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

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Abstract: Apple replant disease (ARD) impacts the economic yield of orchards by physiological 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 pot 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 inducing 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

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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 lead to suppressed tree vigour. The effect is known as apple replant disease (ARD) [1,2]. 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) [3]. 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 [2,3], 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 [4–6]. 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 [2]. In face of the current market development with an increasing demand for fresh fruit and the consequential decreasing lifetime of orchards [7], 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 [8], soil fertilization [9] or soil inoculation by antagonistic microorganisms [10,11] as well as for resistant or tolerant rootstocks [12,13]. 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 [14,15] or integrated pest management [16]. Soil amendments such as composts or mulches, and also biological soil amendments in form of Mycorrhizae or bacterial, as well as fungal biopesticides [17,18] 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 [14].

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. Materials and Methods

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 biologi-

cal 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) [19], formalised by Diehl et al. (2020) [20].

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 (Table 1).

Table 1. Test methodology for proof-of-concept and on-farm field test.

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.

2.2. 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 (Figure 1).

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.

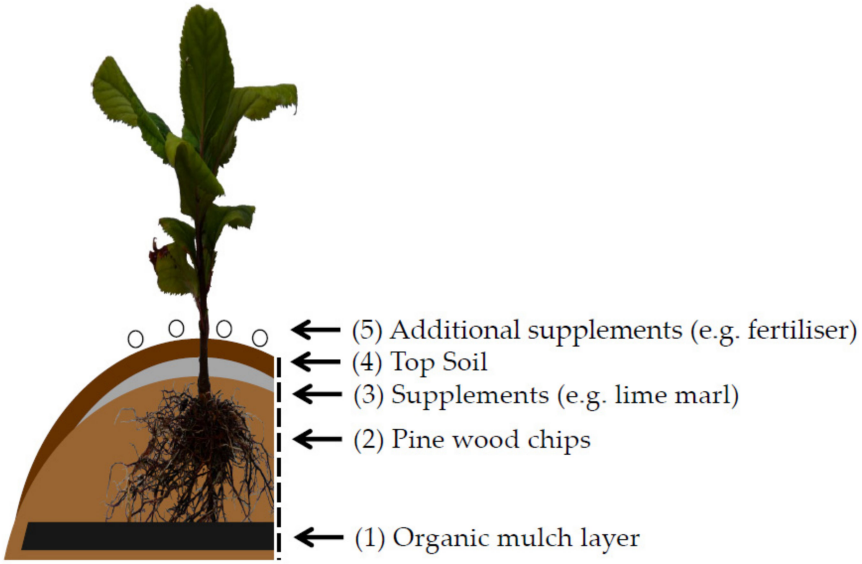

Figure 1. Formalised layers of the MDK ridge for treatment at time of planting. Adapted from Diehl et al., 2020 [20].

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 (Table 2).

Table 2. MDK Pot Experiment on replant soil (May 2016–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) [21]. 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) (Table 3).

Table 3. 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, March 2017, September 2017, January 2018	January 2018
II	nr	MDK (2016)	18		-
III	r	MDK (2009) + MDK (2016)	18		January 2018
IV (control)	r	-	18		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. 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 Hungar 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 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 ration 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 (Figure 2).

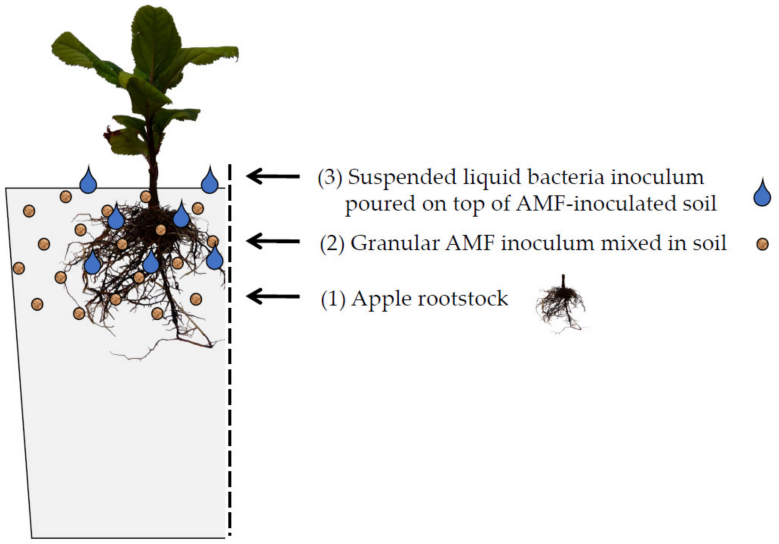

Figure 2. Formalised composition of AMFbac treatment in planting hole at time of planting.

