279	60	9	693	140	23
WS ≤ 50 J ml^{-1}	nr	405	100	14	892	139	31	1203	224	40
	r	679***	81	23	1235***	75	41	1369	122	46
WS ≤ 100 J ml^{-1}	nr	58	25	2	183	82	6	71	23	2
	r	79	25	3	132	57	4	65	16	2
WS > 100 J ml^{-1}	nr	2494	95	83	1645	248	51	1015	146	34
	r	2184***	90	73	1355*	82	45	873*	30	29

Water labile aggregates (WL), water-stable aggregates with binding forces ≤ 50 J mL^{-1} (WS ≤ 50 J mL^{-1}), water-stable aggregates with binding forces ≤ 100 J mL^{-1} (WS ≤ 100 J mL^{-1}) and water-stable aggregates with binding forces > 100 J ml^{-1} (WS > 100 J mL^{-1}) in sieve-size fractions: (a) 125-250 µm, (b) 250-500 µm, (c) 500-1000 µm. Significances calculated between nr and r soil. $\alpha = 0.05$, *** $p \leq 0.001$, * $p \leq 0.05$.

2.1.3.4 Correlations between Soil Fungi and Structural Parameters

We correlated soil fungal densities in moist-field, non-sieved soil with soil structural parameters (MWD and mass distribution of sieve-size fractions), as after soil drying and dry-sieving, the quantification of soil fungal densities for sieve-size fractions did not result in reliable data. As a result, analysis showed correlations in August (Tab. 2.3). Indirect correlations between soil fungal parameters and MWD in a logarithmic scale were due to differing correlations between fungal-parameters and mass distributions of sieve-size fractions. Fungal parameters were indirectly correlated with fraction 2000-6300 µm, and with fractions from 125 to 1000 µm.

Tab. 2.3 Correlation coefficients and regression coefficients (R^2) between soil fungal parameters and MWD and mass distributions of sieve-size fractions.

Soil Structure Parameter	Fungal Parameter	Mar	Apr	Jun	Aug		Oct
	Ag/ITS	- 0.442	- 0.527	- 0.147	- 0.790**	($R^2 = 0.674$)	0.238
MWD	Ag	- 0.624	- 0.482	- 0.154	- 0.895***	($R^2 = 0.825$)	0.102
	ITS	- 0.055	- 0.082	0.154	- 0.671*	($R^2 = 0.570$)	- 0.140
	Ag/ITS	- 0.527	- 0.564	- 0.049	- 0.846		0.294
2000-6300 µm	Ag	- 0.685 ($R^2 = 0.215$)	- 0.500	- 0.140	- 0.951**	($R^2 = 0.827$)	0.137
	ITS	- 0.079	- 0.100	0.084	- 0.629*	($R^2 = 0.562$)	- 0.133
	Ag/ITS	- 0.336	- 0.136	- 0.294	- 0.280		- 0.483
1000-2000 µm	Ag	- 0.445	- 0.227	- 0.217	- 0.399		- 0.238
	ITS	0.045	- 0.327	0.154	- 0.399		0.119
	Ag/ITS	- 0.527	- 0.564	- 0.049	- 0.846**	($R^2 = 0.554$)	0.294
500-1000 µm	Ag	0.309	- 0.245	0.021	0.650*	($R^2 = 0.675$)	- 0.294
	ITS	0.027	- 0.464	0.259	0.545		0.056
	Ag/ITS	0.482	0.182	- 0.133	0.664*	($R^2 = 0.598$)	- 0.385
250-500 µm	Ag	0.770 ($R^2 = 0.021$)	0.191	0.021	0.797**	($R^2 = 0.809$)	- 0.371
	ITS	0.264	0.055	- 0.007	0.762**	($R^2 = 0.688$)	- 0.189
	Ag/ITS	0.418	0.391	0.413	0.804**	($R^2 = 0.635$)	0.154
125-250 µm	Ag	0.564	0.373	0.308	0.867**	($R^2 = 0.745$)	0.186
	ITS	- 0.036	0.227	- 0.203	0.605*	($R^2 = 0.466$)	0.168
	Ag/ITS	0.427	0.409	0.476	- 0.063		0.552
≤ 125 µm	Ag	0.664 ($R^2 = 0.007$)	0.427	0.301	- 0.126		0.406
	ITS	0.182	0.227	- 0.196	- 0.399		0.315

Significances calculated for $\alpha = 0.05$, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$.

For fractions 125-250, 250-500 and 500-1000 µm, non-linear correlations on a logarithmic scale was observed between soil fungal parameters and WS ≤ 50 J mL^{-1} and WS > 100 J mL^{-1}. Ag density was directly correlated with WS ≤ 50 J mL^{-1} in fractions 125-250 and 250-500 µm, whereas it was indirectly correlated with WS > 100 J mL^{-1} in fractions 125-250 and 500-100 µm. Total fungal-density was only directly correlated with WS ≤ 50 J mL^{-1} in fraction 250-500 µm, and indirectly correlated with WS > 100 J mL^{-1} in fractions 125-250, 250-500 and 500-1000 µm. The proportion of Ag among total fungi was only directly correlated with WS ≤ 50 J mL^{-1} and tended to an indirect correlation with WS > 100 J mL^{-1} in fraction 125-250 µm.

2.1.3.5 Correlation between Tree Vigour (CSA), Soil Fungi and Soil Structure

The mean CSA of trees on replant soil of 19.0 cm^2 was significantly lower compared to the mean CSA of reference trees on no-replant soil of 38.5 cm^2 ($p \leq 0.01$). The range of tree vigour on replant soils spanned a minimum of 5.4 cm^2 to a maximum of 33.8 cm^2. For no-replant soil, the range of tree vigour was 34.4 to 39.2 cm^2. Differences in the CSA between no-replant soil and replant soil, but also within soil variants (nr/r), were assumed to be reflected by soil fungal densities and related soil structure. Correlations were observed by a direct comparison of all data between no-replant soil and replant soil (Tab. 2.4, nr + r).

Correlation analysis of soil fungal parameters and soil structural parameters (MWD and mass distribution of sieve-size fractions) with CSA highlights the significant correlations between parameters in August (Tab. 2.4). Indirect correlations on an exponential scale were observed between Ag density, and proportion of Ag among total fungi and CSA. An exponential fitting of regression between MWD and CSA was due to direct correlation of CSA with the mass distribution of fraction 2000-6300 µm and indirect correlation with fractions 250-500 µm and 500-1000 µm.

Tab. 2.4 Spearman's rank correlation coefficient (ρs) among cross-sectional areas (CSA), soil fungal parameters, MWD and mass distributions of sieve-size fractions.

	Soil[1]	March	April	June	August	October
Ag/ITS	nr + r	- 0.109	- 0.392	0.332	- 0.818** ($R^2 = 0.77$)	0.574
	r	- 0.059	- 0.228	0.197	- 0.679	0.418
Ag	nr + r	- 0.273	- 0.469	0.056	- 0.782** ($R^2 = 0.70$)	0.420
	r	- 0.301	- 0.287	0.192	- 0.429	0.117
ITS	nr + r	0.118	- 0.255	- 0.242	- 0.370	- 0.018
	r	- 0.059	- 0.431	0.084	0.679	- 0.059
MWD	nr + r	0.483	- 0.027	0.021	0.595	0.266
	r	0.151	- 0.156	0.360	0.084	- 0.033
2000-6300 µm	nr + r	0.452	0.000	0.109	0.687* ($R^2 = 0.51$)	0.308
	r	0.101	- 0.024	0.460	0.268	- 0.117
1000-2000 µm	nr + r	0.487	- 0.328	- 0.186	0.039	- 0.447
	r	0.426	- 0.359	0.209	- 0.218	0.004
500-1000 µm	nr + r	0.494	- 0.169	- 0.613	- 0.716** ($R^2 = 0.55$)	- 0.550
	r	0.445	- 0.539	- 0.747	- 0.387	- 0.226
250-500 µm	nr + r	- 0.158	0.200	- 0.137	- 0.712** ($R^2 = 0.62$)	- 0.343
	r	- 0.244	- 0.156	- 0.510	- 0.328	- 0.076
125-250 µm	nr + r	- 0.095	0.173	0.161	- 0.487	0.028
	r	- 0.177	0.252	- 0.134	0.109	- 0.259
≤ 125 µm	nr + r	- 0.126	0.183	0.483	0.106	0.441
	r	- 0.226	0.383	0.351	0.029	0.276

[1] No-replant soil (nr) and replant soil (r), significances calculated for $\alpha = 0.05$, ** $p \leq 0.01$, * $p \leq 0.05$.

2.1.4 Discussion

In this study we investigated correlations between the dynamics of soil fungal populations and soil structure (aggregates) in relation to a gradual impact of replanting on tree vigour in a series of time intervals over one growing season; we used not-replanted and replanted soil. Our results show reduced aggregate size and stability, along with decreasing density of total soil fungal DNA (ITS) and increasing density of *Alternaria* group (Ag) for apple trees repeatedly planted on the same site, which suffered a loss of vigour. We found that the density of Ag and soil structure parameters correlate at replant-indicative time intervals – in our study observed in August.

The determination of total fungal densities highlights a replant-related effect in June and August (and shows no replant-related specific behaviour of the total fungal population in March, April or October). One replant-responsive soil fungal group, exemplary for indicating shifts in the soil fungal community, the *Alternaria* group (Ag) (class *Dothideomycetes*, order *Pleosporales*, family *Pleosporaceae*) (Cavael et al., 2020a), continuously increased its density in replant soil over the growing season, resulting in a distinct difference of Ag density between soils in August. On the same site, the Ag was found to be replant-indicative by density of soil fungal population two years earlier (2019) (Cavael 2020a). However, in 2016 an increased Ag density under replant conditions was observed in April with a two-fold greater Ag density in no-replant soil and a four-fold greater Ag density in replant soil as compared to Ag densities found in April 2018. Inter-annual variations have also been reported for the date of maximum growth difference between treated and non-treated replant soils, and for the effect of soil treatments regarding combating replant-affecting soil microbes (Hoestra, 1968).

The formation of aggregates exhibits different dynamics between replant soil and no-replant soil during the growing season. While the degree of aggregation follows similar patterns, the aggregate formation process is changed under replant conditions. The steady degree of aggregation between April and August suggests that aggregate turnover processes are prevented over summer under replant conditions. A decreased aggregation of replant soil has previously been reported for the replanting of peaches (*Prunus persica*) (Lǔ et al., 2019; Zhang et al., 2015). Concomitant with our results, the authors showed that the replant-specific low aggregation was due to a decreased proportion of fraction 2000-6300 µm and increased proportions of fractions 125-250, 250-500 and 500-1000 µm under replant conditions.

Our results show that fractions from 125 to 1000 µm and fraction 2000-6300 µm are replant-sensitive. In contrast, fractions ≤ 125 µm and 1000-2000 µm are replant-inert. The measurements of the mass distribution of sieve-size fractions highlighted differences in the composition of soils

regarding soil structures (aggregates) in the replanted and initial planting area, which are significantly pronounced in August and less strong in October. Our indications of replant-sensitive sieve fractions in size ranges of fine sand (125-250 µm), medium sand (250-500 µm) and coarse sand (500-1000 µm), though not fine sand to silt and clay (≤ 125 µm), are consistent with several studies that state greater replant-related tree vigour suppression in light sandy soils as compared to heavy clay or loamy soils (Fazio et al., 2012; Mahnkopp et al., 2018; Szczygiel & Zepp, 1998; Tewoldemedhin et al., 2011a). This result implies that aggregated particles in size range of small and large macroaggregates (sands) may perform as alternative microhabitats for increased densities of soil fungi.

According to our results, the mass distributions of fractions from 125 to 1000 µm, and fractions 2000-6300 µm are linked to increases of soil fungal densities (Ag) in August. The decrease of soil in fractions 2000-6300 µm and the increase of soil in fractions from 125 to 1000 µm, correlate with an increase in the density of soil fungi (Ag) under replant conditions. This observation is supported by a distinct, though not significant replant-specific behaviour of soil fungi also observed in June, but this could not be related to any change in the mass distributions of sieve-size fractions at that time of the year. Nevertheless, the observations suggest an interaction between soil fungi (Ag) and the formation of soil structures (aggregates) during summer. Soil fungi, in particular filamentous fungi, have a well-documented impact on soil structure by formation or disintegration of aggregates, especially of macroaggregates (> 250 µm) (Lehmann et al., 2017, 2020). The relevance of the correlation analysis is the consideration of potential interactions between soil and the apple under replant conditions over time. The ecology of the soil fungal populations and their association with the soil structure may be the next step in understanding causal interlinkages related to replant disease. For this purpose, we understand our case study as a first step that needs to be further tested by annual duplication in repeated studies in the field.

Wet-sieving and ultrasonication highlighted an increased concentration of less stable soil structures in fractions from 125 to 1000 µm in replant soils in August. Less stability of fractions 250-5000, 500-1000 and 2000-4000 µm, though not in fraction 1000-2000 µm, has previously been reported for the replanting of peaches (*Prunus persica*) (Zhang et al., 2015). Other observations of replant-specific proportions of aggregates with differing stability, notwithstanding 2 h sterilisation due to autoclaving (Lǔ et al., 2019; Zhang et al., 2015), showed a high persistence of aggregate structures under replant conditions, even under extreme abiotic conditions. Persistence of water-stable aggregates for decades to centuries was already proven by Jastrow (1996). The potential persistence of a replant-sensitive aggregate stability class of WS ≤ 50 J mL^{-1} could contribute to the strong

persistence of replant-effects that have been observed also after grubbing and irrespective of catch crops (Van Marle, 1961).

Aggregate-disintegrating processes in smaller and less stable aggregates have been found at a high Ag density. Increased Ag density is linked to less stable aggregates and tree vigour suppression in replant soil. The correlation between Ag density, soil structure and tree vigour means that replant-effects can pass unnoticed for most of the vegetation period and become obvious only in specific time intervals. This is relevant for monitoring replant effects by parameters of the soil.

A steady increase of replant-effects on soil parameters in the summer season may potentially match with the sensitive stage of apple nutrition by root performance. For apple understock M.9, steadily increased growth of root has been reported from June until August (Eissenstat et al., 2006; Psarras et al., 2000). Root flush has also been reported around bloom (Eissenstat et al., 2006), approximately in late April to May in Germany (Kaspar et al., 2015), in line with replant-specific increased Ag density observed in April 2016 (Cavael et al., 2020a). Interestingly, the soil parameters' return to a similar density as was determined at the beginning of the growing season in March before the beginning of dormancy season in October, and then did not differ between no-replant soil and replant soil anymore. This suggests that a replant-effect based on a shifted quantitative composition of soil fungal population is in competition with root growth in soil and de facto diminishes tree vigour by an offset of ontogenetic development, probably due to seasonally limited access to nutrients.

Our observations suggest that differences between replant and no-replant soils are pronounced, but may occur at irregular intervals. This in turn would mean that continuous and densely gridded monitoring of soil (and plant) during the whole growing season of apple would be necessary to detect indicative parameters for apple replant disease. It also shows that one-time sampling of orchard test sites and homogenised soil samples taken at few times only can be misleading in detecting interactions of soil fungi and soil structure (and tree vigour), depending on the time of sampling.

An analysis of interrelations between soil fungi, soil structure and apple tree vigour (suppression) will require continuous and densely gridded monitoring of soil to detect replant effects at indicative time intervals, and needs to be performed on single planting spots rather than with homogenized soil samples.

2.1.5 Conclusions

Soil structure was found to be replant-sensitive by mass distribution of large and small macroaggregates (2000-6300 µm, from 250 to 1000 µm) and large microaggregates (125-250 µm). Small macroaggregates and large microaggregates are less stable under replant conditions. The statistical analyses suggest that specific replant-responsive soil fungi, here the *Alternaria* group (Ag), are involved in replant-related changes in soil structure. Hence, replant-specific aggregate-disintegrating processes seem to be related to densities of soil fungi. A correlation between soil fungi and structure can only be detected at specific time intervals over the growing season. Pronounced differences in soil structure between no-replant soil and replant soil occur together with a selective growth of Ag densities in late summer.

The density of replant-responsive soil fungi (Ag), in particular, is highly correlated with the plant reaction of trees in replant soil, so we conclude that the replant effect is a biologically active process. On the one hand, changes in soil structure contribute to the functional conditions for growth of specific soil fungi, and on the other hand, soil fungi may be involved in the formation of less stable soil aggregates. Our study suggests that the interaction between soil fungi and soil aggregates may be causally linked to interrelations between replant soil and plants.

An analysis of the interrelations between soil fungi, soil structure and apple tree vigour (suppression) will require continuous and densely gridded monitoring of soil to detect replant effects at indicative time intervals, and needs to be performed on single planting spots rather than with homogenised soil samples. In an applied context of the restoration of replant soil, our results provide the first indication that a potentially negative effect of the Ag on soil structure could be managed by good soil aggregators, e.g., mycorrhiza, to restore soil structure under replant conditions.

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Conflicts of Interest: The authors declare no conflict of interest.

2.2 Assessment of growth suppression in apple production with replant soils (Paper 2)

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Abstract:

Apple replant disease (ARD) is a specific apple-related form of soil fertility loss due to unidentified causes and is also known as soil fatigue. The effect typically appears in monoculture production sites and leads to production decreases of up to 50%, even though the cultivation practice remains the same. However, an indication of replant disease is challenged by the lack of specification of the particular microbial group responsible for ARD. The objective of this study was to establish an algorithm for estimating growth suppression in orchards irrespective of the unknowns in the complex causal relationship by assessing plant-soil interaction in the orchard several years after planting. Based on a comparison between no-replant and replant soils, the *Alternaria* group (Ag) was identified as a soil-fungal population responding to replant with abundance. The trunk cross-sectional area (CSA) was found to be a practical and robust parameter representing below-ground and above-ground tree performance. Suppression of tree vigour was therefore calculated by dividing the two inversely related parameters, $Q = \ln(Ag)/CSA$, as a function of soil-fungal proportions and plant responses at the single-tree level. On this basis, five clusters of tree vigour suppression (Q) were defined: (1) no tree vigour suppression/vital (0%), (2) escalating (- 38%), (3) strong (- 53%), (4) very strong (- 62%), and (5) critical (- 74%). By calculating Q at the level of the single tree, trees were clustered according to tree vigour suppression. The weighted frequency of clusters in the field allowed replant impact to be quantified at field level. Applied to a case study on sandy brown, dry diluvial soils in Brandenburg, Germany, the calculated tree vigour suppression was - 46% compared to the potential tree vigour on no-replant soil in the same field. It is highly likely that the calculated growth suppression corresponds to ARD-impact. This result is relevant for identifying functional changes in soil and for monitoring the economic effects of soil fatigue in apple orchards, particularly where long-period crop rotation or plot exchange are improbable.

