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# Apple Growth and Yield in Replant Soils Supplemented by Organic Soil Additives

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**Abstract:** Repeated apple cultivation in the same area leads to apple replant disease (ARD), which can probably be reduced by the use of organic supplements and selected rootstock/variety combinations. Soils at two conventionally and one organically farmed site in north-eastern Germany were tested for ARD in pot trials. In subsequent field trials, the effects of champost, microbially carbonised compost, and coniferous wood shavings piled up like a dam ('Müncheberger Damm' (M)-dam) and of rootstock/variety combinations were tested. On the organic site, only leonardite and champost were tested. The pot trials indicated for all sites that the soil is affected by ARD. After five years, the growth increase in trunks in the M-dam was 20–40% higher than in controls and other treatments, depending on the site. On one site, the yield over four years was a 15.7% increase for M-dam and also for champost compared to controls, on the other site, it was 11.7% and 3.0%, respectively. The M.9 rootstock with the Gala variety had a higher, but insignificant, yield compared to G.11/Gala by 6.7 or 2.6%, depending on the site. No difference in trunk growth or yield with Topaz was observed at the organic farmed site. Further research on M-dam and champost is supported, as both are promising in terms of yield.

**Keywords:** apple rootstock; champost; leonardite; microbially carbonised compost; Müncheberger Damm; replant soil; soil supplements



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## 1. Introduction

Apple orchards are permanent crops with a useful life of 20 years on average. The replanting of apples often happens on sites previously grown on the same or related species, resulting in growth suppression and significantly reduced productivity, known as apple replant disease (ARD). ARD occurs worldwide and on almost all soil types. It typically starts with the uneven growth of young trees. In severe cases, the majority of the trees on the site exhibit poor growth, leading to the death of young trees. Symptoms of apple replant disease extend from the roots to the trunk (including internodes and leaves), accompanied by severe stunting. Typical are the decay of affected roots and the distinct reduction of lateral root development [1–3]. Over the lifetime of an apple orchard, affected trees bear fewer fruits, and this can lead to 50% reduced profitability [4–7]. ARD is observed as a biotic stress induction factor altering photosynthetic activity, antioxidant capacity in leaves, and total phenolic compounds within the roots [8].

Main causes for ARD are attributed to biotic factors and so far mainly to an imbalance in the microbial community in the rhizosphere, including infestations of young plant roots with soil-borne fungi, e.g., *Cylindrocarpon destructans*, *Rhizoctonia solani*, and *Fusarium* spp., Oomycetes like *Pythium ultimum*, *Phytophthora cactorum*, actinomycetes and bacteria like *Bacillus* sp. and *Pseudomonas* sp. [3,9–14]. Occasionally, parasitic nematodes, mainly *Pratylenchus penetrans*, contribute to ARD [5,7,15]. The release of toxic and autotoxic compounds during the decomposition of crop residues can also significantly worsen the conditions for apple replanting [16].

The soil conditions, in particular the clay content, influence the level of expression of ARD. Fazio et al. [17] observed much stronger apple growth in clay soil than in sandy soil, and Mahnkopp et al. [18] ascertained growing problems and subsequent high yield losses of apples cultivated on sandy soils and showed a positive correlation between ARD symptoms and the C/N ratio. Poor soil structure and drainage, excess, or lack of soil moisture, and heavy metal contamination, all responsible for soil structural, pH, and nutrient changes during many years of cultivation, may also contribute to the occurrence of ARD [7,11,19,20]. Since the proportion of clay minerals strongly influences soil fertility, in particular the stabilisation of organic matter and the microbial population [21], an improvement in ARD symptoms can also be expected. Schimmel et al. [22] observed in soil amended with clay a promising effect on apple seedling rootstocks.

Fertilisation that does not meet requirements can also significantly worsen the conditions for apple replanting [16]. Organic fertilisers such as composts, biochar, and dried pine bark, with poorly available nitrogen and often a high C/N ratio, have a suppressive effect and contribute to humus formation and thus to soil improvement and disease suppression. Organic fertilisers with a narrow C/N ratio and/or readily available nitrogen (liquid manure, dry chicken manure), like mineral fertilisers, support the nutrient effect on the plants, but not the build-up of humus. Humus has a specific, multiple positive influence on the physical, chemical, and microbiological properties of soil. Implementing organic amendments, e.g., compost, in nutrient-poor orchards can have positive effects on yield through their ability to supply nutrients, improve soil water holding capacity, and contribute positively to the microbial activity and suppression of soil-borne pathogens [4,23,24].