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) (Table 4).

Table 4. 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 (Table 5).

Table 5. Pot Experiment B (May to September 2020) (16 weeks) (n = 64).

AMFbac Pot Experiment B						
Test Variant	**Soil**	**Treatment** [a,b]	**Understock**	**n**	**Tree Vigour Rating**	**Root Rating**
I	r	AMFbac	Marc	8	May 2020, September 2020	September 2020
II	nr	AMFbac	Marc	8		
III	r	AMF	Marc	8		
IV	nr	AMF	Marc	8		
V	r	-	Marc	8		
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' north west 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) [22]. 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) (Table 6). The data for tree vigour and fine root samples were taken when grubbing-up apple understocks.

Table 6. AMFbac Field Test (April to September 2020) (18 weeks) (n = 48).

AMFbac Field Test						
Test Variant	**Soil**	**Treatment**	**Understock**	**n**	**Tree Vigour Rating**	**Root Rating**
I	r	AMFbac [1]	A2	8	September 2020	September 2020
II	r	AMFbac [2]	A2	8		
III	r	AMF	A2	8		
IV	r	bac [1]	A2	8		
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).

2.4. 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 [23]. 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 [24].

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:

$$\text{growth rate (\%)} = ((t_x - t_0) \times 100)/t_0 \quad (1)$$

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.

2.5. 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.

2.6. 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 de-stained by rinsing in tap water. The mean percentage of root colonisation by mycorrhizal fungi was counted by the grid-line intersection method [25]. 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.

2.7. 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.

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.

3.1. Strategy 1: MDK

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 (Table 7). 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).

Table 7. MDK Pot Experiment-effect of MDK on tree vigour.

MDK Pot Experiment					
Test Variant	**Soil/Treatment**	**Under-Stock**	**CSA (cm^2) 16 May**	**Growth Rate (%) 16 May–17 April**	**Root Rating (0–2) 17 April**
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% (Table 8).

Table 8. MDK Field Test-effect of MDK on tree vigour.

MDK Field Test					
Test Variant	**Soil/Treatment**	**Under-Stock**	**CSA (cm^2) 16 November**	**Growth Rate (%) 16 November to 17 September**	**Root Rating (0–2) 18 January**
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.

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 (Figure 3).