Keywords: orchard management, trunk cross-sectional area, *Alternaria* group, apple production, soil fatigue, apple replant disease

2.2.1 Introduction

Intensive monoculture production involves the continuous replanting of crops at the same location. The consequence is negative plant-soil feedback, compromising the health and sustainable maintenance of production systems due to shifts in the microbial community composition below ground, which are often accompanied by soil nutrient depletion, accumulation of soil-borne pathogens, and the release of phytotoxic and autotoxic compounds during the decomposition of crop residues (Cesarano et al., 2017). The resulting loss in productivity and yield is known as soil fatigue (Wolińska et al., 2018), soil sickness (Cesarano et al., 2017) or replant disease (Nicola et al., 2018).

Although annual crops can be integrated into crop rotation cycles and plot exchange to avoid the impacts of replanting, the fruit orchard production system is particularly affected, as it is characterised by long life cycles of a plantation and long-term maintenance of permanent production sites. Specific apple replant disease (ARD) is found in mother plant plantations, plantations for the cultivation of rootstocks, and plantations for line-out after grafting as well as in orchards. The effect of suppressed growth has been reported in fruit-growing regions worldwide, and it is characterised by alterations in root structure, stunted and uneven growth and an overall reduction in biomass. Indeed, ARD has been found to suppress vegetative and generative performance of apple orchards by up to 50%, to reduce fruit size by up to 10% and to delay the bearing of fruit on trees by 2-3 years (Mazzola, 1998; Mazzola & Manici, 2012; Nicola et al., 2018).

The complex aetiology has been found to be mainly caused by an imbalance between fungi and soil-borne pathogens in the rhizosphere (Franke-Whittle et al., 2015; Mazzola, 1998; Spath et al., 2015; Yim et al., 2013) and to be influenced by certain prevailing environmental conditions such as rootstock or soil treatment (Nicola et al., 2018). Nonetheless, the exact causal determinants have not yet been identified (Tilston et al., 2018; Winkelmann et al., 2019).

In the absence of in-field studies and quantification methods, orchardists are generally not aware of the extent of underperformance in established orchards. To date, comprehensive studies of quantified economic losses or yield decline have not been undertaken beyond the field level, remaining in a stage of reflection on the interaction based on practice (e.g., (Brown et al., 2000; Geldart, 1994). One main hindrance orchardists face in managing ARD is that above-ground replant effects are extremely difficult to identify in the field unless trees on no-replant soil are

present for direct comparison. Effects are most obvious in the first three to five years of cultivation and become almost invisible to the eye with the increasing age of the trees (Rumberger et al., 2007). Growth decline due to ARD is indicated, e.g., by the sum of shoot lengths, shoot dry weight or fine root numbers (Braun et al., 2010; Kelderer et al., 2012; Manici et al., 2013). Alterations in the morphological root structure such as the browning of roots and root tip necrosis have been observed shortly after replanting (Grunewaldt-Stöcker et al., 2019; Lucas et al., 2018), accompanied by inhibited root growth, reductions in growth rate and biomass, and changes in micronutrients (Simon et al., 2020). With no practical method of above-ground screening, an estimation of yield reduction is extremely difficult for orchardists and thus present a challenge to estimating the economic effects of replant for improved orchard management or risk assessment before replantation.

The economic impact of replant effects on apple production sites is of increasing relevance in regions that produce fruit in concentrated areas with limited possibilities for crop exchange due to intensive land use. This situation is exacerbated by a progressive ban of pre-plant chemical fumigation on the grounds of its destructive impact on environment and health (G. S. Brown et al., 2000). Next to management strategies based on the principle of exclusion (e.g., plot exchange, planting in the interrow), producers lack suitable strategies to overcome the effects of replant. Therefore, approximating the underlying process of replant disease by an assessment of symptoms is necessary for estimating ecologic and economic effects, for developing more precise management methods and for monitoring the long-term sustainability of orchard systems.

The trunk cross-sectional area (CSA) is widely used for a practical determination of growth performance in apple trees in field, as it corresponds to root and crown volume, leaf area and yield (Lepsis & Blanke, 2004; Lo Bianco et al., 2012; Westwood & Roberts, 1970). Since replant-related effects are expressed by a depression of vegetative performance, CSA is affected but less directly dependent on orchard management, such as crown pruning. Dendrochronological analyses have shown that the CSA reflects environmental conditions, weather and cultivation methods (Schweingruber, 2012). Therefore, the exact expression of the correlation to above-ground tree area and yield must be validated for agro-climatic conditions, production system and understock-cultivar combination (Lepsis & Blanke, 2004).

Treatments of ARD such as soil disinfection by chemical fumigation (Gur et al., 1991; Hoestra, 1968; Mai & Abawi, 1978; Pitcher et al., 1966) chloropicrin (Spath et al., 2015), disinfection (J. E. Jackson, 1979) or gamma radiation (Yim et al., 2013) provide evidence that replant disease is caused by biological agents in the soil. Changes in the abundance of multiple fungal species including root

endophytic fungi, e.g., *Cylindrocarpon*-like fungi, *Rhizoctonia* and *Fusarium* have been found to correlate with ARD-related plant growth impediments in orchards in central Europe (Franke-Whittle et al., 2015; Manici et al., 2013). Among microflora, different genera of oomycetes (*Pythium*, *Phytophthora*), actinomycetes or bacteria (*Bacillus*, *Pseudomonas*) have been assigned a role in the ARD complex (Tewoldemedhin et al., 2011b,c). However, the relative importance of these organisms varies between study sites.

Nicola et al. (2018) have called for a united standard for reporting soil parameters to account for co-occurrences between environmental impacts microbial interactions with host plants are observed by bio-tests that enable detection of several stages of disease through suppression of vegetative plant performance under different soil treatments (e.g., disinfectants or gamma radiation) (Yim et al., 2013) but not as a function of the condition of the soil. Furthermore, conducting bio-tests under controlled conditions generates high uncertainty regarding the prediction of plant responses in the field, and thus the results are less valid for field conditions (Merwin et al., 2001).

Approaches based on metagenomics have been appropriated for agro-ecosystems when a reductionist approach does not result in clear-cut answers (Cesarano et al., 2017; Wolińska et al., 2018). However, a relation between sequence data and ARD in the microbiomes of replant soils could not be shown (Nicola et al., 2018). Tilston et al. (2018) recently stated that the structural differences of microbial communities in soils replanted with trees – some carrying symptoms, some not – are more subtle than previously thought, suggesting that population abundances rather than the diversity of soil-fungal communities may be causally linked to replant disease.

For low pronunciations of microbial abundance, an approach inspired by eco-typing has been used to analyse genetically determined groups of species. This was proven, e.g., for the *Alternaria* group, re-arranged by sequence data (Fierer et al., 2005; Fraser et al., 2009; Jangid et al., 2008; Lanza et al., 2016; Philippot et al., 2010; Von Mering et al., 2007). Changes in abundance are sensitive and reproducibly detectable, as shown for *Fusarium* and *Alternaria* group (T. Müller et al., 2018). Under field conditions, this approach requires a selection of test sites with identical cultivation management differing only in terms of soil (replant/no-replant).

The objective of this study was to establish an algorithm for estimating growth suppression linked to replant-related effects in an orchard site under real production conditions. This was done using a responsive replant-sensitive fungi group and CSA for a comparison of trees cultivated in the direct vicinity on no-replant and replant soils under identical cultivation management.

2.2.2 Methods

2.2.2.1 Experimental design

We selected two orchards (A, B) on sandy brown, dry and warm diluvial soils in a lowland area located in Brandenburg, a state in north-eastern Germany. Brandenburg has approximately 900 ha of commercial apple production area, with an average of 24.1 t/ha yield in 2011 to 2016 (Amt für Statistik Berlin-Brandenburg, 2017).

Orchard A (Altlandsberg, longitude: 52.626263, latitude: 13.804264) was a commercial production site established in 2009 and planted with tall spindles from apple scions of Malus × domestica ROHO 3615 EVELINA® cultivated on understocks of M9 on no-replant and replant soils in direct proximity. For the analysis, we selected four plots of 18 trees each on no-replant soil (Plots A1.1 to A1.4) and four plots of 18 trees each on replant soil (Plots A2.1 to A2.4).

Orchard B (Müncheberg, longitude: 52.520496, latitude: 14.127071) was located within a test-station for apple cultivation specialising in the conservation of cultivars. As is characteristic for a conservation and test-station, B1 to B3 contained various top-varieties of apple. All were cultivated on the understock A2 using 'Oeschberg cultivation'. B4 contained 32 trees of the 'Granny Smith' top-variety cultivated on understock M9 (B4.1) followed by 32 trees of 'Elstar (rot) Michielsen'® top-variety cultivated on understock M9 (B4.2) planted on no-replant soil in 1988. This was followed by 32 trees of 'Braeburn Schneider'®/M9 (B4.3) and 'Braeburn Lochbuie'®/M9 (B4.4) planted in 2001 on replant soil. An overview of the experimental settings is presented in Tab. 2.5.

Tab. 2.5 Overview of the experimental design.

Plot	Year	Cultivar	No. of trees	Initial soil	Management
Orchard A: Commercial Plantation					
A1.1	2009	Evelina®/M9	18	No-replant	
A1.2	2009	Evelina®/M9	18	No-replant	Tall-spindle
A1.3	2009	Evelina®/M9	18	No-replant	cultivation
A1.4	2009	Evelina®/M9	18	No-replant	
A2.1	2009	Evelina®/M9	18	Replant	
A2.2	2009	Evelina®/M9	18	Replant	Tall-spindle
A2.3	2009	Evelina®/M9	18	Replant	cultivation
A2.4	2009	Evelina®/M9	18	Replant	

Plot	Year	Cultivar	No. of trees	Initial soil	Management
Orchard B: Experimental and Conservation Test-Station					
B1	1984	Various/A2	16	No-Replant	Oeschberg cultivation
B2	2009	Various/A2	32	Replant	
B3.1	2010	Various/A2	72	No-Replant	
B3.2	2010	Various/A2	159	Replant	
B4.1	2001	Granny Smith/M9	32	No-Replant	Tall-spindle cultivation
B4.2	2001	Elstar (rot) Michielsen®/M9	32	No-Replant	
B4.3	2001	Braeburn Schneider®/M9	32	Replant	
B4.4	2001	Braeburn Lochbuie®/M9	32	Replant	

2.2.2.2 Selection and measurement of plant growth-related parameters

We selected two plant growth-related parameters and one soil-fungal parameter based on the background literature summarised in the introduction. All samples and measurements were taken in April 2016 in orchard A and in February/March 2016 in orchard B:

- above-ground tree area – indirectly related to the tree crown and productive leaf mass
- cross-sectional area of the tree trunk – as a function of the trunk circumference and related to tree vigour and potential yield
- total soil-fungal DNA, *Fusarium* and *Alternaria* group (Ag) abundance.

2.2.2.3 Above-ground tree area

The above-ground tree area was sampled by photographic imaging by projecting the volume of the tree above the surface of the ground to a unit area [Nikon D800; 1/800 s exposure times, ISO-200 film speed, focal length 28 mm, 300 dpi × 300 dpi, 24-bit colour depth]. Images were cropped to a standardised unit area of 20.0 × 16.0 cm showing the tree stem from the soil surface upwards. The number of pixels in defined colour ranges was determined using the program Krypten (Herolab GmbH Laborgeräte, Wiesloch, Germany): positive (= tree area), negative (= background), unassigned (= disorders: positive - negative overlap, ≤ 5%).

2.2.2.4 Trunk cross-sectional area (CSA)

The trunk circumference of a single tree was measured using a standard folding rule at 40.0 cm above the soil surface and a millimetre tapeline. For each trunk circumference, the trunk cross-sectional area (CSA_{40}) was calculated by $CSA = \frac{\pi}{4} * (trunk\ circumference)^2$. (2.3)

The highest values of Spearman's rank correlation coefficient were between the CSA at 40.0 cm above the soil surface (CSA_{40}) and the tree area ($\rho s = 0.786\text{-}0.949$), which was thus significantly independent of the tree age, type of top-variety and understock, and cultivation on no-replant or replant soil.

2.2.2.5 Abundance of soil-fungal populations

Soil cores were sampled in 10.0 cm intervals to a depth of 60.0 cm from the soil surface at 10.0 cm from the tree trunk, one for each tree across the test plots and rows (A1-2, B1-4). This was conducted using a standard Puerckhauer soil sampler 100.0 cm in length and 5.0 cm in diameter. The cores were stored at 4° C.

Total DNA was extracted from 0.5 g soil using the NucleoSpin soil kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The total amounts of purified DNA were assessed using a NanoDrop 1000 micro-volume spectrophotometer following the NanoDrop ND-1000 standard protocol (Kisker Biotech GmbH & Co. KG, Steinfurt, Germany).

Total fungal DNA was amplified using the highly conserved fungal rRNA gene primers (ITS1F CTTGGTCATTTAGAGGAAGTAA and ITS4 TCCTCCGCTTATTGATATGC) previously described (Gardes & Bruns, 1993; White et al., 1990). Quantification of total fungal DNA in a sample was determined by SYBR green fluorescence qPCR (QuantStudio 12 K flex, Applied Biosystems) using an external standard curve. PCRs (20 µl) contained 5 µl of template DNA qPCR HRM-mix (3 mM MgCl2, Solis BioDyne, Tartu, Estonia) 100 nM of ITS1F primer, and 500 nM ITS4 primer. The PCR thermal protocol consisted of an initial 15 min denaturation step at 95° C, 32 amplification cycles of 95° C for 30 s, 55° C for 30 s, 72° C for 60 s, and a final extension step of 72° C for 10 min.

For the quantification of the *Alternaria* group, standard curves were generated based on dilution series of DNA from *Alternaria tenuissima* GH50t (efficiency > 0.91 and $R^2 > 0.998$). The fungus was stored in the culture collection of microorganisms of the working group 'Fungal Interactions' at the Leibniz Centre of Agricultural Landscape Research Müncheberg. The primers and probes used for detection of Ag were as described by Grube et al. (2015), as follows (5'-3'):

AltF (forward) TCTTTTGCGTACTTCTTGTTTCCTT); AltR (reverse) TTACTGACGCTGA TTGCAATTACA); AltP (probe) TGGGTTCGCCCACCACTAGGACA).

The PCR conditions were adapted to the qPCR mix (3 mM MgCl2, Solis BioDyne, Tartu, Estonia) and a two-step PCR: 10 min at 95° C followed by 45 cycles of 95° C for 15 s and 64° C for 45 s. In silico tests of DNA sequences (software package DNA star, DNASTAR, Inc., Madison, WI, United States) resulted in the detection of all genetically defined species of Ag according to Lawrence et al. (2013) and (Woudenberg et al., 2013, 2015).

The detection of *Fusarium* species was based on the region between primers Fa+7 and Ra+6 of the translation elongation factor gene TEF1 (Karlsson et al., 2016). Probe and primers were selected using the software package DNA star (DNASTAR, Inc., Madison, WI, United States): S FUS pl probe 50-CAATAGGAAGCCGC T GAGCTCGG TAAG GGTTC-3, Fa pl3 (forward) 5'-TACCCCGCCACTCGAGCG-3', FUS pl (reverse) 5'-TTGAGCTTGTCAAGAACCCAGGCG-3'. The PCR cycles included 95° C for 10 min followed by 45 cycles of 95° C for 15 s, 65° C for 20 s and 72° C for 30 s (T. Müller et al., 2018).

In the *Alternaria*-specific qPCR, *Fusarium* strains were used as negative control and vice versa. A set of strains was selected from *A. tenuissima* (GST09t, GH50t, and At220) and *A. alternata* (GST37a) (Kahl et al., 2015; M. E. H. Müller et al., 2012) as well as *Fusarium* strains of the species *F. graminearum* (Fg23 and Fg486) and *F. culmorum* (Fc13 and Fc493) partly characterised (Fc13 and Fg23) by Korn et al. (2011). Different strains of plant-associated fungal species were used as a negative control: reference strains of *Verticillium* (CBS 130603, CBS 130339, CBS 130340, DSM 12230, and CBS 447.54), *Gibellulopsis* (CBS 747.83), *Trichoderma* spp. (St365), and *Fusarium* (Korn et al., 2011).

2.2.2.6 Statistical analysis

A tree-specific value for growth suppression was determined by dividing the tree-specific ln(Ag) by the tree-specific vegetative plant performance, as represented by CSA_{40}, relating tree vigour suppression for the single tree: $Q = \ln(Ag)/CSA_{40}$.

A k-means cluster analysis was performed to evaluate the suppressive effect on trees in replant soils by clusters with greatest possible distinction between the combined soil-fungal and plant growth parameters (as algorithm Q). As the data did not follow normal distribution, Spearman's rank correlation coefficients (ϱs) were calculated for the total data set ($n = 375$). Significant correlations were accepted at $p < 0.05$. The analyses were conducted using IBM SPSS Statistics 22.

2.2.3 Results

2.2.3.1 Correlation between plant growth related parameters

The CSA measured 40 cm above the soil surface was employed as a sensitive plant physiological parameter based on significant high correlations between CSA_{40} and the above-ground tree area. The correlation between CSA_{40} and the above-ground tree area across all trees of orchard B (B1, B2, B3 and B4) showed a logarithmic progression between both plant growth parameters, with an increase in the above-ground tree area for each increase in CSA_{40} ($\varrho s = 0.892$) (Fig. 2.4). The increase in CSA_{40} plateaued towards a maximum at a relationship of 200 cm^2 to 200 thousand pixels/unit area. The differences in tree age had little effect on the correlation between CSA_{40} and the above-ground tree area. Although the planting years differed by 26 years, the vegetative plant performance of single trees overlapped within a range of CSA_{40} from 150 to 220 cm^2 and the above-ground tree area from 140 thousand to 200 thousand pixels/tree area.