It is known that apple rootstocks have different levels of ARD tolerance and that rootstock genotypes alter the microbial community structure [24,25]. Up to now, East Malling rootstocks (M.9 and M.26) have mainly been used for professional cultivation in Germany and Europe. In the USA, a large-scale breeding programme focused on disease resistance to biotic stress factors and soil-borne diseases that occur in all apple-growing regions. For some years now, the Geneva® rootstock series G.11 has been described as promising for replanting [26].

In the north-eastern region of Germany, Brandenburg, with the dilluvial sands (light loamy sand) typical of the region, the complex syndrome of ARD in apple orchards has become increasingly severe over the last two decades. Stunted growth and lower yields can be seen as early as two to three years after replanting young apple trees. Practicable control strategies to overcome the replant disease are limited. Feasible options are soil amendment via incorporation of different types of compost, cultivating tolerant apple rootstocks, and using modified crop management like adaptation of the cultivation method 'Müncheberger Damm' (M-dam), which already has achieved a positive effect on stone fruit and blueberry production [27]. This special form of cultivation is based on dam layering, including substrate culture with coniferous wood shavings (grain size 0–40 mm) around the apple tree. The substrate layering imitates the natural biological metabolic processes of mixed forests and fosters root growth [28].

Our study evaluated the effects (five years) of soil treatments with compost, microbially carbonised compost, and coniferous wood shavings as M-dam on newly planted apple trees at three sites, two of which are conventionally managed and one organically managed, located in orchards where apples have been grown previously. Our hypothesis was that the soil treatments would improve the development of young apple trees and increase their yield. We also investigated the influence of the rootstocks M.9 and G.11 and hypothesised that yield and stem growth would be higher on G.11. First, we carried out bioassays to determine whether the soils were contaminated with ARD and to find a classification of ARD grade.

## 2. Materials and Methods

### *2.1. Site Description and Soil Parameters*

For the field trials, two apple orchards with conventional cultivation, site A (52°18′20.9″ N 14°26′44.9″ E) and site B (52°16′37.5″ N 14°27′05.7″ E), and one with organic cultivation, site C (52°16′37.6″ N 14°27′40.8″ E) in northeast Germany, were selected for new plantings. The areas are classified as replanting areas since apples have been growing for 25 years (sites A and C) and 38 years (site B) in these areas. Cereals were grown in 2015 and 2016 after clearing the apple trees. Before preparing the sites for the trials from each site, 16 samples from the top soil layer (0–30 cm) were taken with a soil probe (18-mm diameter) and pooled with thorough mixing to analyse soil properties, like clay content, organic substance (OS), pH-value, nutrients, and C/N ratio (Table 1). For sample preparation, the soil samples were dried and ground to <2 mm prior to analysis. The determination of soil parameters and nutrients was performed according to DIN-standards (Supplementary Table S1). Plant-available extraction of magnesium was performed with calcium chloride solution; phosphorus and potassium were extracted using double lactate; total C, N, and S were determined by dry combustion in a CNS analyser (Vario EL cube, Elementar, Hanau, Germany); pH was measured using calcium chloride.

**Table 1.** Soil properties, pH-value, and nutrients in soil (mg/100 g) of sites A, B, and C.

| Site | Clay (%) | Soil Class | OS (%) | pH | C/N Ratio | N Total | C Total | S Total | P | K | Mg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | (mg/100 g Soil Dry Matter) | | | | | |
| A | 8.0 | lS | 1.3 | 6.0 | 10.1 | 78.8 | 800 | 13.2 | 9.3 | 12.2 | 9.3 |
| B | 5.9 | lS | 2.0 | 5.7 | 11.1 | 111.7 | 1235 | 15.8 | 5.0 | 12.1 | 10.3 |
| C | 7.0 | lS | 1.5 | 6.0 | 11.8 | 82.3 | 973 | 20.3 | 7.1 | 10.3 | 6.5 |

OS: organic substance; lS: slightly loamy sand. N (nitrogen), C (carbon); S (sulphur); P (phosphorus), K (potassium), and Mg (magnesium).