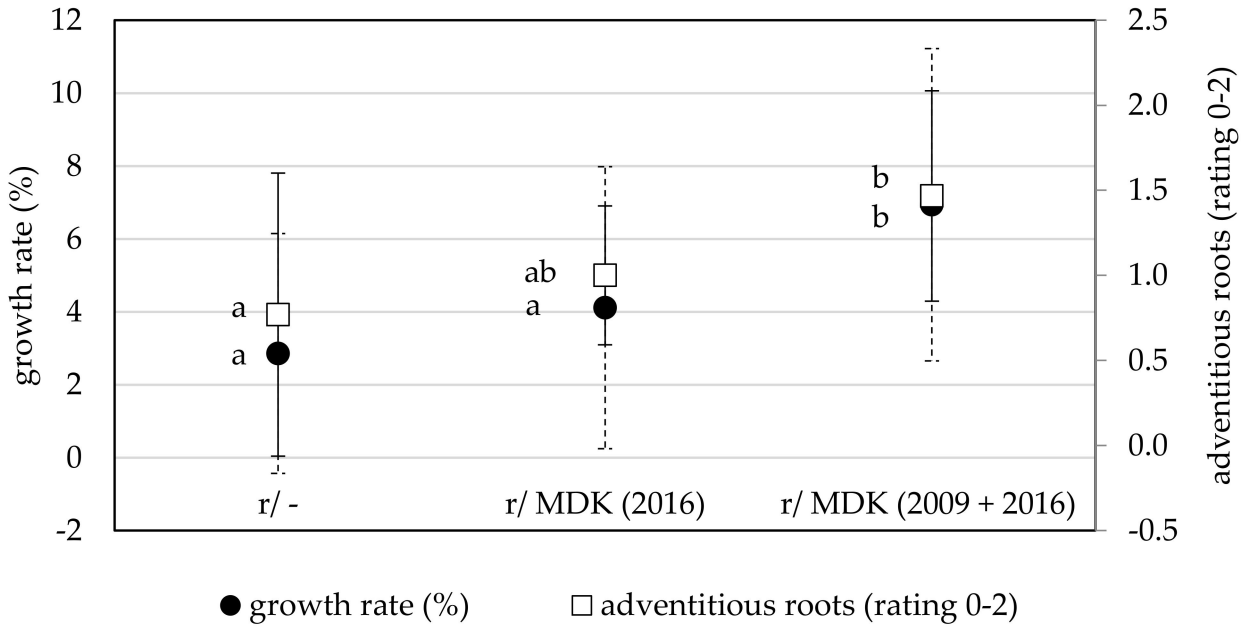

Figure 3. MDK Field Test - Parallels between vegetative growth (%) (17 November to 18 January) and rating of adventitious, January 2018. Significances calculated between test-variants, $\alpha = 0.05$, relative standard deviation.

3.2. Strategy 2: AMFbac

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 (Figure 4).

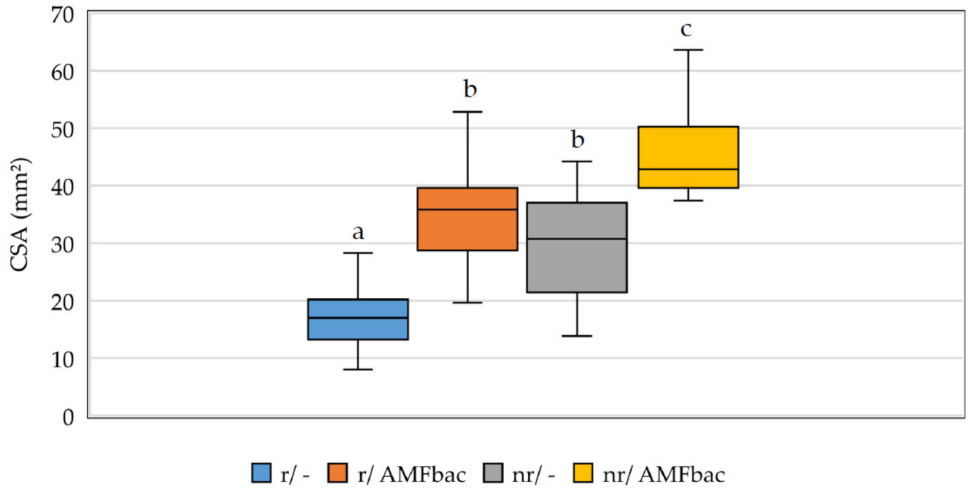

Figure 4. AMFbac Pot Experiment A-CSA (mm^2), November 2017 (week 32), $\alpha = 0.05$.

The replant effect was fully mitigated in the AMFbac treatment (Figure 4). 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 (Table 9). 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 ($p \geq 0.05$).

Table 9. AMFbac Pot Experiment A-Growth rate of CSA and degree of mycorrhizal root colonization.

AMFbac Pot Experiment A						
Test Variant	**Soil/Treatment**	**Under-Stock**	**CSA (mm^2) 17 April**	**Growth Rate (%) April–17 June (week 1 to 9)**	**Growth Rate (%) April–17 November (week 1 to 32)**	**Root Colonisation (%) 17 November (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 = 0.5)	+ 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 (Table 9). 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.

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 (Figure 5a). The AMFbac treatment was found to suppress growth rates of understocks by −6.7% (Marc) and 13.5% (B9) ($p \leq 0.10$) (Figure 5a,b).