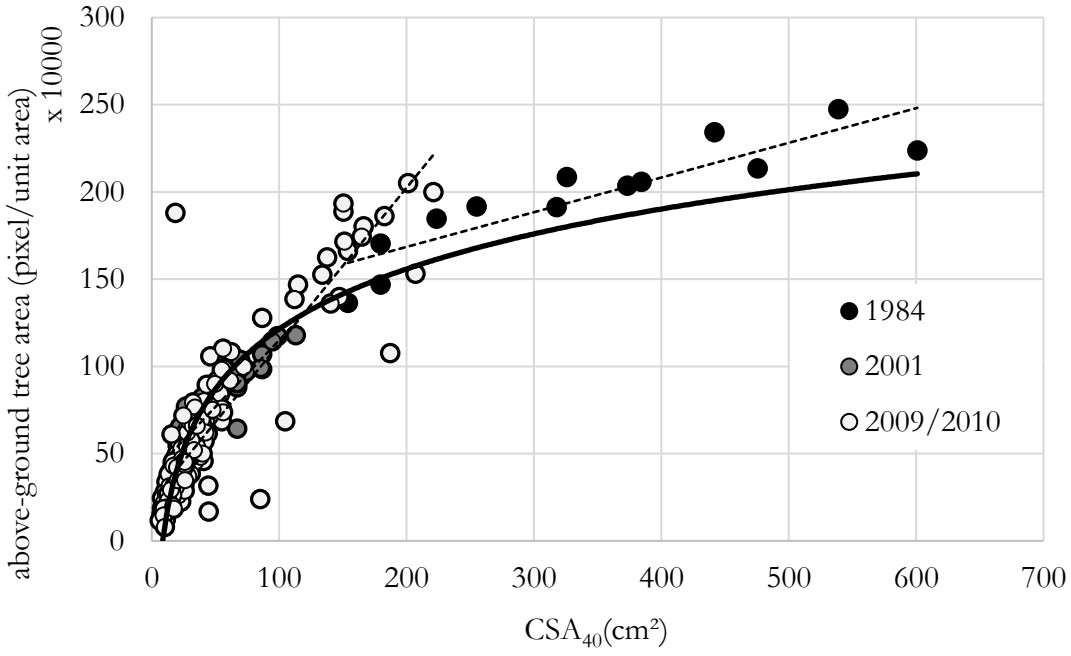

Fig. 2.4 Correlation between CSA_{40} (cm^2) and the above-ground tree area according to tree age.

The cultivation of trees had no effect on the correlation coefficient between CSA_{40} and the above-ground tree area. Trees cultivated on no-replant soil and replant soil showed a similar correlation coefficient ($\varrho s = 0.613$, respectively $\varrho s = 0.676$) (Fig. 2.5). There was a high correlation between both plant growth parameters across all top-varieties ($\varrho s = 0.894$).

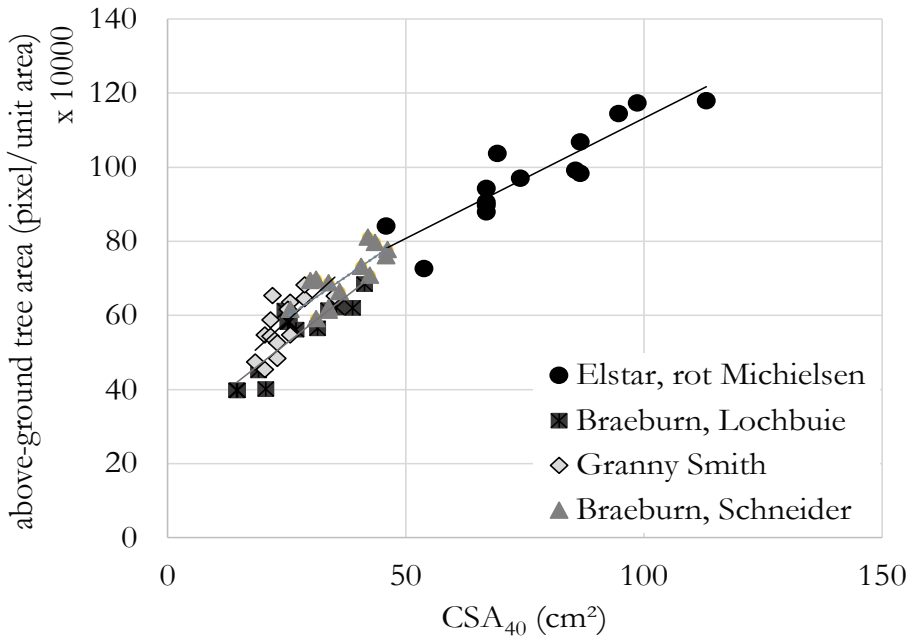

Fig. 2.5 Correlation between CSA_{40} (cm^2) and tree area depending on the type of top-variety.

2.2.3.2 Comparison of parameters related to plant growth on no-replant and replant soils

The mean CSA_{40} of the trees cultivated on no-replant soil was significantly higher compared to the trees cultivated on replant soil ($p \leq 0.001$). With a mean CSA_{40} for no-replant soil of 24.1 cm^2 compared to 14.5 cm^2 for replant soil, the difference was 50%. A shift to a lower mean CSA_{40} was observed, which was linked to a 50% reduction in the range of variation; this shifted from 40.1 cm^2 on no-replant soil to 19.3 cm^2 on replant soil. Similarly, the CSA_{40} for no-replant soil showed a coefficient of variation of CV = 37.4% compared to the much less distributed CV = 27.4% for replant soil. For no-replant soil, as well as for replant soil, the distribution of CSA_{40} was based on the strong heterogeneity of CSA_{40} for trees within direct vicinity. Thus, a thick tree could stand beside a very weak tree without a trend of decreasing or increasing CSA_{40} along rows in an orchard. A stable mean of CSA_{40} was given by 18 trees. The frequency distribution of trees sorted according to CSA_{40} for no-replant and replant soils is shown below for orchard A (Fig. 2.6).

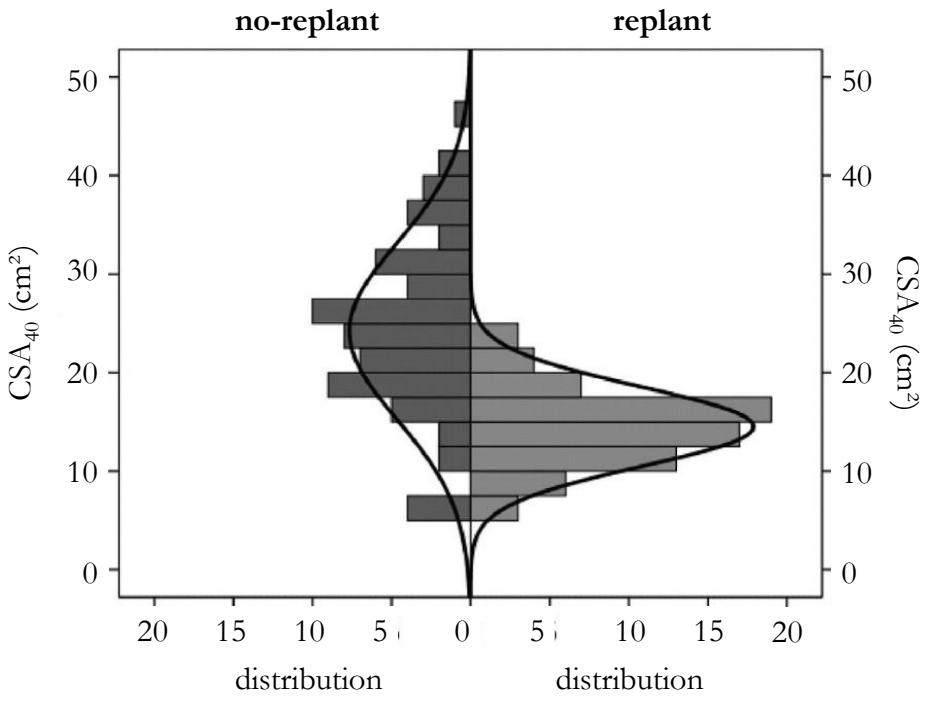

Fig. 2.6 Distribution of CSA_{40} (cm^2) on no-replant soil and replant soil (Orchard A).

2.2.3.3 Abundances of soil-fungal populations on no-replant and replant soil

Abundances of the soil-fungal populations were quantified for plots A1.2, A1.3, A1.4 and B1.4 on no-replant soil and for plots A2.1, A2.3 and B3.2 on replant soil in soil layer 0-20 cm.

Increased abundances of the total fungal DNA were found on replant soils (Tab. 2.6), with the mean abundance of total fungal DNA being significantly increased ($p \leq 0.01$) and twice as high as that for no-replant soil on orchard A. The increase in abundance was less strongly pronounced for orchard B. The abundance became more heterogeneous in replant soil with a high CV in orchards A and B (respectively, 79.6%, 94%) compared to no-replant soil with a CV of approximately 35%. Conversely, total extractable soil DNA was significantly decreased ($p \leq 0.05$) on replant soils, with a mean soil DNA of 95.8 ng/µl (SD = 31.4 ng/µl). Replant soils also showed stronger heterogeneity, CV = 32.8%, compared to no-replant soil, with a mean of 121.9 ng/µl (SD = 10.1 ng/µl) and CV = 8.3%.

Tab. 2.6 Mean abundances of total fungal DNA and Ag and proportion of Ag on total fungal DNA in no-replant and replant soil in orchards A and B.

Orchard	Soil-variant	total fungal DNA (genome/g soil)	CV (%)	Ag (genome/g soil)	CV (%)	Ag/total fungal DNA (%)
A	no-replant	5.6×10^{5a} (SD = 2.1×10^5)	37.8	67^a (SD = 73)	109.0	0.01^a (SD = 0.01)
A	replant	10.7×10^{5b} (SD = 8.5×10^5)	79.6	2706^b (SD = 3507)	129.6	0.35^b (SD = 0.49)
B	no-replant	7.8×10^5 (SD = 2.7×10^5)	35.0	322^a (SD = 269)	83.5	0.04^a (SD = 0.04)
B	replant	9.1×10^5 (SD = 8.6×10^5)	94.0	3025^b (SD = 3960)	130.9	0.35^b (SD = 0.37)

Indicated significances ($p \leq 0.05$) refer to the comparison within (not between) orchards.

No differences were observed in the abundance of *Fusarium* between no-replant and replant soil for either orchard A or orchard B. However, significant differences between soil variants (no-replant/replant) were observed for the *Alternaria* group (Ag). Mean abundance of the Ag was significantly increased ($p \leq 0.05$) to more than nine-fold in the replant soil in orchard B. In orchard A, the increase in Ag abundance was found to be forty times as high ($p \leq 0.001$) in replant soil as compared to no-replant soil. This result was accompanied by considerable heterogeneity with regard to the Ag abundance in no-replant soil and replant soil (CV = 109.0% in orchard A and CV = 83.5% in orchard B for no-replant soil, and CV = 129.6% and CV = 130.9% for replant soil).

With the increase in Ag, the share of Ag in the total fungal DNA changed. In no-replant soil, the share of Ag in total DNA was found to be vanishingly small, at 0.01-0.04%. In replant soil, however, the share of Ag was approximately 0.35%, in both orchards. Orchard A and B showed no differences in total fungal DNA and Ag in no-replant soil and likewise no differences in the fungal abundances in replant soil ($p \geq 0.05$).

Additionally, abundances of Ag were quantified in intervals of 10 cm to a depth of 60 cm from the soil surface for no-replant and replant soil (Fig. 2.7). A main proportion of total Ag to a soil depth of 60 cm was amassed at soil depths 0-10 cm and 10-20 cm. For orchard B, an Ag proportion of 97% in no-replant soil and 87% in replant soil was observed at a soil depth of 0-20 cm. At the same soil depth, a proportion of 93% in replant soil was found for orchard A. An exception was a small Ag proportion of only 27% at a soil depth of 0-20 cm in the no-replant soil of orchard A. In addition, a tenfold increase in Ag abundance to 60 cm soil depth in replant soil was found to be

mainly induced at soil depths of 0-20 cm; thus, soil depths of 20-60 cm were subsequently disregarded in ensuing analyses.

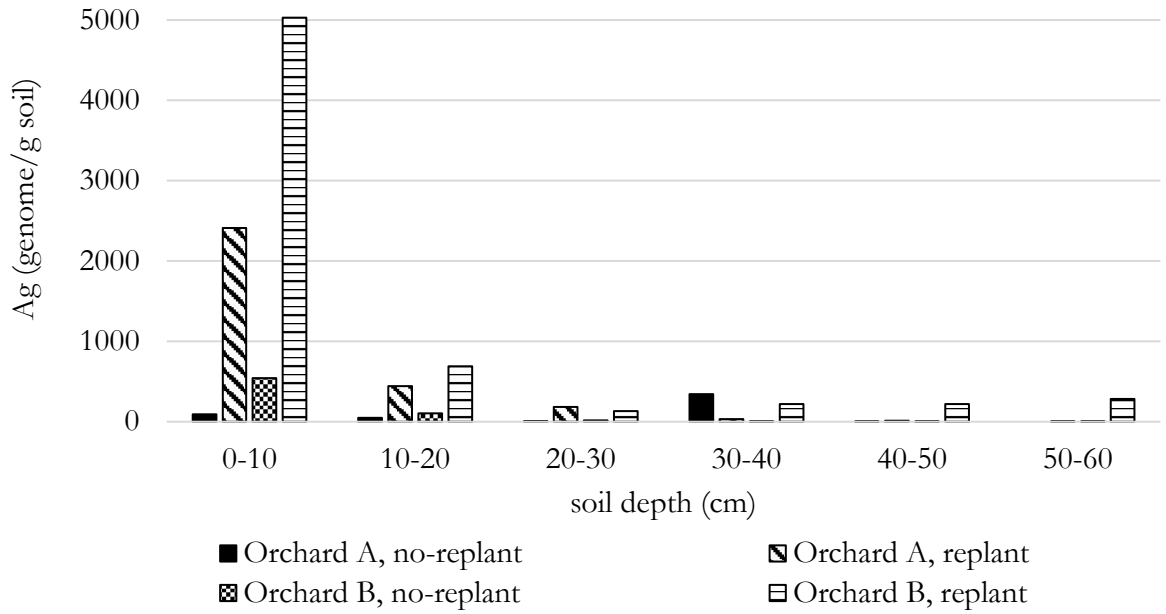

Fig. 2.7 Distribution of Ag abundance in 10 cm intervals from the soil surface to 60 cm soil depth.

2.2.3.4 Growth suppression at the single-tree level

The abundance of Ag was found to be an appropriate parameter reflecting shifts in the abundance of soil-fungal population on replant soil, thereby contrasting CSA_{40} in an inverse progression. This result was most pronounced in the top soil layer (0-20 cm below ground), thereby corresponding to the main body of the rhizosphere and the greatest surface for interaction between soil microbial fauna and shallow-rooted apple trees.

In combining contrasting Ag abundance with CSA_{40}, we formed the quotient $Q = \ln(Ag)/CSA_{40}$. Thus, the tree-specific Ag abundance in soil was normalised for tree-specific vigour. This resulted in a tree-specific relation between Ag and CSA_{40} represented by the algorithm (Q). As Q increases, the CSA_{40} decreases, with reduced variation. Ln(Ag) showed an inverse progression, and the linear adjustment of CSA_{40} and ln(Ag) exhibited a moderate goodness of fit ($R^2 = 0.69$ $R^2 = 0.56$). Both data sets tended towards an indirect correlation ($R^2 = -0.36$) (Fig. 2.8).

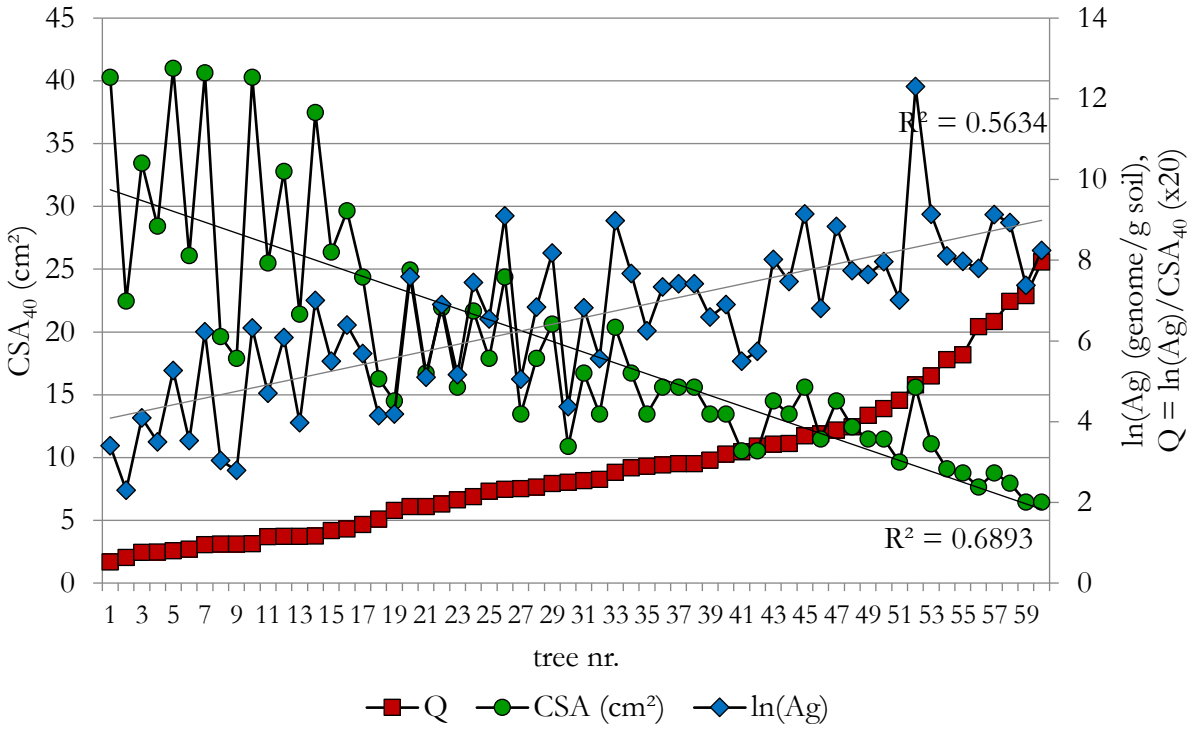

Fig. 2.8 Tree-specific indication of tree vigour suppression by the algorithm $Q = \ln(Ag)/CSA_{40}$. Inverse progressions of ln(Ag) and CSA_{40} for a subset of 60 trees from no-replant and replant soils of orchards A and B sorted by increasing Q. Each value Q represents one tree.

A k-means cluster analysis was performed based on the data set of Q for all trees, resulting in five clusters with significant differences in ln (Ag) and CSA_{40} (Fig. 2.9, Tab. 2.7).