For determining the occurrence of micro-organisms among other plant pathogenic ones, some of the soil samples were sent to an external laboratory (Scientia Terrae Research Institute, Antwerp, Belgium). The presence and abundance of pathogenic fungi were analysed there using the DNA Multiscan technique, which uses an array of genus- and species-specific DNA fragments to detect and quantify a variety of mainly plant-related pathogenic fungi. The intensity of the DNA hybridisation signal is scored from 0 (not detected) to 6 (strongest signal). DNA hybridisation signals were found for Alternaria, Fusarium, Pythium, and Verticillium. Signal intensities were highest for *Pythium* spp., *P. sylvaticum*, *P. ultimum*, and *Fusarium* spp. At site B, all detected microorganisms were found with more or less medium to high signals; at site A, no *Verticillium* spp. and in site C, no *Alternaria* spp. and *P. ultimum* were detected (Supplementary Table S2A).

Soil contamination with migratory root nematodes was investigated by the Baermann funnel method, a classical nematology technique, isolating active nematodes from the substrates. The nematodes were determined via microscopy and DNA sequencing analysis through the official national plant protection organisation of Brandenburg (LELF). Their number was not classified as high for all sites (Supplementary Table S2B).

### *2.2. Pot Trials—For Testing ARD and Additives in Soils*

To find out whether growth and yield decline in the selected sites are related to soil sickness and to find ARD classification grades, bioassays in pots were carried out with soils from site A = soil A, site B = soil B, and site C = soil C in the greenhouse (Table 2). The experiments were completely randomised in each case.

For the first pot trial, some of the soils A, B, and C were steam sterilised in a heater for one hour at 100 °C and mixed with the nonsterilised soils in mixing ratios similar to Otto and Winkler [29]: (i) nonsterilised soils, (ii) sterilised soils, soil mixtures in a volume ratio ($v/v$) of nonsterilised to sterilised soil (iii) 1:2 and (iv) 2:1. M.26 apple seedlings (source: Leibniz University Hannover, Germany) with 10 cm shoot length were planted

in 1.2 L pots and fertilised with nutrient solution per litre of soil (N: 200 mg; P: 60–80 mg; K: 200 mg; Mg: 50–90 mg; S: 30–80 mg, Fe: 10 mg; B: 2 mg; Zn: 2 mg; Cu: 1 mg; Mo: 0.5 mg). The number of replicates was 10. Plants were grown by natural daylight in a greenhouse from the beginning of April to the beginning of June, and the temperature was set at 22 °C/18 °C at 12–14/12–10 h day/night rhythm. Shoot length was measured 2, 4, 6, and 8 weeks after planting to calculate the increase in shoot growth (GI). The dry matter (DM) of the shoots and roots and the total biomass as a sum of both were determined at the end of the experiment, after 8 weeks.

**Table 2.** Overview of performed pot trials.

| | **1. Pot Trial** | **2. Pot Trial** |
|---|---|---|
| Treatments | Nonster soil;<br>Ster soil, 100 °C, 1 h;<br>Nonster: ster = 2:1 (*v*/*v*);<br>Nonster: ster = 1:2 (*v*/*v*) | Nonster soil;<br>Ster soil 50 °C;<br>Ster soil 100 °C |
| Root stock | M.26 | 'Bittenfelder Sämling' |
| Site | A, B, C | A, B, C |
| Test specimen | 10 | 8 |
| Measurements | GI, shoot, and root DM | GI, shoot, and root DM |
| Model | two-factorial | two-factorial |
| Experiment duration | 8 weeks | 8 weeks |

Nonster = nonsterilised; ster = sterilised; 1 h = one hour; (*v*/*v*) = volume per volume; GI = growth increase; DM = dry matter.