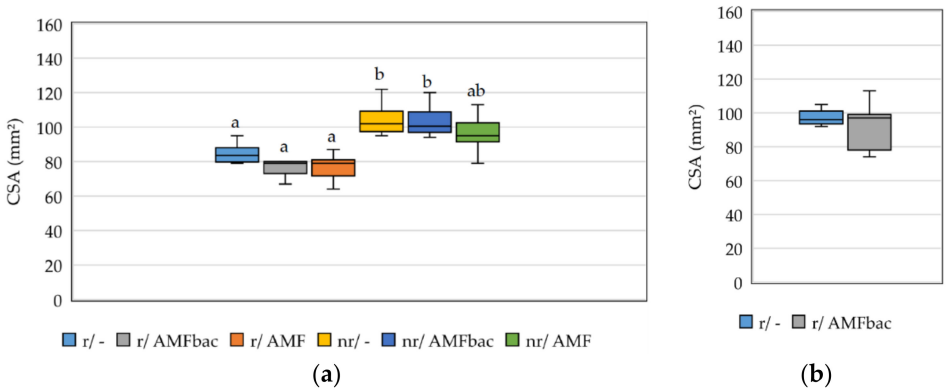

Figure 5. AMFbac Pot Experiment B-CSA (mm^2) apple understocks' type (**a**) Marc and (**b**) B9, September 2020 (week 16), $\alpha = 0.05$.

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 ($p \leq 0.10$) (Table 10).

Table 10. 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 (mm^2) 20 May**	**Growth Rate (%) May–20 September (week 1 to 16)**	**Root Colonisation (%) 20 September (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 (rel. SD = 0.1)	+ 50.1 ^{xy} (rel. SD = 0.4)	4.5 (rel. SD = 1.3)
VII	r/AMFbac	B9	81.4 (rel. SD = 0.2)	+ 17.2 (rel. SD = 1.0)	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.

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 (Figure 6, Table 11). This effect could only be observed in the full application of AMFbac, but neither for only AMF nor only bac treatment.

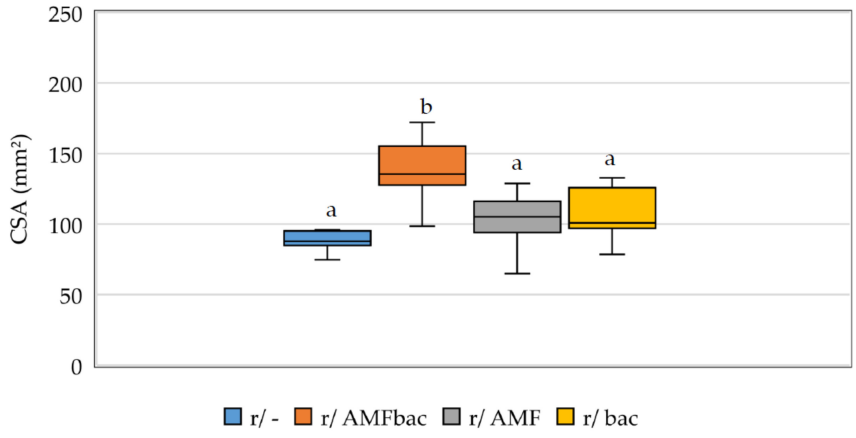

Figure 6. AMFbac Field Test-CSA (mm^2), September 2020 (week 18), $\alpha = 0.05$.

Table 11. AMFbac Field Test-Mean CSA (mm^2) and degree of mycorrhizal root colonization.

AMFbac Field Test				
Test Variant	**Soil/Treatment**	**Under-Stock**	**CSA (mm^2) 20 September (Week 18)**	**Root Colonization (%) 20 September (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 (Table 11). 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.

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.

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 [26]. 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 [27–29]. Mulching can likewise increase fine feeder root biomass with greater root density and root extending into the mulch itself [4]. This response is related to the non-systemic localised response of trees to replant soil [30]. 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 [20].

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 [31–33]. 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.

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 [34]. Effects of AMF to mitigate (apple) replant disease are presented in detail by Lü and Wu (2018) [17]. According to these studies, AMF affects and regulates soil biotic factors that are causally linked to ARD, e.g., soil and root microflora [35], 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 [36]. Soil aggregation processes are altered under replant conditions resulting in an aggregate disintegration and reduction of aggregate stability [37]. 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 [38]. 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 [39,40].

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 [41]. Perhaps with continuously improving technology for genetic analysis of AMF, the impact of AMF can be determined more precisely over time [42]. 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. Uthkede and Smith (2000) report antagonistic relationships between *G. interadices* and *B. subtilis*, as well as *E. agglomerans* in apple replant soil [43]. 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 [17].

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 [44], 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) [45]. 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 ($\pm$6) years of plantation lifetime, the long-term effects have to be considered. Uthkede 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 [43]. These results indicate that AMFbac has a potential to raise tree vigour over time by long-term effects.

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 [4,46,47]. 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 [48].

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.

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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