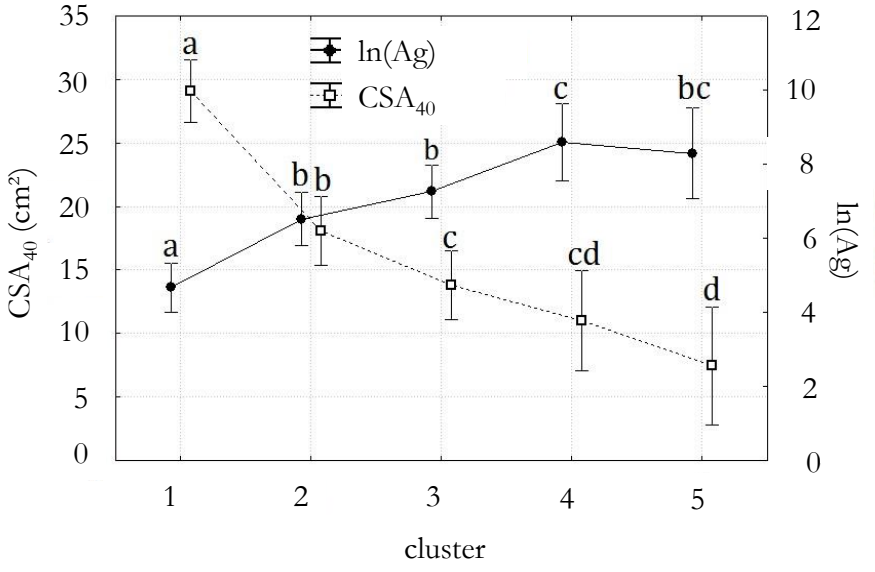

Fig. 2.9 Significance test for the five groups differentiated by k-means cluster analysis of the Q data.

Tab. 2.7 Mean Q per cluster and significant differences in CSA_{40} and ln(Ag) between clusters.

Cluster	Q	Q 95% CI	CSA_{40} (cm^2)	95% CI	ln(Ag) (genome/g soil)	95% CI
(1)	0.16	[0.15, 0.17]	29.1^{a}	[26.6, 31.6]	4.7^{a}	[4.0, 5.3]
(2)	0.36	[0.38, 0.35]	18.1^{b}	[15.4, 20.8]	6.5^{b}	[5.8, 7.2]
(3)	0.53	[0.59, 0.48]	13.8^{c}	[11.1, 16.5]	7.3^{b}	[6.5, 8.0]
(4)	0.78	[1.07, 0.64]	11.0^{cd}	[7.1, 15.0]	8.6^{c}	[7.5, 9.6]
(5)	1.11	[2.54, 0.79]	7.5^{d}	[2.8, 12.1]	8.3^{bc}	[7.1, 9.5]

[a] to [d] indicate significant differences ($p \leq 0.05$).

Tree vigour suppression for each cluster was well separated. Cluster 1 represented trees that were not affected by replant and were considered vital (1). Tree-specific tree vigour suppression can be derived from ln(Ag) and (CSA_{40}) in relation to cluster 1. The greatest difference between clusters was found between no tree vigour suppression (vital) (1) and escalating tree vigour suppression (2), with a reduction in tree vigour of almost 38%. The higher clusters of tree vigour suppression were followed by progressively lower additional reductions in CSA_{40} (Tab. 2.8).

Tab. 2.8 Cumulative reduction in CSA_{40} per cluster of tree vigour suppression.

Tree vigour suppression	Reduction of CSA_{40} (%)	Yield equivalence (t/ha)
(1) vital	0.0	80
(2) escalating	−37.8	49.8
(3) strong	−52.6	37.9
(4) very strong	−62.2	30.2
(5) critical	−74.2	20.6

2.2.3.5 Validation of ARD impact levels in orchard

The sampling of CSA_{40} was repeated in November 2016 for a subset of trees in orchard A (A1.2, A1.3, A2.3). CSA_{40} was increased for each cluster within the vegetation period. A moderate good logarithmic correlation was shown compared to the tree-specific cluster value measured in April 2016 ($R^2 = 0.79$). After one vegetation period, the mean CSA_{40} still differed between clusters and tree vigour, with decreasing tree vigour accompanying an increasing level of replant impact. Thus, the trees remained within their specific cluster of tree vigour suppression (Tab. 2.9).

Tab. 2.9 Mean CSA_{40} (cm^2) and cumulative reduction in CSA_{40} per cluster for the example of orchard A (replant and no-replant) (November 2016 and September 2017).

Tree vigour suppression	Mean CSA_{40} (cm^2)	Reduction of CSA_{40} (%)	
		November 2016	September 2017
(1) vital	37.04	0.00	0.00
(2) escalating	20.97	−43.4	−45.4
(3) strong	15.82	−57.3	−50.3
(4) very strong	12.08	−67.4	−69.3
(5) critical	8.15	−78.0	−71.5

2.2.3.6 Growth and yield suppression at the field level

For plots of no-replant soil and replant soil, the percentage frequency of clusters of trees in the field was determined. Subsequently, we were able to assess a replant impact at the field level for the example of the two case study locations. Although the trees on no-replant soil all showed no tree vigour suppression (1), only 11% of trees on replant soil were vital. Overall, replant effects suppressed tree vigour considerably, with the highest distribution of clusters found to be escalating (2) and strong (3) tree vigour suppression, amounting to 32%. The remaining trees fell into very strong (4) and critical (5) tree vigour suppression clusters. In terms of the CSA and Ag abundance (described in Sections 3.2 and 3.3), the five clusters were heterogeneously distributed across the replant field, with no spatial concentration of clusters across trees or rows.

The total effect of replanting was calculated by adding the weighted reduction in tree vigour for all clusters. The result showed a replant impact of - 46% in the reduction of tree vigour compared to no-replant soil (Tab. 2.10). Based on the calculation of replant impact at the field level, the mean yields produced in our case study were calculated to be approximately 40 t/ha when replanting.

Tab. 2.10 Growth suppression at the field level shown by the distribution of cluster (in % of total area), the weighted reduction in tree vigour (weighted reduction of CSA_{40}) and the calculated yield reduction.

Tree vigour suppression	Distribution of clusters (%)	Weighted reduction of tree vigour (%)	Calculated yield reduction (reference 80 t/ha)
(1) vital	10.6	0.0	8.5
(2) escalating	31.9	−12.1	15.9
(3) strong	31.9	−16.8	12.1
(4) very strong	14.9	−9.3	4.5
(5) critical	10.6	−7.9	2.2
Total growth (%) and yield (t/ha) suppression on field level (%)		**−46.1**	**43.2**

2.2.4 Discussion

The potential yield for this case study location, as stated by the experience of the orchardist, is approximately 80 t/ha (pers. comm. Günzel, 2017). The calculated strong suppression of tree vigour according to our results, 46%, halves this potential yield, thereby supporting the ARD impact of 50% stated in a previous study (G. S. Brown et al., 2000). Overall, the regional mean yields under optimal weather conditions in Brandenburg are approximately on par with the calculated mean yield under replant conditions in our study (35-40 t/ha) (Amf für Statistik Berlin-Brandenburg, 2018). This unexpectedly low mean yield across production sites in the region may be related to differences in orchard management but also in part to already widespread growth suppression due to replanting at a number of sites. With a large share of apple production sites in Brandenburg under more or less intensive use over many years, comprehensive monitoring research would be required to address the articulated concern of farmers in the region regarding replant impact in the larger production area.

The methodological approach shown in this study can be applied to assess and estimate replant-related suppression of tree vigour according to the relation of CSA_{40} and a replant-sensitive soil-fungal population, independent of causal agents, thereby contributing to a practical handling of replant disease in the field, even though the cause itself is unknown. A significantly different frequency distribution of tree vigour represented by the cross-sectional area of the trunk on replant sites adds up to approximately 50% compared to vigour at no-replant sites.

The comparison of trees between no-replant and replant soils within an orchard shows that the CSA_{40} achieves a lower average with a narrower scatter on replant soils. By contrast, no-replant soils are characterised by the strong variability of CSA_{40} and the appearance of particularly vital trees, which fully exploit the site-specific potential and therefore achieve a large CSA_{40}. The appearance of vital trees in close proximity to ARD-symptomatic trees, both cultivated on replant soil, was recently reported again by Tilston et al. (2018). The overlap in the ranges of the CSA_{40} across stocks and the variability of trees in the direct vicinity emphasise the need for an assessment of replant-related growth suppression at the individual tree level. Furthermore, the direct comparison of trees assigned to no-replant and replant soil shows that trees with the same CSA_{40} occur in both soil variants, so that a tree cannot be clearly associated with replant effects by CSA_{40} alone.

Fungal abundances of the class *Dothideomycetes* have previously been associated with former apple tree stations on commercial and intensively managed orchards (Deakin et al., 2018; Mark Mazzola et al., 2015; Tilston et al., 2018). Increased fungal abundances of the order *Pleosporales* are related to

phenotypical expression of replant effects (Tilston et al., 2018). Abundances of the *Alternaria* group (Ag) (class *Dothideomycetes*, oder *Pleosporales*, family *Pleosporacea*) were strongly increased in replant soil in this study, with proven differences in the *Alternaria* group (Ag) abundance on a logarithmic scale between no-replant and replant soil. Abundances of Ag are here related to the phenotypical expression of replant effects on the single-tree level due to an inverse progression between Ag abundance and CSA_{40}. The percentage increase in Ag abundance was more strongly pronounced than the percentage increase in total fungal DNA. This results in an increased proportion of Ag in total fungal DNA in the replanting soil. Therefore, the Ag was derived as a replant-sensitive responding population. The possible function of the Ag within the system requires further investigation. As long as individual members of the community in replant soil, as well as potential interactions, are still unknown, replant-sensitivity of the Ag can be attributed to neither primary nor secondary processes and functions (saprophytic/necrotrophic/pathogenic) in replant soil. The increase in the total fungal DNA was not compensated due to increased Ag abundance, suggesting that further populations are involved in replant-related effects in soil in a responsive manner. The methodological approach can, in principle, be adapted to other site-specific replant-sensitive soil-fungal populations.

By employing a formula or an algorithm, changes in soil-fungal abundances and depression in growth (directly) and yield (indirectly) are taken into account. The significant differences between clusters of tree vigour suppression found in this study allow the calculation of growth suppression for each cluster in relation to vital trees. Based on the potential yield given for a specific cultivar (top-variety/understock) and a specific location (soil, climate) on no-replant soil (for which a quick estimation by orchardists can be obtained from public statistics and variety trials conducted at experimental test sites), the potential yield reduction at field level can be calculated by adding the reductions in tree vigour in each cluster.

Assessing replant-related growth suppression based on the quotient $Q = \ln(Ag)/CSA_{40}$ is found to be appropriate within and across orchards. The positive correlation between CSA_{40} and the above-ground tree area confirms the earlier findings of Russo et al. (2007), thus proving the relationship between CSA and potential yield. In a direct comparison between no-replant and replant, CSA_{40} is found to be an appropriate parameter for replant-related growth suppression. However, the sensitivity of CSA_{40} suggests that the estimation of replant effects in relation to yields is more meaningful under similar soil climatic conditions and more precise when performed on trees of the same age and under the same cultivation method.

Statistical risk analysis is highly relevant for perennial tree crop systems in weighing potential gains and losses among orchards on a given farm (Mouron et al., 2006). With replanting being the most expensive item among orchard investments, previous and future seasons need to be factored into any assessment of production potential (Cerutti et al., 2013). In the absence of suitable detection methods for ARD before replanting, a classification of tree vigour suppression based on the suggested indication reveals the non-visible effects of replant in a standing orchard. The algorithm can thus be used to predict and assess actual as well as potential effects on growth and yield in an established orchard as well as the risk from replanting a plantation before clearing. Following the argument of Cerutti et al. (2014), relative values can be a good matrix of comparison for fruit yield under ARD constraints in different locations (no-replant and replant), production areas (across Germany) or cultivation methods. The algorithm for estimating growth and yield suppression can be applied within a production region where conditions are consistent for several orchards and the data for several orchards can be combined for reference and validation.

2.2.5 Conclusion

Replant-related effects of tree vigour suppression can be calculated at the individual tree level by combining a replant-sensitive soil-fungal parameter and the trunk cross-sectional area. Each individual tree can then be associated with a cluster representing a specific level of growth suppression. Based on the classification into clusters, the relative distribution of tree vigour suppression can be evaluated by the frequency of clusters in the field, from which an overall replant effect can subsequently be calculated for a specific region.

Growth suppression at the level of the single tree is related to different microbial communities depending on the location and condition of the site. The replant effect appears in different strengths between individual trees and proceeds discontinuously within rows. This conforms to differences in the abundance of fungal populations in the soil. Due to the significant correlation of the variability with the CSA_{40} at the level of single trees and the clear differentiation between no-replant and replant soils, the algorithm (Q) can be derived as replant related.

This algorithm is particularly applicable for the early years of production in a plantation as well as the full production phase in an established orchard. With data calculation becoming increasingly common in on-site assessments, the approach is usable for identifying trees for early treatment of replant-related growth suppression. As more precise farming methods are being developed, single-tree management can be applied where replant impact is high.

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2.3 Assessment of agro-ecological apple replant disease (ARD) management strategies: organic fertilisation and inoculation with mycorrhizal fungi and bacteria (Paper 3)

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Abstract:

Apple replant disease (ARD) impacts the economic yield of orchards by physical and morphological suppression of apple trees on replanted soils. The complexity of replant disease caused by a plethora of biological interactions and physical properties of the soil requires complex management strategies to mitigate these effects. Based on expert recommendations, we selected two management strategies linked to agroecological principles of (a) organic fertilisation with a specific mulch composition (MDK) and (b) biofertilisation with arbuscular mycorrhizal and bacterial strains (AMFbac), applied by a composition of existing products. For both management strategies we provide a proof-of-concept, by plot and field experiments. Both treatments have the potential to mitigate ARD effects on plant vigour. ARD effect was fully mitigated by MDK treatment in the short-term (one year) and was mitigated by up to 29% after seven years of MDK treatment (long-term). MDK provides an additional substrate for root growth. AMFbac has the potential to mitigate ARD effects on plant vigour but with non-replicable plant-beneficial effects in its current form of application. Thereby our results show a principal potential to mitigate economic effects but not to overcome replant disease including effects. While the MDK treatment is found resource intensive but reliable, the AMFbac treatment was found more user-friendly.

Keywords: tree vigour, soil-plant interaction, soil management, agro-practices, Müncheberger Dammkultur, soil fatigue, apple orchards, microbial inoculation, replant soil

2.3.1 Introduction

Intensive apple production in the form of monoculture plantations in densely used orchard areas is associated with degradation processes in the soil-plant-system that leads to suppressed tree vigour. The effect is known as apple replant disease (ARD) (Manici et al., 2013; Mazzola & Manici, 2012). The term refers to the harmfully disturbed physiological and morphological reaction of apple plants to soils linked to the frequency of replant, amongst others (e.g., tree nurseries have a higher probability to be affected by ARD as compared to permanent plantations) (Winkelmann et al., 2019). The current understanding in research is that ARD cannot be explained by a direct single cause or deficit, neither biologically nor physically nor environmentally determined by the plant-soil-climate related ecosystem of the plant. More probably, it is related to a range of soil biotic factors which are regulated by abiotic factors (Mazzola & Manici, 2012; Winkelmann et al., 2019), and it is therefore highly complex and site-specific. Above all, ARD is difficult to diagnose and overcome. From a farmer's perspective this poses foremost an economic problem. Visibly decreased vegetative performance above and below ground as well as decreased generative performance up to 50% are reported from commercial production sites (Brown et al., 2000; Mazzola, 1998; Van Schoor et al., 2009). Particularly in the first years after replant, symptomatic tree vigour suppression and stunted growth can lead to a 2-3-year delay in fruit bearing (Mazzola & Manici, 2012). In face of the current market development with an increasing demand for fresh fruit and the consequential decreasing lifetime of orchards (Cerutti et al., 2011), the economic impact on the viability of a plantation can be significantly impacted by ARD. The main aim of the producing farmer is therefore to manage ARD when it appears in the field, ideally in such a way that the economic impact is mitigated.

While chemical fumigation of soils before planting was largely phased out in food production systems due to environmental concerns and human health impacts, there is a growing interest for thermal, biological and cultural measures such as biofumigation (Hanschen & Winkelmann, 2020), soil fertilization (Wilson et al., 2004) or soil inoculation by antagonistic microorganisms (Line et al., 2005; Liu et al., 2018) as well as for resistant or tolerant rootstocks (Reim et al., 2019; Robinson et al., 2012). As yet, singular control measures aiming for a direct effectuation have not resulted in a reliable and transferable management strategy for remediation in different locations and settings. We assume that the complex nature of ARD requires more complex measures that affect the interaction between replant soil and plant. Such approaches include e.g., biofertilisation with living organisms or organic fertilisation based on agroecological principles (Nicholls & Altieri, 2016; Wezel et al., 2014) or integrated pest management (Sharma et al., 2020). Soil amendments such as composts or mulches, and also biological soil amendments in form of Mycorrhizae or bacterial, as

well as fungal biopesticides (Lü & Wu, 2018; Watson, 2018) increasingly gain interest as alternative ARD management strategies. In this context, the intrinsic knowledge and practical experience of farmers is a relevant input, which can in principle lead to innovative measures in orchard management.

An increasing number of projects is currently promoting in-field research using transdisciplinary approaches and on-farm testing. Particularly in agro-ecologic research, the integration of traditional knowledge and practical experience of farmers and practitioners is expected to improve the search for a design of new and alternative cultivation measures. The overall aim is to improve production by using the knowledge of ecosystem functions and services, e.g., to maintain soil fertility, to substitute pesticides or to improve the efficiency of fertilisers (Nicholls & Altieri, 2016).

In this study, we explore two complex management strategies for ARD control that were selected upon recommendation and personal experience of experts and farmers. The largely intrinsic knowledge was formalised and applied in an experimental test setting, as is commonly used in pre-selection studies before repeated field trial testing. We analysed the ARD management strategies for two questions: (1) how does the application effectuate ARD impact, and (2) what practical lessons can be learned from applying the strategies in conventional and intensive orchard production?

An inductive methodological approach can lead to new insights into the ecological mechanisms of the strategies and their impact on soil-plant relationships in replanted orchards. Furthermore, statistical analysis and observation may bring forth practical knowledge for a further design of viable ARD management strategies and their socio-technical integration into orchard management.

2.3.2 Materials and Methods

2.3.2.1 Selection and Formulation of the ARD Management Strategies

We conducted open expert interviews with experts, farmers and consultants in Brandenburg and Schleswig-Holstein, Germany with the aim to identify of ARD management in production systems. Two ARD management strategies were selected that could be described comprehensively, based on individual experiences and explorative applications in conventional orchard production sites. Both were perceived to have a positive impact on ARD by the respective experts (Workshop on "Soil fatigue and management strategies to overcome ARD in apple production", Esteburg Jork, 7 March 2018). Both management strategies were systematized, described in terms of a formulated application and checked for test-trial applicability in interaction with the respective farmers and consultants.

1. The 'Müncheberger Dammkultur' (MDK). The 'Müncheberger Dammkultur' is a specific substrate composition using pine wood chips to imitate natural biological metabolic processes that take place in mixed woodlands. The 'Müncheberger Dammkultur' is named after the location of its emergence at the Müncheberger Field Station for Fruit Genetic Resources in Brandenburg (Germany) where the treatment was developed over 20 years on a test site and was applied to apple orchards, cherry orchards and blueberries. For improved readability, the treatment is hereinafter abbreviated with MDK. The application of the MDK for apple orchards followed the instruction of Schwärzel (2013), formalised by Diehl et al. (2020).
2. A composite of biological soil amendment products containing arbuscular mycorrhiza species (AMF) and bacterial strains (bac), hereinafter named AMFbac after its principal components. The application of AMFbac followed the instructions of M. Tauschke (maize experiments, unpublished) and was formalised by Cavael et al. (this publication).