In the second pot trial, soils A, B, and C were either (i) nonsterilised or steam sterilised at (ii) 50 °C or (iii) 100 °C. Seedlings of the variety 'Bittenfelder Sämling' (Lodder-UNTERLAGEN GmbH, 48249 Dülmen, Germany) with a shoot length of 10 cm were planted in 2.5-L pots filled with 2 L of the respective soils. Cultivation, fertilisation, and recording of the test characteristics were carried out in the same way as the first pot trial. The number of replicates was 8. Plants were grown by natural daylight and temperature in an open-sided greenhouse from the beginning of June to the beginning of August. Additionally, necrotic symptoms on roots were evaluated after 12 weeks. Roots were washed and assessed visually according to the following assessment levels of necroses: 1 = 0–10%; 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; 5 = 75–100%. Samples of steam sterilised (100 °C) and nonsterilised soils A, B, and C were taken randomly from three pots per treatment and analysed for DNA multiscan (see above).

### *2.3. Field Trials*

In 2017, the two-factorial trial (soil treatment and rootstock/variety combinations) including four replicates was set up as a split-block design at sites A and B (Scheme 1). The planting distance within a row was 0.875 m, with a row spacing of 3.5 m. One-year-old trees, 20 Gala, or 8 GalaRed (biologically produced; Huber-Brugger nursery; Terlano, Italy), were planted according to each factor combination and replicate. The soil treatment factor included four levels: control, champost (mushroom substrate from a biological production based on wheat straw and chicken manure; 30 t/ha) (Biopilzhof, Emstek, Germany), microbially carbonised compost (mc-compost; 30 t/ha), and 'Müncheberger Damm' (M-dam). Mc-compost was produced inhouse according to the description of Walter Witte [30]: straw-rich cow manure was composted at least for 24 months under anaerobic conditions to let micro-organisms remain active for humic acid buildup. The pH-values, C/N ratio, and macro- and micronutrients of the organic supplements were analysed in the laboratory according to the above-mentioned extraction methods for soil properties (Table 3). For the construction of the M-dam, coniferous wood shavings (80 L per running metre) were spread at a height of 20 cm and a width of 50 cm in a row. The rootstock/variety factor included four levels at site A: Gala/M.9, GalaRed/M.9, Gala/G.11, and GalaRed/G.11. The two levels at site B were Gala/M.9 and Gala/G.11. At site C, on an organically managed farm, a

single-factor block trial (four replicates) was carried out with the variety Topaz on M.9 and the soil treatments, control, champost (30 t/ha), and leonardite—biologically standardised PERLHUMUS® granules (2 kg/planting hole; Grevenbroich, Germany) (Scheme 1). The mc-champost and M-dam treatments are not certified for organic cultivation. Leonardite, a high-quality humus-based soil conditioner, was selected as a substitute.

**Site A**

| **Treatment** | **Rootstock/variety combinations** | | | | **Treatment** | **Rootstock/variety combinations** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | M.9/ | M.9/ | G.11/ | G.11/ | | M.9/ | M.9/ | G.11/ | G.11/ |
| **Block 1** | GalaRed | Gala | GalaRed | Gala | **Block 3** | GalaRed | Gala | GalaRed | Gala |
| Control | | | | | M-dam | | | | |
| Mc-compost | | | | | Champost | | | | |
| M-dam | | | | | Mc-compost | | | | |
| Champost | | | | | Control | | | | |
| | G.11/ | G.11/ | M.9/ | M.9/ | | G.11/ | G.11/ | M.9/ | M.9/ |
| **Block 2** | GalaRed | Gala | GalaRed | Gala | **Block 4** | GalaRed | Gala | GalaRed | Gala |
| M-dam | | | | | M-dam | | | | |
| Control | | | | | Mc-compost | | | | |
| Champost | | | | | Control | | | | |
| Mc-compost | | | | | Champost | | | | |

**Site B**

| **Treatment** | **Rootsock/variety combinations** | | **Treatment** | **Rootsock/variety combinations** | |
|---|---|---|---|---|---|
| **Block 1** | M.9/Gala | G.11/Gala | **Block 3** | M.9/Gala | G.11/Gala |
| Control | | | M-dam | | |
| Mc-compost | | | Champost | | |
| Champost | | | Mc-compost | | |
| M-dam | | | Control | | |
| **Block 2** | G.11/Gala | M.9/Gala | **Block 4** | G.11/Gala | M.9/Gala |
| M-dam | | | M-dam | | |
| Control | | | Mc-compost | | |
| Champost | | | Control | | |
| Mc-compost | | | Champost | | |

**Site C** **Rootstock/variety combination** M9/ Topaz

| **Treatment** | | | |
|---|---|---|---|
| **Block 1** | **Block 2** | **Block 3** | **Block 4** |
| Champost | Leonardite | Control | Leonardite |
| Leonardite | Control | Champost | Champost |
| Control | Champost | Leonardite | Control |

**Scheme 1.** Schemes of the experimental design of sites A, B, and C. M-dam ('Müncheberger Damm'); mc-compost (microbially carbonised compost).

**Table 3.** PH-value and nutrients (g/kg) of used organic supplements, champost, microbially carbonised (mc-)compost, coniferous wood shavings for M-dam, and leonardite.

| Organic Supplement | pH | C/N Ratio | N Total | C Total | S Total | P | K | Mg |
|---|---|---|---|---|---|---|---|---|
| | | | | | (g/kg Dry Substance) | | | |
| Champost | 6.8 | 13.8 | 20.70 | 285.97 | 17.95 | 2.50 | 20.95 | 1.45 |
| Mc-compost | 6.9 | 10.7 | 14.19 | 152.50 | 3.16 | 2.82 | 16.09 | 0.77 |
| M-dam | 5.2 | 75.0 | 5.53 | 414.50 | 2.05 | 9.25 | 17.75 | 0.86 |
| Leonardite | 7.2 | 56.0 | 6.20 | 347.50 | 40.10 | 0.02 | 0.05 | 2.82 |

N (nitrogen), C (carbon); S (sulphur); P (phosphorus), K (potassium), Mg (magnesium).

The orchards were cultivated, fertilised, and watered as usual, and plant protection was in accordance with good farming practices under the support of a cultivation advisor. Treatment effects were evaluated each year, starting in May. The average trunk diameters (d) of the trees per treatment and rootstock/variety combination were measured at 0.20 m above the grafting point using a calliper gauge (two measurements per trunk). The arithmetic means of two diameter measurements (d) were calculated and applied for trunk cross-sectional areas (CSA) based on the formula CSA = $\pi$ ($\frac{1}{2} \times d$)$^2$. From 2020 onward, the yield of 5 trees for each treatment × rootstock/variety combination was recorded separately in September.

### 2.4. Statistical Analyses

The two-factorial pot experiments were evaluated for possible treatment, variety, site, and interaction effects using ANOVA in Statistica® v13.5 (TIBCO Software Inc., Palo Alto, CA, USA). Significant differences were tested with the Tukey test, with $p \leq 0.05$ set as the threshold for statistical significance. The data from the two-factorial strip-block field trials were analysed for possible treatment, variety, or interaction effects using the F-test. Mixed models were fitted via the 'PROC MIXED' procedure in SAS 9.4 (SAS Institute, Cary, NC, USA). Pairwise significant differences between treatments were determined using the Tukey test ($p \leq 0.05$). Variance homogeneity and the normal distribution of the residuals were previously controlled.

Statistical evaluation was performed for sites A and B as a two-factorial strip plot with 4 soil treatments and rootstock/variety combinations in 4 blocks (=replicates). Site C was evaluated statistically as a one-factorial experiment. The Kruskal–Wallis test was used to compare the scoring of root necroses.

## 3. Results

### 3.1. Pot Trials

In the first pot trial, the interaction between soils A, B, and C and the four treatments was only significant in shoot mass and total biomass (Table 4). Across all three soils, the root DM and the GI of the plants showed a higher value in steam sterilised soil and in the soil with a mixing ratio of nonsterilised and sterilised soils at 1:2 ($v/v$) (Table 5). For soil A, no significant differences were found between nonsterilised and sterilised soils, nor for the mixtures. Shoot DM and total biomass of M.26 plants were higher in steam-sterilised soils B and C and also in the mixing ratio of nonsterilised and sterilised soils C at 1:2 ($v/v$) (Table 5).