Both applications were first tested in a pot experiment. Based on auspicious results, the strategies were subsequently adapted and tested on-farm in a field test (Tab. 2.11).

Tab. 2.11 Test methodology for proof-of-concept and on-farm field tests.

Strategy 1: MDK		**Strategy 2: AMFbac**		
Pot Experiment	**Field Test**	**Pot Experiment A**	**Pot Experiment B**	**Field Test**
$n = 6$	$n = 90$	$n = 72$	$n = 64$	$n = 48$

By taking up management strategies from practitioners for research analysis, and testing these in the actual environment of their production system, we had to account for differences in test locations, soil types and various types of apple understocks (vigorous/dwarfing). The experimental setup, however, allowed for an analysis of the strategies within the orchard site under actual cultivation practice and real-world conditions. The experiments thus provided for a proof-of-concept under multifactorial influences as found in a commercial orchard. The pot experiments provided for an initial effect analysis. The field tests were expected to show whether the strategies effectuate tree vigour specifically in the area of an ARD onset in the orchard.

2.3.2.2 Strategy 1: Müncheberger Dammkultur (MDK)

The MDK treatment consisted of a layered composition of substrates applied to the ground surface of the planting spot of trees. A bottom lining of hardly bio-degradable organic mulch layer containing white peat and black peat with a high proportion of clay (BP Substrate, Kammlott GmbH) was applied in a loose fill of 10 L per running meter. This biofilm was covered with a layer of pinewood chips, adding 60-80 L per running meter. The woodchip layer was supplemented with lime marl by 150 g/tree. A top layer of 10 L per running meter of soil (1-2 cm height) was taken from the orchard to stabilise the ridge. Lastly, the MDK composition was supplemented with magnesium-nitrate fertilizer (Magnisal, Haifa Chemicals Ltd., Haifa Bay, Israel) to 36 g/tree (Fig. 2.10).

For the pot experiment the mulch was layered on a 10.0 cm layer of replant soil, separated by a thin, root-permeable plastic foil. The top layer of soil for stabilisation was omitted in the pot experiment.

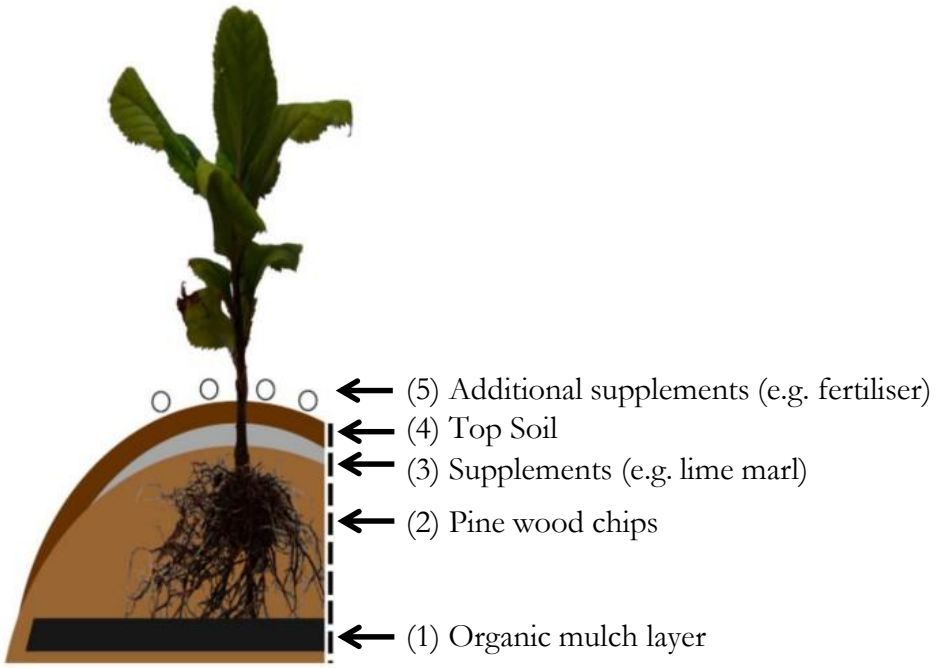

Fig. 2.10 Formalised layers of the MDK ridge for treatment at time of planting. Adapted from Diehl et al., 2020.

Sampling Design for Müncheberger Dammkultur (MDK)

MDK Pot experiment

The pot experiment was set up in May 2016. The experiment consisted of six trees from plant material of the top-variety Topaz cultivated on understock M9 and planted into 70 L pots. The trees were grafted nursery trees at stage of sale for production. Three trees were treated with MDK, three were left untreated. Plant vigour rating was conducted periodically over one year, data presented are sampled in May 2016 and April 2017 (Tab. 2.12).

Tab. 2.12 MDK Pot Experiment on replant soil (May 2016 to April 2017) (n = 6).

MDK Pot Experiment				
Test Variant	**Soil**	**Treatment**	***n***	**Tree Vigour Rating**
I	r	MDK (2016)	3	May 2016, April 2017
II	r	-	3	

MDK Field Test

The field test was set up in November 2016 on an intensively managed commercial fruit orchard in north-eastern Germany, located approximately 50 km east of Berlin (Altlandsberg: longitude: 52.62623, latitude: 13.804264). The orchard comprised a variety of fruit trees, including different varieties of dessert apples. The site is characterised by sandy brown, dry and warm diluvial Eutric Retisols (Geoabruptic, Arenic, Aric) and Geoabruptic Luvisols (Arenic, Aric, Cutanic) (according to World Reference Base for Soil Resources, WRB) (LBGR, 2021). We selected a section of mature orchard spanning replant (r) and no-replant (nr) soil in direct vicinity. The section was uniformly cultivated with tall spindles from apple scions ROHO 3615 EVELINA® on apple understock M9 since 2009.

We analysed three test variants of MDK treatment. Two test variants compared MDK treatment on replant and no-replant soil, applied in November 2016 to the soil of mature trees (test variant I and II). A third test variant used trees which had been initially treated with MDK at the time of planting in 2009 and were treated again in 2016 (test variant III). The aim was to use this old stock of MDK treatment to identify differences between single and repeated applications of MDK as well as short-term (0.5-1.5 years) and long-term (7 years) effects in a mature stock of trees. Each variant was tested on 18 trees (n = 18) with two control variants (IV and V) (Tab. 2.13).

Tab. 2.13 MDK Field Test (November 2016 to January 2018) ($n = 90$).

MDK Field Test					
Test Variant	**Soil**	**Treatment**	***n***	**Tree Vigour Rating**	**Root Rating**
I	r	MDK (2016)	18	November 2016,	January 2018
II	nr	MDK (2016)	18	March 2017,	-
III	r	MDK (2009) + MDK (2016)	18	September 2017,	January 2018
IV (control)	r	-	18	January 2018	January 2018
V (control)	nr	-	18		-

No data for no-replant soil.

Tree vigour was assessed at the end of the growing season in November 2016 for an evaluation of growth levels in mature trees seven years after planting. Further data were collected three times over the course of one year. Observation of root morphology of mature trees became possible in January 2018, due to the uprooting of several hundred trees on replant soil by a cyclone in October 2017 (Xavier).

2.3.2.3 Strategy 2: Inoculation with Arbuscular Mycorrhiza Fungi (AMF) and Amendment with Bacterial Strains (bac) (AMFbac)

The AMFbac treatment consists of a mixture of granular or liquid compositions of mycorrhizal strains containing *Rhizoglomus irregulare* (Blaszk, Wubet, Renker & Buscot) Sieverd, G.A. Silva & Oehl, *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler and *Funneliformis caledonium* (T. H. Nicolson & Gers.) C. Walker & A. Schüßler (INOQ Agri and INOQ Advantage, INOQ GmbH). The inoculation of bacterial strains contains a composition of bacterial strains in liquid form: *Azospirillium lipoferum*, *Azotobacter cinelandit*, *Bacillus megaterium*, *Bacillus circulans*, *Micrococcus roseus*, *Pseudomonas fluorescens*, and *Bacillus subtillis* (BactoFil® A10, AGRO.bio Hungary Kft.).

Inoculation of AMFbac was performed during planting of apple understocks. The AMF granular was mixed with soil and added to the root area at a concentration of 33.0 mycorrhizal units per cm^3 soil. The INOQ Agri was found ready for use with mycorrhizal units of 145 mL^{-1} (Pot Experiment A). The INOQ Advantage with mycorrhizal units 550 million mL^{-1} was mixed at a ratio of 1:5 with Vermiculit. The mixture was then mixed with expanded clay (Leca® 0.5-2.5 mm) at a ratio of 1:16 to achieve 33.0 mycorrhizal units per cm^3 of soil (Pot Experiment B). The bacterial inoculum was applied to achieve 0.001 mL inoculum per cm^3 soil and suspended with H_2O in a ratio of 1:200.

For the field test, we used a different product for the same principal composition of AMFbac. The AMF granular contain composition of mycorrhizal strains *Funneliformis caledonium*, *Funneliformis mosseae*, *Rhizoglomus irregulare* in a ratio of 1:1:1 (MITAK GmbH, Paulinenaue, Germany) The liquid bac composition contained a humic substance-based bacteria suspension (without specification of bacteria composition) (GeoHumat, GeoFert GmbH) and a liquid composition of bacterial strains *B.velenzensis*, *B.licheniformis* and *B.amyloliquefaciens* (ABITEP GmbH Berlin, Germany). Bacterial inoculums were suspended with H_20 in a ratio of 1:10.

The AMF granular was mixed with soil and filled in the planting hole immediately before planting apple understocks. Bacterial liquid inoculum was poured on top of the AMF-inoculated soil after planting (Fig. 2.11).

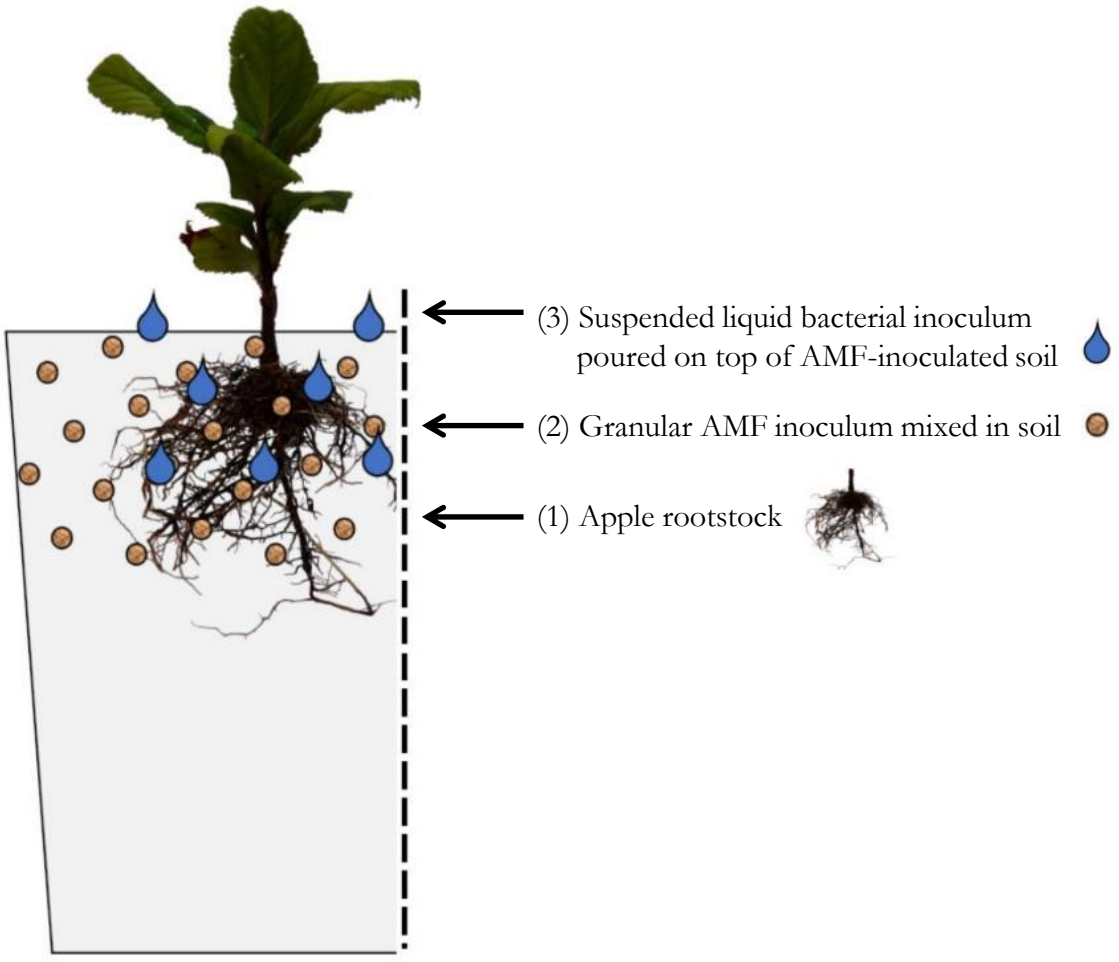

Fig. 2.11 Formalised composition of AMFbac treatment in planting hole at time of planting.

Sampling Design for Arbuscular Mycorrhiza Strains and Bacterial Strains (AMFbac)

AMFbac Pot experiment

Two pot experiments (A, B) were set up for the AMFbac treatment. Replant soil was taken from a test-station for apple cultivation located east of Berlin (Müncheberg, longitude: 52.520496, latitude: 14.127071). No-replant soils were taken in close vicinity to this test-station from a long-standing fallow (A) and non-apple cultivated cropland (B). Soil was taken after removing the top 2.0 cm of surface soil.

Pot Experiment A was set up in April 2017. It consisted of 36 trees cultivated in 1.5 L pots under greenhouse conditions for one vegetation period using in-vitro propagated understocks of type Bittenfelder Sämling (vigorous) and type M26 (dwarfing) (Tab. 2.14).

Tab. 2.14 AMFbac Pot Experiment A (April to November 2017) (32 weeks) ($n = 72$).

AMFbac Pot Experiment A						
Test Variant	**Soil**	**Treatment** [a,b]	**Understock** [c]	***n***	**Tree Vigour Rating**	**Root Rating**
I	r	AMFbac	BS	9	April 2017, June 2017, November 2017	November 2017
II	r	AMFbac	M26	9		
III	nr	AMFbac	BS	9		
IV	nr	AMFbac	M26	9		
V	r	-	BS	9		
VI	r	-	M26	9		
VII	nr	-	BS	9		
VIII	nr	-	M26	9		

[a] AMF: INOQ Agri (INOQ GmbH, Germany). [b] bac: BactoFil® (AGRO.bio Hungary Kft.), [c] types of apple understock: Bittenfelder Sämling (BS), M26.

Pot Experiment B was set up in May 2020. It was set up under open field conditions using generative propagated understocks of type Marc (dwarfing) and B9 (dwarfing) before grafting cultivated in 10 L pots. In Pot Experiment B the AMF as a single inoculant (without amendment of bac) was tested additionally (Tab. 2.15).

Tab. 2.15 Pot Experiment B (May to September 2020) (16 weeks) ($n = 64$).

AMFbac Pot Experiment B						
Test Variant	**Soil**	**Treatment** [a,b]	**Understock** [c]	***n***	**Tree Vigour Rating**	**Root Rating**
I	r	AMFbac	Marc	8		
II	nr	AMFbac	Marc	8		
III	r	AMF	Marc	8		
IV	nr	AMF	Marc	8	May 2020,	September
V	r	-	Marc	8	September 2020	2020
VI	nr	-	Marc	8		
VII	r	AMFbac	B9	8		
VIII	r	-	B9	8		

[a] AMF: Advantage (INOQ GmbH, Germany). [b] bac: BactoFil® (AGRO.bio Hungary Kft.).

For an analysis of the effectiveness of AMFbac, data of tree vigour were collected when setting up the experiments and at the end of experimental period. In Pot Experiment A, tree vigour was additionally collected after two months of experimental period. Fine root samples for analysis of root colonisation by mycorrhizal fungi were taken at the end of experimental period.

AMFbac Field Test

The field test was set up in April 2020 in the region 'Kehingen' northwest of Hamburg (Balje, longitude: 53.828248, latitude: 9.135356). In this district apple is cultivated on tidal marshes (Fluvisol, according to soil World Reference Base for Soil Resources, WRB) (NIBIS Kartenserver. Niedersächsisches Bodeninformationssystem., 2020). A previous apple orchard was chosen for field testing, which was cultivated for several years until grubbing-up trees in the end of 1980s. The former orchard was used as crop land since. In total, 48 apple understocks of the type A2 (vigorous) before grafting were planted in row. AMFbac treatment was tested (I and II) starting in April 2020). Additionally, the amendment of bac as a single inoculant was tested without the additional amendment of AMF (III) (Tab. 2.16). The data for tree vigour and fine root samples were taken when grubbing-up apple understocks.

Tab. 2.16 AMFbac Field Test (April to September 2020) (18 weeks) ($n = 48$).

AMFbac Field Test						
Test Variant	**Soil**	**Treatment[a,b]**	**Understock[c]**	***n***	**Tree Vigour Rating**	**Root Rating**
I	r	AMFbac[1]	A2	8		
II	r	AMFbac[2]	A2	8		
III	r	AMF	A2	8	September	September
IV	r	bac[1]	A2	8	2020	2020
V	r	bac[2]	A2	8		
VI	r	-	A2	8		

AMF: *Funneliformis caledonium*, *Funneliformis mosseae*, *Rhizoglomus irregulare*, bac[1]: GeoHumat (GeoFert GmbH, Teterow, Germany). Bac[2]: *B.velenzensis*, *B.licheniformis*, *B.amyloliquefaciens* (ABITEP GmbH, Berlin, Germany).

2.3.2.4 Tree Vigour Rating

Trunk circumference was measured by a standard rule 40.0 cm above soil surface on grafted trees. This parameter was found as an appropriate parameter reflecting tree vigour (Cavael et al., 2020a). On (non-grafted) understocks we measured the circumference of the root collar 1.0 cm above soil surface according to quality rating used for plant material in nurseries (Büttner, 2004).

Cross-sectional area (CSA) was calculated per trunk and root collar circumference. To rule out possible irregularity of CSA on different test plants at the start time of the experiments the percentage growth rate of CSA was calculated using the formula:

$$growth\ rate\ (\%) = \left((t_x - t_0) \times 100\right) \Big/ t_0 \qquad (2.4)$$

where t_x is the time of sampling. The total of subsets of understock represented the baseline for CSA and thus as a baseline for the growth rate of each test variant.