**Table 4.** *p*-values for site, treatment, and interaction effects on shoot and root dry mass (DM), total biomass, and growth increase in pot trials 1 and 2 as calculated from the variance analysis.

| | Shoot DM | Root DM | Total Biomass | Growth Increase |
|---|---|---|---|---|
| Pot trial 1 | | | | |
| Treatment | 0.000 * | 0.000 * | 0.000 * | 0.000 * |
| Site | 0.032 * | 0.377 | 0.046 * | 0.005 * |
| Site × treatment | 0.008 * | 0.066 | 0.008 * | 0.060 |
| Pot trial 2 | | | | |
| Treatment | 0.000 * | 0.000 * | 0.000 * | 0.000 * |
| Site | 0.003 * | 0.081 | 0.004 * | 0.063 |
| Site × treatment | 0.701 | 0.188 | 0.631 | 0.424 |

* Significant at $p \leq 0.05$.

**Table 5.** Dry matter (g per plant) from shoots, roots, and total biomass and growth increase (cm per plant) of young seedlings of apple rootstock M.26 grown eight weeks in pots with soils A, B and C, either nonsterilised (nonster), steam sterilised at 100 °C (ster) or mixed in ratios 1:2 or 2:1 ($v/v$) (pot trial 1).

| Effect | Shoots (g/Plant) | Roots (g/Plant) | Total Biomass (g/Plant) | Growth Increase (cm/Plant) |
|---|---|---|---|---|
| Soil treatment | | | | |
| Nonster | 4.82 ± 0.29 | 0.79 ± 0.06 b | 5.60 ± 0.33 | 15.48 ± 0.96 b |
| Ster | 7.49 ± 0.23 | 1.17 ± 0.04 a | 8.66 ± 0.27 | 23.12 ± 0.45 a |
| 1 nonster: 2 ster | 6.18 ± 0.35 | 1.07 ± 0.07 ab | 7.25 ± 0.41 | 19.94 ± 1.02 a |
| 2 nonster: 1 ster | 5.02 ± 0.38 | 0.86 ± 0.07 b | 5.88 ± 0.44 | 16.34 ± 1.24 b |

**Table 5.** *Cont.*

| Effect | Shoots (g/Plant) | Roots (g/Plant) | Total Biomass (g/Plant) | Growth Increase (cm/Plant) |
|---|---|---|---|---|
| HSD ($p = 0.05$) | 1.07 | 0.22 | 1.25 | 3.19 |
| Source site of soil | | | | |
| A | 6.12 ± 0.21 | 0.97 ± 0.05 a | 7.10 ± 0.25 | 20.06 ± 0.64 a |
| B | 5.54 ± 0.39 | 0.94 ± 0.06 a | 6.48 ± 0.44 | 17.41 ± 1.10 b |
| C | 6.21 ± 0.39 | 1.04 ± 0.07 a | 7.24 ± 0.45 | 19.20 ± 1.14 ab |
| HSD ($p = 0.05$) | 0.84 | 0.17 | 0.97 | 2.49 |
| Site/soil treatment | | | | |
| A/nonster | 5.64 ± 0.40 a | 0.87 ± 0.10 | 6.50 ± 0.48 a | 18.09 ± 1.30 |
| A/ster | 6.99 ± 0.25 a | 1.08 ± 0.06 | 8.07 ± 0.30 a | 23.13 ± 0.65 |
| A/1 nonster: 2 ster | 6.09 ± 0.43 a | 1.00 ± 0.12 | 7.09 ± 0.53 a | 20.28 ± 1.04 |
| A/2 nonster: 1 ster | 5.86 ± 0.47 a | 0.97 ± 0.11 | 6.84 ± 0.54 a | 19.08 ± 1.39 |
| HSD ($p = 0.05$) | 2.47 | 0.52 | 2.87 | 7.36 |
| B/nonster | 4.12 ± 0.30 b | 0.74 ± 0.06 | 4.85 ± 0.34 b | 13.29 ± 1.29 |
| B/ster | 7.89 ± 0.51 a | 1.24 ± 0.10 | 9.13 ± 0.58 a | 23.50 ± 0.86 |
| B/1 nonster: 2 ster | 5.25 ± 0.56 b | 0.92 ± 0.09 | 6.17 ± 0.63 b | 17.06 ± 1.91 |
| B/2 nonster: 1 ster | 3.72 ± 0.58 b | 0.71 ± 0.11 | 4.42 ± 0.66 b | 12.63 ± 2.38 |
| HSD ($p = 0.05$) | 2.47 | 0.52 | 2.87 | 7.36 |
| C/ nonster | 4.59 ± 0.63 b | 0.75 ± 0.12 | 5.34 ± 0.73 b | 14.75 ± 2.02 |
| C/ster | 7.52 ± 0.37 a | 1.19 ± 0.06 | 8.71 ± 0.41 a | 22.72 ± 0.85 |
| C/1 nonster: 2 ster | 7.37 ± 0.70 a | 1.33 ± 0.14 | 8.70 ± 0.81 a | 22.79 ± 1.99 |
| C/2 nonster: 1 ster | 4.43 ± 0.62 b | 0.78 ± 0.15 | 5.20 ± 0.76 b | 13.88 ± 2.36 |
| HSD ($p = 0.05$) | 2.47 | 0.52 | 2.87 | 7.36 |