2.3.2.5 Root Morphology Rating in MDK

Understocks were qualitatively rated by a scoring model with a range from 0 (no adventitious roots) over 1 (small and thin adventitious roots of an herbaceous habitus) to 2 (strong and pronounced adventitious roots). The root of each understock was assigned a full number for rating.

2.3.2.6 Measurement of Root Colonisation by Mycorrhizal Fungi

Root colonisation by mycorrhizal fungi was monitored by staining fresh roots. The roots were rinsed several times with tap water, cleaned by shaking (100 stroke min^{-1}) in 50° C heated 10% (wt/vol) KOH for 15 h and then rinsed again several times with tap water to ensure transparent roots suitable for staining. Cleaned roots were boiled for 3 to 4 min in 0.05% methyl blue lacto glycerol and roots were destained by rinsing in tap water. The mean percentage of root colonisation by mycorrhizal fungi was counted by the gridline intersection method (Brundrett et al., 1996). A total of 100 root segments were observed per understock and counting of root colonisation was repeated three times per understock. The mean degree of root colonisation per understock was calculated. The rate of root colonisation by mycorrhizal fungi on fine roots was determined for each test variant, here equalling each subset of understock.

2.3.2.7 Statistical Analysis

The data for all trees, respectively understock vigour parameters above ground (CSA, growth rate) and below ground (root morphology), as well as data for root colonisation by mycorrhizal fungi were analysed using ANOVA (analysis of variance) and significant differences between test-variants were calculated by Tukey post-hoc test. $p < 0.05$ was accepted as significant.

As datasets of plant parameters did not follow a normal distribution, Spearman's rank correlation coefficient (ρs) was calculated for correlations between CSA growth rate and root colonisation by mycorrhizal fungi. Significant correlations were accepted at $p < 0.05$. All statistics were conducted using IBM SPSS Statistics 22.

2.3.3 Results

The baseline data for trees in replant soils in the pot experiments and field tests showed significantly decreased tree vigour associated with ARD. We calculated an overall replant-related growth suppression of - 25% to - 50% in the field before treatment, measured by tree vigour rating and growth rate calculation based on CSA.

2.3.3.1 Strategy 1: MDK

2.3.3.1.1 Tree Vigour Rating

The Pot Experiment showed a considerable tree vigour promoting effect of MDK treatment on replant soil, with growth rates almost doubling (Tab. 2.17). Root development was observed strong with many adventitious roots in MDK (rating of 2 on average), whereas no adventitious roots were observed in replant soil (rating of 0.5 on average).

Tab. 2.17 MDK Pot Experiment – effect of MDK on tree vigour.

MDK Pot Experiment					
Test Variant	**Soil/ Treatment**	**Under-stock**	**CSA (cm²) 16 May**	**Growth Rate (%) 16 May - 17 Apr**	**Root Rating (0-2) 17 Apr**
I	r/MDK (2016)	M9	3.7	+ 3.4	2.0
II	r/-	M9	3.2	+ 1.7	0.5

The Field Test showed a positive effect of the MDK treatment not only in the plants treated in November 2016, but also in the plants which had been previously treated at the time of planting in 2009 (long term effects).

When comparing tree vigour before treatment in November 2016, we identified different tree vigour rates in trees on replant as compared to no-replant soils by 50%. The effect of the MDK treatment in 2009 at the time of planting proved to have a positive effect after seven years, raising CSA significantly higher as compared to trees on replant soil, thus mitigating the effect of ARD by 29% (Tab. 2.18).

Tab. 2.18 MDK Field Test – effect of MDK on tree vigour.

MDK Feld Test					
Test Variant	**Soil/ Treatment**	**Under-stock**	**CSA (cm²) 16 Nov**	**Growth Rate (%) 16 Nov - 17 Sep**	**Root Rating (0-2) 18 Jan**
I	r/MDK (2016)	M9	13.0	+ 15.3	1.0 [ab]
II	nr/MDK (2016)	M9	26.9	+ 9.2	-
III	r/MDK (2009) + MDK (2016)	M9	19.1	+ 15.7	1.5 [b]
IV	r/-	M9	14.2	+ 10.7	0.8 [a]
V	nr/-	M9	28.2	+ 14.2	-

Characters indicate statistical significance. Significances calculated between test variants, $\alpha = 0.05$.

One year after treatment with MDK an increase of CSA by 15.3% and an annual growth rate similar to trees on no replant soil was measured. The repeated MDK treatment likewise raised growth levels to similar levels as in trees on no replant soil. Overall, annual growth rate was 5% stronger on trees treated with MDK as compared to trees without MDK treatment.

The MDK treatment conducted on trees in no-replant soil reduced the annual growth rate of trees by about 5%. The reductions shifted tree vigour to similar rates as found on trees in replant soil.

2.3.3.1.2 Root Morphology Rating

The MDK treatment on replant soil led to considerably more adventitious roots, raising the rating of the root morphology from 0.8 to 1.0 on average in treated trees, and to 1.5 in the repeated application. The same result was observed in the pot experiment where root morphology also significantly improved in treated plants.

We observed that the adventitious roots avoided the layer of replant soil by growing into the ridge layer of the MDK treatment. Only minor growth of roots could be observed in the replant soil. An overlay of root morphology data with CSA data from the field test showed parallels between root growth and vegetative growth in MDK treatment on replant soils (Fig. 2.12).

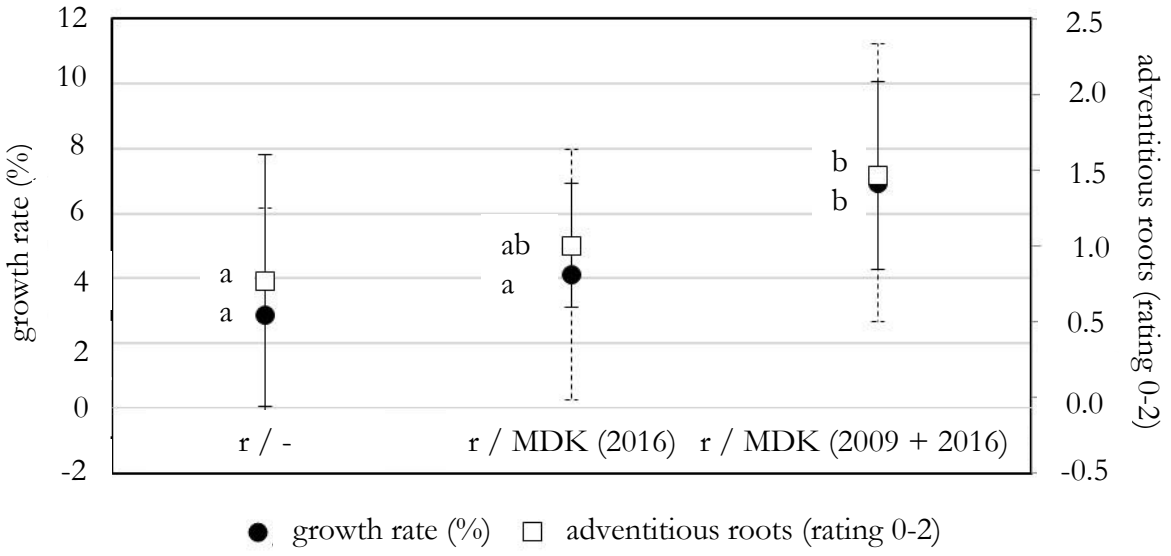

Fig. 2.12 MDK Field Test – Parallels between vegetative growth (%) (17 November to 18 January) and rating of adventitious, January 2018. Significances calculated between test-variants, $\alpha = 0.05$, relative standard deviation.

2.3.3.2 Strategy 2: AMFbac

2.3.3.2.1 AMFbac Pot Experiment

A Tree Vigour A replant effect was observed nine weeks after planting. The tree vigour suppression remained visible over the 32 weeks of testing. With a mean CSA of 18.0 mm^2 on replant soil, the tree vigour in replant soil was found significantly lower (- 39.8%) as compared to trees in no-replant soil (Fig. 2.13).

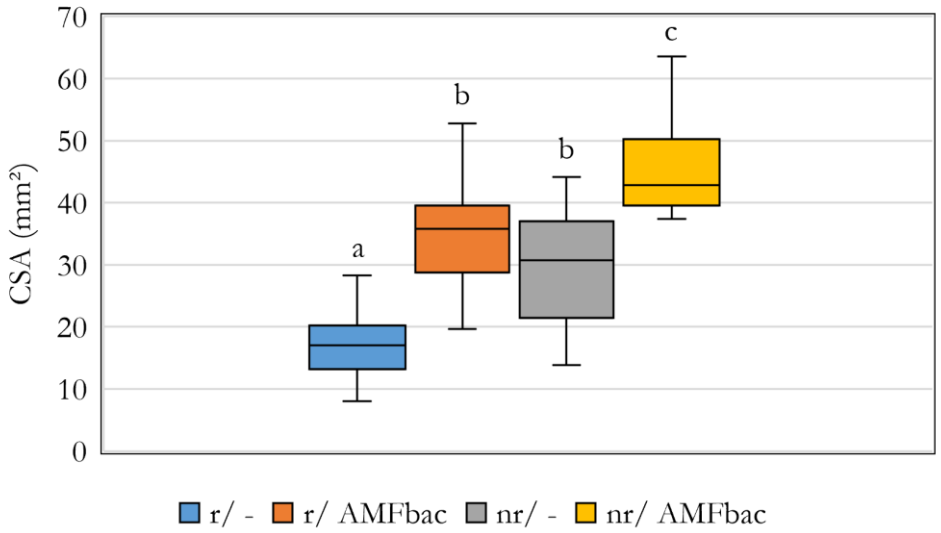

Fig. 2.13 AMFbac Pot Experiment A – CSA (mm^2), November 2017 (week 32), $\alpha = 0.05$.

The replant effect was fully mitigated in the AMFbac treatment (Fig. 2.13). Growth rates doubled in week 9 and increased to approximately triple growth rates after 32 weeks. CSA of 36.7 mm^2 was doubled as compared to trees in replant soil.

The treatment overall resulted in stronger growth rates as compared to no-replant soil (Tab. 2.19). On no-replant soil an effect of treatment of about + 75.4% increase of growth rate was observed ($p \leq 0.05$). However, this effect of treatment on no-replant soil was less strong than observed by an increase of growth rate by + 187.3% on replant soil. The type of understock had no effect on plant or fungal parameters ($p \geq 0.05$).

Tab. 2.19 AMFbac Pot Experiment A – Growth rate of CSA and degree of mycorrhizal root colonization.

AMFbac Pot Experiment A						
				Growth Rate (%)		Root Colonisation (%)
Test Variant	**Soil/ Treatment**	**Under-stock**	**CSA (mm²) 17 Apr**	**Apr - 17 Jun (week 1 - 9)**	**Apr - 17 Nov (week 1 - 32)**	**17 Nov (week 32)**
I+II	r/ AMFbac	BS, M26	5.6 (rel. SD = 0.4)	+ 206.7 ^{c} (rel. SD = 0.4)	+ 634.6 ^{z} (rel. SD = 0.5)	54.7 ^{m} (rel. SD = 0.2)
III+IV	nr/ AMFbac	BS, M26	5.5 (rel. SD = 0.4)	+ 304.1 ^{b} (rel. SD = 0.3)	+ 774.2 ^{z} (rel. SD = 0.3)	38.5 ^{lm} (rel. SD = 0.4)
V+VI	r/-	BS, M26	6.6 (rel. SD = 0.6)	+ 95.0 ^{a} (rel. SD = 1.0)	+ 220.9 ^{x} (rel. SD = 0.7)	25.2 ^{kl} (rel. SD = 0.6)
VII+VIII	nr/-	BS, M26	6.5 (rel. SD = 0.5)	+ 271.9 ^{bc} (rel. SD = 0.6)	+ 441.3 ^{y} (rel. SD = 0.6)	5.9 ^{k} (rel. SD = 0.6)

Characters indicate statistical significance. Significances calculated between test variants for respective analysis period, $\alpha = 0.05$.

Root Colonization

The replant effect was also observed in the fine root colonisation by mycorrhizal fungi (Tab. 2.19). Root colonisation was about five-times greater in replant soil than in no-replant soil.

The treatment significantly raised the root colonisation in replant and no-replant soils. In replant soil, the degree of mycorrhizal root colonisation was more than twice as much after treatment. In no-replant soil, the effect of treatment was much lower (+ 552.5% on nr, + 117.1% on r), but still higher than compared to no treatment.

2.3.3.2.2 AMFbac Pot Experiment B

Tree Vigour

The replant effect was observed by a significantly lower mean CSA (- 21.0%) on replant soil than on no-replant soil 16 weeks after planting. The AMFbac treatment in this experiment had a negligible effect on replant soil. AMF treatment (without bac) raised growth rates on no-replant soil by 10.8%, but the full AMFbac treatment had no effect (Fig. 2.14a). The AMFbac treatment was found to suppress growth rates of understocks by - 6.7% (Marc) and 13.5% (B9) ($p \leq 0.10$) (Fig. 2.14a, b).

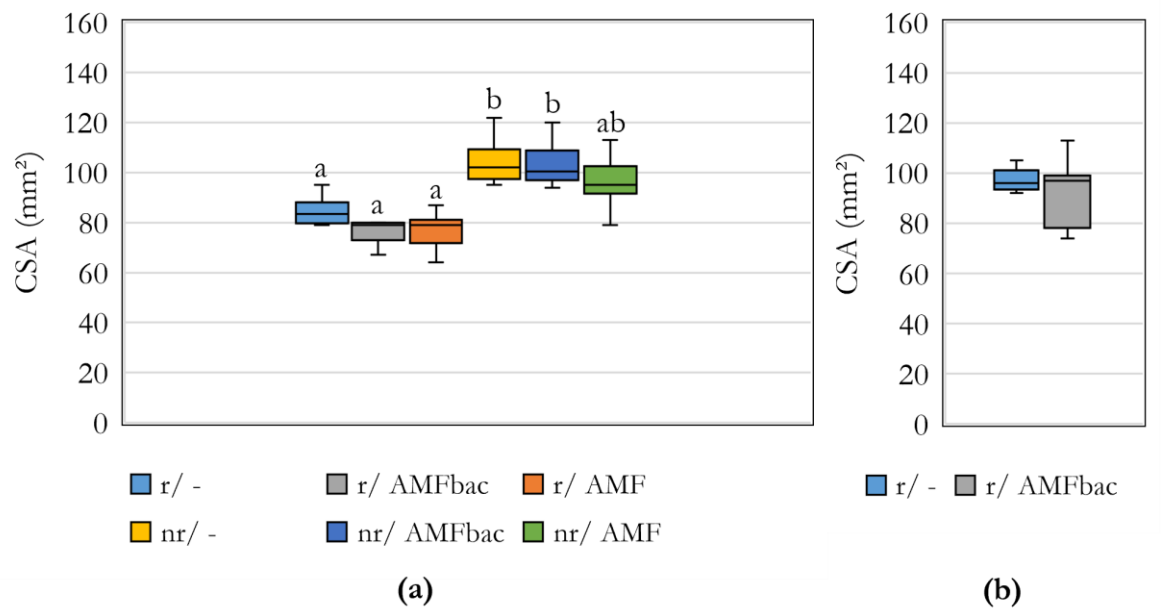

Fig. 2.14 AMFbac Pot Experiment B - CSA (mm^2) apple understocks' type (a) Marc and (b) B9, September 2020 (week 16), $\alpha = 0.05$.

Root Colonization

An increase of root colonisation by mycorrhizal fungi was observed in replant soil. This is in line with previous results from Pot Experiment A. The root colonisation in Pot Experiment B was found three times higher in replant soil as compared to no-replant soil ($p \leq 0.10$) (Tab. 2.20).

Tab. 2.20 AMFbac Pot Experiment B – Growth rate of CSA and degree of mycorrhizal root colonization.

AMFbac Pot Experiment B					
Test Variant	**Soil/ Treatment**	**Under-stock**	**CSA (mm²) 20 May**	**Growth Rate (%) May - 20 Sep (week 1 - 16)**	**Root Colonisation (%) 20 Sep (week 16)**
I	r/AMFbac	Marc	64.8 (rel. SD = 0.3)	+ 23.5 ^{x} (rel. SD = 0.9)	15.0 (rel. SD = 0.8)
II	nr/AMFbac	Marc	65.9 (rel. SD = 0.3)	+ 55.9 ^{xy} (rel. SD = 0.6)	8.4 (rel. SD = 1.2)
III	r/AMF	Marc	58.9 (rel. SD = 0.1)	+ 33.3 ^{xy} (rel. SD = 0.5)	22.0 (rel. SD = 1.0)
IV	nr/AMF	Marc	60.9 (rel. SD = 0.1)	+ 60.9 ^{y} (rel. SD = 0.3)	2.5 (rel. SD = 0.8)
V	r/-	Marc	64.4 (rel. SD = 0.1)	+ 30.2 ^{xy} (rel. SD = 0.4)	28.9 (rel. SD = 0.7)
VI	nr/-	Marc	70.9	+ 50.1 ^{xy}	4.5

AMFbac Pot Experiment B					
Test Variant	**Soil/ Treatment**	**Under-stock**	**CSA (mm²) 20 May**	**Growth Rate (%) May - 20 Sep (week 1 - 16)**	**Root Colonisation (%) 20 Sep (week 16)**
VII	r/AMFbac	B9	(rel. SD = 0.1) 81.4 (rel. SD = 0.2)	(rel. SD = 0.4) + 17.2 (rel. SD = 1.0)	(rel. SD = 1.3) 17.8 (rel. SD = 0.8)
VIII	r/-	B9	78.0 (rel. SD = 0.0)	+ 30.7 (rel. SD = 0.8)	34.1 (rel. SD = 0.3)

Characters indicate statistical significance. Significances calculated between test variants for sampling date/analysis period, with exception of test variants differing by type of understock, $\alpha = 0.05$.

The AMFbac treatment halved root colonisation in replant soil. This contrasted the previous results from Pot Experiment A. The effect was observed for both understocks (Marc, B9). The effect was not found significant due to a high coefficient of variation.

In no-replant soil, the AMFbac treatment led to a mean degree of root colonisation by mycorrhizal fungi twice as high than without treatment. AMF treatment (without bac) on no-replant soil led to a decrease of root colonisation as compared to non-treated soil.

2.3.3.2.3 AMFbac Field Test

The inoculum product had no significant effect on plant or fungal parameters ($p \geq 0.05$). Therefore, hereinafter we did not differentiate bacterial inoculums (bac^1, bac^2) for analyses of field test.

Tree Vigour

AMFbac treatment significantly increased the CSA of understocks on replant soil by + 55.0% at 18 weeks after planting (Fig. 2.15, Tab. 2.21). This effect could only be observed in the full application of AMFbac, but neither for only AMF nor only bac treatment.

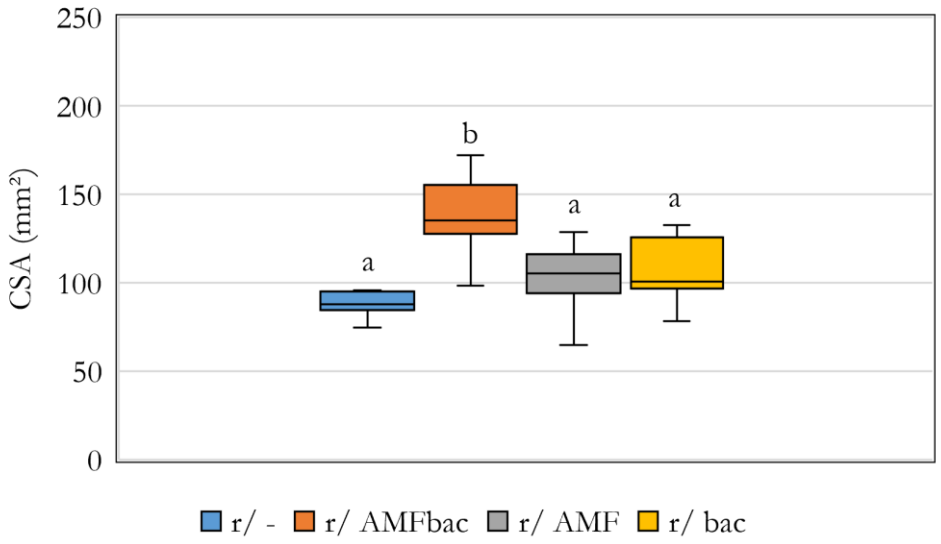

Fig. 2.15 AMFbac Field Test – CSA (mm²), September 2020 (week 18), $\alpha = 0.05$.

Tab. 2.21 AMFbac Field Test – Mean CSA (mm²) and degree of mycorrhizal root colonization.

AMFbac Field Test				
Test Variant	**Soil/ Treatment**	**Under-stock**	**CSA (mm²) 20 Sep (week 18)**	**Root Colonisation (%) 20 Sep (week 18)**
I + II	r/AMFbac	A2	140.5 [b] (rel. SD = 0.2)	11.3 (rel. SD = 0.6)
III	r/AMF	A2	102.9 [a] (rel. SD = 0.2)	13.7 (rel. SD = 0.5)
IV + V	r/bac	A2	108.8 [a] (rel. SD = 0.2)	9.2 (rel. SD = 0.6)
VI	r/-	A2	91.2 [a] (rel. SD = 0.2)	10.5 (rel. SD = 0.6)

Characters indicate statistical significance. Significances calculated between test variants, $\alpha = 0.05$.

Root Colonization

The degree of root colonization by mycorrhizal fungi did not differ between AMFbac-treated and non-treated replant soil (Tab. 2.21). The rate of root colonization by mycorrhizal fungi after AMFbac treatment was found at 11.3%, with similar rates observed in AMF treatment and slightly less in bac treatment (without AMF) ($p \geq 0.05$).

No correlated linkage could be determined between AMF colonisation and tree vigour in any of the AMFbac experiments, neither in replant nor in no-replant soil.

2.3.4 Discussion

By analysing two ARD management strategies which were proposed by practitioners (experts and farmers) based on their observations in the field, we can now shed some light on the mechanisms and practical lessons as well as research questions for both strategies.

2.3.4.1 Müncheberger Dammkultur (MDK): Effectuation and Impact on ARD

The growth response of treated apple trees indicates the potential of MDK to fully mitigate the impact of ARD in the short-term and to mitigate and manage the ARD-impact by up to 29% in the long-term (seven years after treatment), as apple trees can recover their natural growth potential with this strategy. The long-term response is in line with other mulching strategies, e.g., using various types of composts, resulting in increased growth and shoot elongation of + 2% to 26% as compared to non-treated controls in the short-term (Franke-Whittle et al., 2019). An initial treatment at planting time can maintain growth rates at higher levels (+ 34.5%) over several years. Repeated treatment shows improved results, raising the growth rates to more or less no-replant levels. Treatment of mature plants are shown to improve growth rates, however, detrimental effects caused by ARD in previous years cannot be regained.

Mulching strategies mix substrates into the replant soil, whereas the MDK treatment adds substrate as a groundcover on replant soil. Thus, the MDK provides an additional substrate for better and favourable root penetration, thereby impacting the growth direction of roots to the substrate ridge. Similar observations of root proliferation are documented for mulching (Forge et al., 2008; Forge & Kempler, 2009; Yao et al., 2005). Mulching can likewise increase fine feeder root biomass with greater root density and root extending into the mulch itself (Van Schoor et al., 2009). This response is related to the non-systemic localised response of trees to replant soil (Lucas et al., 2018). Root penetration of the MDK ridge reduces further penetration of replant soil, as the MDK ridge provides nutrition as well as beneficial soil-climate conditions to the plant (Diehl et al., 2020).

The positive effects of MDK treatment are replant specific. In practical terms, MDK treatment is not suitable for a comprehensive precautionary treatment of mature trees and can only be recommended for application on mature trees in soils demonstrably affected by ARD. Profitable MDK treatment thus requires unambiguous testing of soils for ARD in order to achieve improvements in ARD-affected substrates that equal natural no-replant soils. Whether a precautionary treatment at the time of planting has the same detrimental effect needs to be proven in further research.

In contrast to strategies of larger-scale soil replacement strategies involving the excavation of ARD-contaminated soil and the replacement with topsoil from nearby locations (a strategy sometimes applied in the course of large-scale orchard replantation in intensive production areas in northern Germany), the MDK treatment is applied as an additional groundcover on natural topsoils. Thus, it is considered non-invasive, i.e., it does not impact the layers or functional characteristics of the site-specific soil system. However, similar to soil exchange, the MDK treatment allows for an autarkic artificial soil system that enables the reuse and/or regeneration of orchard sites over time. For reasons of soil conservation, but also material input and resources, it is prioritised over soil replacement.

The application is knowledge intensive and requires the movement of considerable amounts of substance materials. All materials are easily accessible in principle, but require additional financial and staff resources.

The results for the MDK treatment described here are considered applicable for orchards in Central Europe. An application of the treatment in other regions, that strongly differ e.g., by temperatures, rainfall events or windstorm events the MDK treatment needs to be tested and may require local-specific adaptions.

Comparable systems can be found in container cultivation, in greenhouses and nursery substrates for soil-free cultivation based on pine wood chips (Jackson & Wright, 2009; Owen et al., 2016; Wright et al., 2006). The pot experiments for MDK treatment show, that cultivation in alternate substrates can be conducted independent of location. The MDK treatment allows both for container cultivation and open field cultivation.

2.3.4.2 Arbuscular Mycorrhiza Strains and Bacterial Strains (AMFbac): Effectuation and Impact on ARD

The AMFbac treatment comprises the inoculation of soils with biological amendments at the time of planting. The treatment is less knowledge intensive, as the formula for the liquid amendment can be acquired by marketed products. All materials are easily accessible and can be handled easily.

AMF are used as fungal agents for the biological control of replant disease in a variety of horticultural crops (Huang et al., 2003). Effects of AMF to mitigate (apple) replant disease are presented in detail by Lü and Wu (2018). According to these studies, AMF affects and regulates soil biotic factors that are causally linked to ARD, e.g., soil and root microflora (Jamiołkowska et al., 2017), as well as abiotic factors that modulate the impact of ARD, e.g., soil physiochemical

conditions. Furthermore, it is reported to influence soil aggregation processes (Rillig & Mummey, 2006). Soil aggregation processes are altered under replant conditions resulting in an aggregate disintegration and reduction of aggregate stability (Cavael, et al., 2020b). The mitigation of the ARD impact was observed by AMFbac treatment, however, was not observed when microbial inoculants were applied as single inoculum. Our result is in line with Gąstoł and Domagała-Świątkiewicz (2015) who presented best productivity of replanted apple treated with microbial consortium of a variety of AMF species and bacterial strains. Our results do not point to any mode of action of the bacterial inoculum. It can be assumed that bacterial strains here may perform as mycorrhiza helper bacteria (MHB) that stimulate the formation of mycorrhizal symbiosis, or else, positively impact the functioning of mycorrhizal symbiosis (Frey-Klett et al., 2007; Garbaye, 1994).

The effect of AMFbac treatment is not replicable according to our results. The effect on tree vigour rates vary considerably, with significantly positive effects in Pot Experiment A, negative effects in Pot Experiment B, and positive effects in the Field Test. Due to the multiple factors influencing the experiments in our approach, we cannot point out a distinct causal limitation. Previous studies find differing impacts of AMF on fungal species and soil types (Ridgway et al., 2008). Perhaps with continuously improving technology for genetic analysis of AMF, the impact of AMF can be determined more precisely over time (Van der Heijden et al., 2015). Practitioners in our study relied on various products assuming a non-composition-specific reaction. However, the alignment of the formula composition with the soil properties in situ is not fully understood as yet, and the formular composition may have to be adapted to different soil-climate systems.

The differences between inoculums (AMF, bac) and their interaction with species on site are not yet fully understood. Utkhede and Smith (2000) report antagonistic relationships between *G. interadices* and *B. subtilis*, as well as *E. agglomerans* in apple replant soil. For a successful treatment of ARD, we therefore recommend further research of inoculum compositions to optimise the interaction between AMF and bacteria, and their interaction with the plant.

Since the AMFbac treatment is inoculated, it is considered an invasive treatment at least to the biological properties of the soil. Further research of the impact on biological as well as soil physical and chemical properties is advisable, and may influence future inoculation methods (Lü & Wu, 2018).

The AMFbac treatment is non-specific for replant soil. The relation of higher mycorrhizal colonisation but lower growth rate in replant soil is upheld after treatment. Previous studies report that AMF inoculation has higher effects on apple seedlings when treated in no-replant soils as compared to replant soils (Mehta & Bharat, 2013), suggesting that mycorrhizal colonisation of

roots is not a requirement for tree growth performance but rather a tree vigour promoting effect. Accordingly, the AMFbac treatment has no significant effect on root colonisation, but raises tree vigour in general. The lower efficiency of mycorrhiza in replant soil can be attributed to a thin mycorrhizal formation in the root cortex and penetration into the central cylinder of roots on replant soil as reported by Aldea (1998). Overall, this means that trees both in replant and in no-replant soil can potentially be improved by raising the tree vigour via AMFbac treatment. The effect, however, is stronger on replant soils as compared to no-replant soils.

Our study is limited to the analysis of short-term effects of AMFbac treatment covering one vegetation period (32 weeks). The effects on tree vigour are relevant for plantations with a rapid replant frequency of apple understocks as well as top varieties in tree nurseries. In long-term plantations with traditionally 18 (± 6) years of plantation lifetime, the long-term effects have to be considered. Utkhede and Smith (2000) report long-term effects of AMF inoculation after six years, as well as effects of inoculation with bacterial strains on the vegetative and generative performance of trees under replant conditions. These results indicate that AMFbac has a potential to raise tree vigour over time by long-term effects.

2.3.4.3 Practical Lessons Learnt

Irrespective of causal mechanisms in replant soil, the question remains whether the strategies can maintain profitable orchard site locations. While MDK is suitable for applying at planting stage and repeated applications at later stages, the AMFbac treatment is, in principle, suitable for an application before orchard planting (on replant and no-replant soils) and also for tree nurseries.

For treatment of trees in commercial apple production (fruit plantation or tree nurseries), reliable effects are required. Thus, the rate of performance restoration as well as security of application need to be assessed for each strategy. This includes profitability and viability of the treatments. Being non-invasive, replicable and applicable in a formalised way, the MDK treatment fulfils these requirements to a large extent. It can be applied at the time of planting and shows short-term as well as long-term effects that mitigate ARD especially in the first 1-3 crucial years, when ARD impediments are particularly detrimental to the profitability of the orchard (Atucha et al., 2011; Neilsen et al., 2004; Van Schoor et al., 2009). At the same time, mature trees with ARD impediments can be treated to reinvigorate plant growth.

The AMFbac treatment requires less resources and material inputs, and by way of application is simple in handling. A recent study on acceptance rates of farmers for biofertilisation with living microorganisms, shows that the acceptance rate is not so much determined by user-friendliness or

economic factors, but rather by usefulness in terms of compatibility of use and relative benefit. The study identifies a 68% acceptance rate for the use of microorganisms as an ARD management strategy for farmers in the same orchard regions as focussed in this study (Petzke, 2019).

The AMFbac treatment used in this study (in its current stage of formalised application) shows compatibility of use and a relative benefit in the Field Test and Pot Experiment A. However, due to the lack of effect in Pot Experiment B, the usefulness of the strategy is not found replicable in effect. Based on our results, we believe the AMFbac treatment has the potential in principle to mitigate ARD effects by enhancing tree vigour. However, at this point it cannot be derived to which conditions the AMFbac treatment beneficially affects the soil-plant interactions.

2.3.5 Conclusions

Neither the MDK ridge treatment nor the AMFbac inoculation treatment overcome replant effects per se. However, they can mitigate growth suppression, and thus economic effects of the orchard to a certain extent. MDK treatment provides an alternative substrate for root growth, thereby indicating that trees react to the physical properties of the replant soil by growing into the ridge. AMFbac treatment leads to enhanced growth rates on replant and no-replant soils. Both treatments can be applied irrespective to the strength of the ARD effect. The MDK treatment is not site specific and, by adding to any surface layer available, can be transferred to other locations and sites for further testing. It can, in principle, be also applied for soil-less cultivation in greenhouses. AMFbac treatment has practical benefits such as easy application and low resources input. However, the effects of the formula compositions on soils and understock variants need to be resolved.

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3. Discussion

The overall subject of this work is to contribute to a practical handling of orchards impacted by apple replant disease, even though the causal agents of the disease itself are unknown. To this end, the work looks at the impact of replant disease on tree vigour, thereby targeting the main hindrances fruit growers are faced with in managing replant disease – the estimation and mitigation of replant impact on orchard performance.

3.1 Impact of replant disease on tree vigour

In this work, an impact of replant disease on tree vigour by its suppression in order of 20 to 50% on soils, representative for one soil-climate region (lowland area in Brandenburg, Germany) is shown. Calculated suppression of tree vigour is represented in range of replant-related growth and yield suppression previously stated by Brown et al. (2000) and recently attested by Pscheidt and Ocamb (2021). By monitoring tree vigour in a series of time intervals it is found that replant-related suppressive tree vigour is due to suppression in tree growth rate by 25 to 30%. In breaking down tree growth rates to ecological periods of tree growth, Diehl et al. (2020) figured out that growth rate of replanted trees is suppressed in time intervals that generally perform as vegetation period in apple production (summertime in nemoral zone), and physiological tree growth shifts into wintertime (generally dormancy period of vegetation). Deficits in tree growth rate during summertime are not caught up during wintertime, thus tree vigour suppressive effect is adversely felt.

The replant-related suppression of tree vigour is observed on recently planted tree stocks (within one year after planting), as well as on mature trees in established orchards (more than six years after planting). Replant disease has been reported to stress juvenile phase of apples (non-fruiting) and thus, to delay adult phase (fruiting) for two to three years (Mazzola, 1998), whereas effects of replant on mature trees in established orchards are often not adversely perceived. In accordance with previous research (Weiß et al., 2017), results from this work suggest that tree vigour is affected by replant disease immediately after planting and continuously with progression of tree rooting in soil. Therefore, to estimate the impact of replant disease in perennial production-systems previous as well as future seasons need to be factored.

Tree vigour is significantly suppressed and achieves a lower average with a narrower scatter on replant sites than on no-replant sites, suggesting that trees cannot fully exploit their site- and type-specific growth potential under replant conditions. However, the comparison between replant and

no-replant sites shows an overlap in the range of tree vigour. Therefore, comparison of means is found less meaningful to estimate replant impact on orchard performance. Within replant sites suppression of tree vigour is differently pronounced between trees, resulting in a heterogeneous distribution of replant-symptomatic and non-symptomatic trees in direct vicinity over orchard (Simon et al., 2020; Tilston et al., 2018). The variability of tree vigour in direct vicinity and the overlap in the ranges of tree vigour across stocks (no-replant/replant) emphasises the need for an estimation of replant impact on orchard performance by quantification of replant-related tree vigour suppression at the individual tree level. Trees cannot be associated with replant by tree vigour as a single measure. In field, many other factors beside the disease will affect growth and yield even at a small scale. Therefore, next to impediments in tree vigour a quantification needs to consider further replant-indicative parameters, e.g. done by qPCR analysis (this study) or other means of soil scan to be developed in future.

3.2 Estimation of site-specific replant impact on tree vigour

To estimate the impact of replant at an aggregated tree level, an algorithm is established to indicate and quantify replant-related suppression of tree vigour according to the relation of plant- and replant-sensitive soil parameters irrespective of causal agents of the disease, thereby contributing to a practical handling of replant in field, even though the causal agents itself are unknown.

By applying the algorithm $Q = \ln(Ag)/CSA$, instead of single parameter CSA, the replant impact is estimated in ecological terms. Based on the quotient Q each tree is categorised into one cluster. Clusters represent categories of disease severity by gradual impact on tree vigour. For each cluster, losses in tree vigour, and related fruit yields (Lepsis & Blanke, 2006), are calculated in relation to vital trees, that fully exploit the site- and type-specific growth and yield potential. The calculation can be based on expected tree vigour, e.g. experiences under disease free conditions, or actual tree vigour on no-replant sites where conditions are consistent (Brown & Keane, 1997).

The approach allows for an early quantification as early as one year after planting (juvenile phase of apple trees), as well as on established orchards (adult phase of apple trees). By linking impediments in tree vigour with shifts in replant-sensitive soil fungal population the actual state of replant impact on tree vigour is quantified. A cluster-specific growth potential enables to predict tree performance for at least one vegetation period. Further analysis will be required to exercise if classification of trees remain valid also for several vegetation periods.

By adding cluster-specific tree vigour suppression by clusters' frequency distribution in field the actual replant impact on orchard performance is precisely estimated. An estimation of replant

impact on orchard performance is required to assess the economic impact of replant disease and to determine benefits of potential management strategies (Brown & Keane, 1997). An economic assessment can be performed, e.g. by calculation of contribution margins as a measure of profitability of apple production under replant conditions (Cavael, 2016). The quantification of replant impact for individual trees provides fruit growers with a well-founded choice which trees need to be treated by soil management strategy. A more homogenized tree vigour over plantation, resulting from a selective treatment of soil, will simplify tree management thereby contributing to a resource- and cost-efficient management of replanted orchards.

3.3 Mitigation of replant impact on tree vigour

Two biological soil management strategies, AMFbac treatment and MDK treatment, are evaluated for their potential to mitigate replant impact on tree vigour. Both treatments (alternative formula compositions) have previously been reported for their plant and yield promoting potential in fruticultural systems (Emmanuel & Babalola, 2020; Schwärzel, 2013; Todeschini et al., 2018), and have been recommended by experts upon personal experiences for their potential to mitigate suppressed tree vigour, yet have not been systematically tested in orchard field conditions. In this study, treatments are explored as non-chemical alternatives to manage replant impact on tree vigour. Treatments are presumed to enhance tree vigour under replant conditions by modulating soil structure and related microbiome (AMFbac treatment) (Hao et al., 2021; Rillig & Mummey, 2006; Santoyo et al., 2021) and by creating an alternative soil environment for fibrous root systems by organic layer irrespective of the quality of the origin soil (MDK treatment).

Both treatments, AMFbac and MDK, are identified as promising non-chemical alternatives for managing replant impact on tree vigour under production conditions of German apple production regions. In comparison with chemical methods (Nyoni et al., 2019), biological soil-management strategies tested in this study are equal in preventive effectiveness by initial treatment of soil at time of replant. The long-term monitoring of treated trees, however, indicates that the effect of biological soil management is not of long-lasting nature. To maximise tree vigour under replant conditions a regular treatment throughout the life cycle of orchard will be required (Van Schoor et al., 2012).

Only a few post-planting management strategies exist for replanted orchards so far (Bradshaw, 2016). In this study, the MDK treatment is identified as applicable to mitigate replant impact on tree vigour when treating replant-symptomatic mature trees in established orchards. Irrespective of the phase an apple tree is in (juvenile/adult) trees can recover their natural (site- and type-

specific) vigour, by shifting back their physiological growth into summertime (Diehl et al., 2020). An adequate dormancy period of apple trees is significant for quantity and quality of fruitification and thus, highly relevant for economic viable production of dessert fruits (Else et al., 2019).

A lack of sufficient chilling hours in winter period is also known as a consequence of climate change. While replant leads to a delay in tree's dormancy period, climate change leads to lack of early cold in wintertime and earlier phenological timings in springtime (Fraga & Santos, 2021; Singh et al., 2016). So far, little is known about the effects of climate change under specific conditions of fruit production and under replant conditions. Nevertheless, it could be considered that MDK treatment might be one part of an integrated management for enhancing climate change adaption and mitigation of apple production under specific conditions of replant affected orchards.

Analyses of AMFbac treatment is performed for one vegetation period. Gąstoł and Domagała-Świątkiewicz (2015) report tree vigour enhancing effects by composite treatment of AMF and bacterial strains after four vegetation periods. These results indicate that biofertilisation can be applied in tree nurseries with a rapid replant frequency (one to two years), as well as in long-term effected, perennial orchards. On orchards, the AMFbac treatment has the potential to mitigate replant impact on tree vigour especially in the first one to three years when replant impediments are particularly detrimental to the profitability of orchard (Atucha et al., 2011; Neilsen et al., 2004; Van Schoor et al., 2009). An interest in the AMFbac treatment is shown by a commercial nursery specialised in apple understock production in Dülmen (region Münster, Germany). An AMFbac treatment on replanted production-site of this nursery was set up in 2019 (pers. comm., Eulenstein & Lodder, 2019), and results are pending.

The general land use competition (Haberl et al., 2014) and an increasing intensification of fruit production in specialised fruit growing regions will further limit an access of fruit growers to non-replanted production-sites. Results of this study indicate that replant-related suppression in tree vigour and yields can be mitigated in short-term and managed in long-term by biological soil management. However, despite an enhancement of growth rate by treatment up to 100%, losses in tree vigour caused by replant disease that have already been manifested cannot be regained. In order to ensure an economical sustainable apple production on replant sites fruit growers can either manage soil by initial treatment, e.g. AMFbac or MDK treatment, by treatment of soil as soon as replant impact on tree vigour rise, e.g. MDK treatment, or else by AMFbac and MDK treatment as part of an integrated (replant) pest management.

3.4 Implementation in fruticultural practice

The methodological approach to estimate replant impact on tree vigour, presented in this study, is parameter-open. If further analyses indicate plant- and soil-parameters, especially parameters causally linked to replant disease, this can also be included or substitute present parameters into the algorithm. An indication of ARD-linked soil-parameters is expected within the project BonaRes (Modul A): ORDIAmur (Overcoming Replant Disease by an Integrated Approach) in a timely manner (ordiamur.de).

For identification of replant-linked plant- and soil parameters the indication and quantification of replant impact on single tree level might be of special interest. The categorisation of individual trees into clusters allows for an investigation of replant impact on tree vigour linked to dynamics of soil parameters, that does not become obvious by comparison of less differentiated samples of no-replant and replant soils from different orchards/plots. The comparison of homogenised soil samples is found limited and less significant due to large variability in soil structure and physiochemical properties (Negassa et al., 2019; Tola et al., 2017), and thus microbial structures even at a small scale (Buckley & Schmidt, 2001; Naveed et al., 2016). In this study, a gradual impact of replant on tree vigour is shown along with disproportional increase in replant-sensitive soil fungi (Ag) on total soil-fungal DNA (ITS), as well as soil-aggregate disintegrating processes in smaller and less stable aggregates. The analysis of soil-structure under replant conditions, in this case, is limited to trees representing strongest, medium and lowest tree vigour on replant sites. If further analyses strengthen this observation, purposeful management strategies for overcoming replant disease in soil can be derived, e.g. treatment of mycorrhizal fungi affecting soil aggregate dynamics (this study; Wang et al., 2021; Zydlik et al., 2021) or other means of soil management to be developed in future. For analyses and assessment of management strategies to mitigate replant impact on tree vigour the approach for estimation replant impact presented in this study can be used.

For practical implementation in commercial apple production the approach to estimate site-specific impact of replant on tree vigour is consuming resources. A quantification according to the relation of plant- and replant-sensitive soil parameters requires a densely gridded monitoring of soil (and plant) during the whole growing season of apples. For soil analyses agricultural microbiology services are available. An extensive implementation in fruticultural practice will require progress in microbial soil scanning, e.g. by soil testing kits for quick on site-testing of soils.

For a practical handling, the estimation of replant on tree vigour is simplified when quantifying the suppression of actual tree vigour or tree growth rate by CSA as a single parameter, instead of the

relation between CSA and replant-sensitive soil-fungal abundances. In this simplified form the approach will be used to assess several ARD mitigation strategies on performance of apple understocks on replanted nursery production-sites within project BonaRes (Modul A): ORDIAmur (ordiamur.de). Using the CSA as single parameter presupposes that state of replant (no-replant/replant) is known and further detrimental influences on tree vigour can largely be ruled out. A quantification of replant impact by CSA can be performed by fruit growers during vegetation period and vegetation dormancy as well. To estimate replant impact on orchard performance growth data of a minimum of 18 trees in direct vicinity has to be taken for considering heterogeneous distribution of more or less replant-affected trees over plantation.

Results of this study indicate that the approach to estimate replant impact on tree vigour can be applied within a production region where conditions are consistent for several orchards and data of orchards can be combined for reference or validation between orchards (Fig. 3.1; A and M). Orchards in other regions may be affected by replant disease in another extent, e.g. due to differing soil and climate conditions. Therefore, results of this study are not to be generalised. However, the approach to quantify replant impact based on the indication algorithm can principally be transferred to orchards in different locations. For comparison of replant impact on orchard performance between regions the region-specific replant impact needs to be estimated.

An interest in the approach was shown by fruit growers of an orchard located in Gambach (Wetterau), a southwestern region in Hessen, Germany (Fig. 3.1; G). The approach is found as a suitable measure for estimation of replant impact on performance of this orchard alike. Therefore, the approach is assumed to be transferable to orchards with differing soil and climate conditions in different fruit growing regions. Should further analysis strengthen this point, then this would be an important step toward an estimation of replant impact comparing different fruit growing regions.

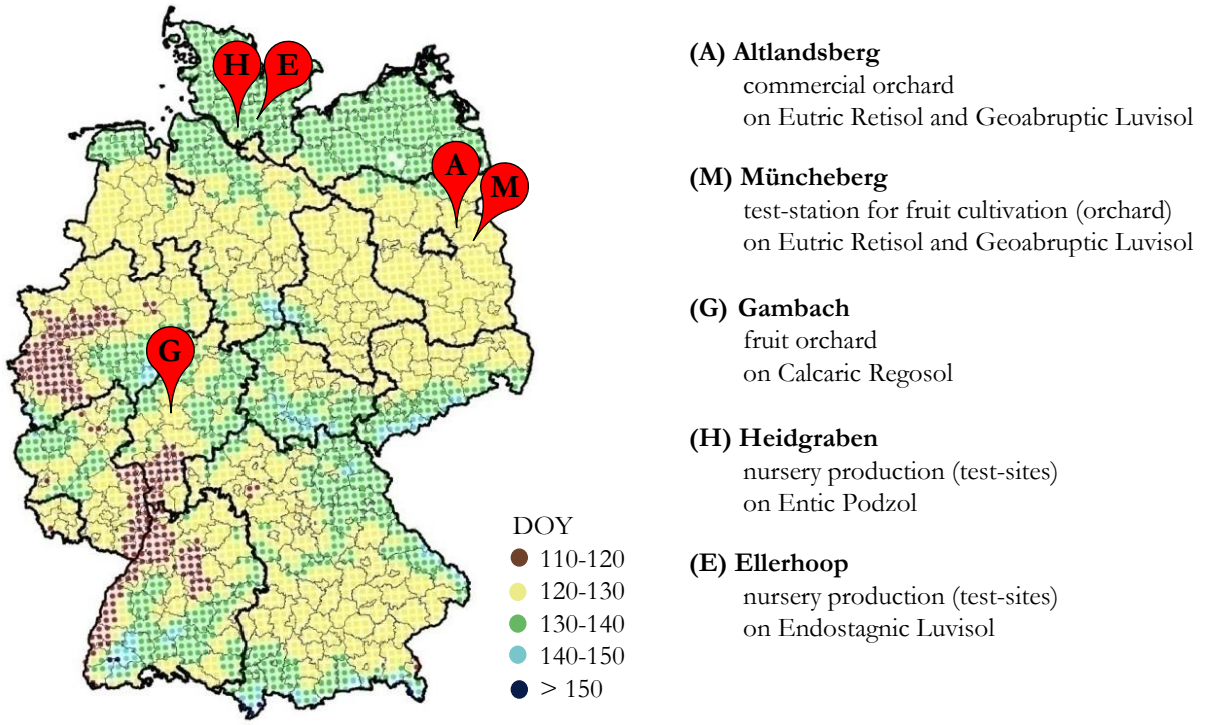

Fig. 3.1 Test-sites of nursery production and orchards differing in soil-climate conditions; soil classification according to World Reference Base (WRB), climate represented by phenology of apple – middle beginning of flowering by days of year (DOY) (Map: Chmielewski, 2009; edited).

The impact of replant on tree vigour is of special significance for nurseries specialised in fruit tree production. Short-term rotation of nursery trees each one to two years leads to a frequent replanting of production sites. The approach was assessed for the estimation of site-specific replant impact on nursery production-sites (ordiamur.de). On test-sites, located in the region Pinneberg (Schleswig-Holstein, Germany) (Fig. 3.1; H and E) the approach is found principally as an appropriate measure. However, on nursery production-sites understock vigour and replant-sensitive soil-fungal population may be less pronounced than on perennial production system of orchards. The algorithm $Q = \ln(Ag)/CSA$ was not proper to quantify replant impact on understock vigour across localities of nursery production-sites (Lentzsch et al., 2019).

The replant disease is known for a wide variety of horticultural crops, including pome and stone fruits (e.g. Traquair, 1984), berries (e.g. Jagdale et al., 2013), as well as floricultural crops such as roses (e.g. Baumann et al., 2020). These crops have in common, that the causalities of the culture-specific replant disease are largely unknown and thus, the handling of replant effects challenges growers. The culture-specificity of replant disease suggests that replant-causally linked agents differ between cultures. This study is focused on the estimation and mitigation of replant effects on apple

fruit production. The methodological approach presented in this study is nevertheless assumed to be transferable on other replant affected cultures. A transformation of the methodological approach will require an identification of culture-specific replant-sensitive plant- and soil-parameters.

Summing up, the methodological approach is an appropriate measure for site-specific estimation of replant impact in different localities and stages of apple production (nursery/orchard). For an estimation across localities and production stages further research should still address the detection of additional replant-indicative parameters of cross-site validity (transfer function). On a larger scale the comparative estimation of replant impact enables to identify particular replant-sensitive fruit growing regions, that require an intensive soil-management of replanted production sites thereby contributing to a preservation of fruit growing regions by sustainable apple production when fruit growers are aware of the replant impact and have the possibility for a timely and systematic management of replanted sites. At present, results of this study contribute to a practical handling of replant disease while fruit growers can estimate the impact of replant in its present extent and also help to mitigate losses on already established orchards.

4. Conclusion

Commercial fruit growers are faced with a wide range of challenges to maintain an economically viable fruit production while meeting demand for sustainable land use at the same time. One basic challenge in apple fruit production is the 'Apple Replant Disease' (ARD) related to losses of vegetative and generative yields. Replant disease strongly limits an economically feasible production of apple and sustainable use of soils cultivated by apple, for several decades.

In this study, a hands-on approach for estimation of replant impact on orchard performance and its mitigation by biological soil management strategies is submitted. By in-field studies on fruit orchards the impact of replant disease is examined by conditions of commercial fruit production.

Plant reaction of apple trees in replant soil by suppression of tree vigour is highly correlated with abundances of soil fungal population (*Alternaria* group, Ag) and densities of soil aggregate size fractions (125-100 µm, 2000-6300 µm) as well as stabilities of large micro- and small macro-aggregates (125-250 µm, 250-500 µm) at specific time intervals during vegetation period. Result suggest that soil aggregation processes related to densities of soil fungal population may be causally linked to the interaction of apple tree and replant soil. An analysis of the interaction between apple

tree and soil requires a continuous and densely gridded monitoring of soil performed on single planting spot.

To estimate impact of replant on tree vigour, the knowledge of the causal agents of the disease is not of urgent need. In this study, for quantification of replant impact an indication algorithm is established according to the relation of plant-parameter (CSA) and replant-sensitive soil-parameter (abundance of Ag). The quantification of replant effects based on the indication algorithm is applicable for the early years of production on plantation as well as the full production phase in established orchards.

Within orchards, the replant effect appears in different strengths between individual trees and proceeds discontinuously within rows. By quantification of replant effects on single tree level the site-specific replant impact on tree vigour and related losses of fruit yields are precisely estimated on orchard level.

Replant impact on tree vigour can be mitigated by biological soil management strategies. In this study, two biological soil management strategies: AMFbac treatment and MDK treatment are tested. The MDK treatment has not been associated previously with mitigation of replant impact on apple tree vigour. Both treatments could replace chemical soil treatments mitigating replant disease. In contrast to usual mitigation strategies, tree vigour is enhanced by initial treatment of soil (AMFbac treatment, MDK treatment) at time of planting, as well as by treatment of soil on established orchards (MDK treatment). Replant impact on tree vigour is mitigated or can be fully prevented by initial and additionally repeated (regular) treatment of soil. By this, replant affected orchards can be cultivated without additional losses in yield by the end of economically determined useful life. This is of particular importance in the context of an increasing intensification in apple production system and sustainable land use in traditional fruit growing regions within limited geographical areas. By treatment of suitable soil management strategies replant-related losses in fruit yield can be mitigated in the long term, and thus, regional fruit production can be stabilised by improvement of soil health and fertility.

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List of Publications

This dissertation is based on the following peer-reviewed paper. The following numbering reflects the order of the paper presented in this thesis.

Cavael, U., Tost, P., Diehl, K., Büks, F., Lentzsch, P. (2020). Correlations of soil fungi, soil structure and tree vigour on an apple orchard with replant soil. *Soil Syst*, *4* (4), 70-85. DOI: 10.3390/soilsystems4040070

Cavael, U., Diehl, K., Lentzsch, P. (2020). Assessment of growth suppression in apple production with replant soils. *Ecol Indic*, *109*, 105846. DOI: 10.1016/j.ecolind.2019.105846

Cavael, U., Lentzsch, P., Schwärzel, H., Eulenstein, F., Tauschke, M., Diehl, K. (2021). Assessment of agro-ecological apple replant disease (ARD) management strategies: organic fertilisation and inoculation with mycorrhizal fungi and bacteria. *Agronomy*, *11*(2), 272-289. DOI: 10.3390/agronomy11020272

Additional peer-reviewed publications (co-) authored in the course of this PhD-study:

Cavael, U., Diehl, K., Lentzsch, P. (2020). Impact assessment of apple replant disease (ARD) by indication at single tree level in orchards. IX International Symposium on Soil and Substrate Disinfestation. *Acta Hortic*, *1270*, 281-294. DOI: 10.17660/ActaHortic.2020.1270.34

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Declaration in accordance with § 8 of the General Provisions for Doctoral Degrees at the University of Kassel dated 14.07.2021.

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Erklärung über den Eigenanteil an den veröffentlichten oder zur Veröffentlichung vorgesehenen wissenschaftlichen Schriften innerhalb meiner Dissertationsschrift gemäß § 7a der Besonderen Bestimmungen des Fachbereiches Ökologische Agrarwissenschaften zu den Allgemeinen Bestimmungen für Promotionen an der Universität Kassel (AB-PromO) vom 16.01.2019.

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2. **Nummerierte Aufstellung der eingereichten Schriften (Titel, Autoren, wo und wann veröffentlicht bzw. eingereicht):**

   1. Titel: Correlations of Soil Fungi, Soil Structure and Tree Vigour on Apple Orchards with Replant Soil.
      Autoren: Ulrike Cavael, Philipp Tost, Katharina Diehl, Frederick Büks, Peter Lentzsch
      Journal: Soil Systems
      Veröffentlicht: Dezember 2020

   2. Titel: Assessment of growth suppression in apple production with replant soils.
      Autoren: Ulrike Cavael, Katharina Diehl, Peter Lentzsch

Journal:	Ecological Indicators
Veröffentlicht:	Februar 2020

3. Titel: Assessment of Agro-Ecological Apple Replant Disease (ARD) Management Strategies: Organic Fertilisation and Inoculation with Mycorrhizal Fungi and Bacteria.

Autoren:	Ulrike Cavael, Peter Lentzsch, Hilmar Schwärzel, Frank Eulenstein, Marion Tauschke, Katharina Diehl
Journal:	Agronomy
Veröffentlicht:	Januar 2021

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