1 nonster: 2 ster or 2 nonster: 1 ster is the respective mixed volume ratio of nonsterilised and sterilised soils. ± Standard error; different letters denote significant differences in each column at $p \leq 0.05$; if interactions were significant; if interactions were not significant, then for main effects; HSD = honest significant difference.

In the second pot trial, no interaction between site and treatment was observed (Table 4). Soil sterilisation at 100 °C had the highest promoting effect on shoot and root DM, total biomass, and GI of shoots (Table 6). Soil A had the highest and soil C had the lowest growth results for the characteristics of shoot DM and total biomass (Table 6).

**Table 6.** Dry matter (DM) (g per plant) from shoots, roots, and total biomass and growth increase (cm per plant) of young apple seedlings 'Bittenfelder Sämling' grown eight weeks in pots with soils A, B, and C, either steam sterilised (ster) at 50 °C or at 100 °C or nonsterilised (nonster) (pot trial 2).

| Effect | Shoot DM (g/Plant) | Root DM (g/Plant) | Total Biomass (g/Plant) | Growth Increase (cm/Plant) |
|---|---|---|---|---|
| Treatment | | | | |
| Nonster | 4.21 ± 0.37 b | 1.45 ± 0.09 b | 5.65 ± 0.43 b | 29.64 ± 1.96 b |
| Ster 50 °C | 4.67 ± 0.34 b | 1.60 ± 0.09 b | 6.27 ± 0.42 b | 30.29 ± 1.88 b |
| Ster 100 °C | 6.66 ± 0.29 a | 2.08 ± 0.11 a | 8.74 ± 0.37 a | 42.28 ± 1.36 a |
| HSD ($p = 0.05$) | 1.09 | 0.32 | 1.32 | 5.89 |
| Source site of soil | | | | |
| A | 6.06 ± 0.43 a | 1.87 ± 0.11 a | 7.93 ± 0.53 a | 37.10 ± 2.07 a |
| B | 5.02 ± 0.39 ab | 1.66 ± 0.12 a | 6.68 ± 0.48 ab | 33.57 ± 2.21 a |
| C | 4.59 ± 0.34 b | 1.63 ± 0.10 a | 6.22 ± 0.42 b | 31.90 ± 2.02 a |
| HSD ($p = 0.05$) | 1.09 | 0.32 | 1.32 | 5.89 |

± Standard error; different letters denote significant differences in each column at $p \leq 0.05$; HSD = honest significant difference.

Using DNA Multiscan, *Fusarium* spp. and *Pythium* spp. were only detected in the samples of nonsterilised soils. The DNA hybridisation signals in samples of nonsterilised

soil A were higher than in samples of soils B and C (Supplementary Table S3). Assessment of root necroses after 12 weeks based on the percentage of dark brown necroses formed showed that the lowest root necroses were in soil samples of all three sites sterilised at 100 °C. The 50 °C sterilisation did not have a sufficiently positive effect on the reduction of root necroses in the soils of the three sites (Figure 1a,b).


(**a**) (**b**)

**Figure 1.** (**a**) Images of apple roots 'Bittenfelder Sämling' grown in nonsterilised soil (**i**), sterilised at 50 °C (**ii**), and at 100 °C (**iii**) in soil of site B after 12 weeks of growth in pots (pot trial 2); (**b**) median of root necroses on apple roots 'Bittenfelder Sämling' grown in nonsterilised (nonster) and at 50° and 100 °C sterilised soils of the sites A, B, and C soils (pot experiment 2) with classifications 1 = 0–10%, 2 = 10–25%; 3 = 25–50%; 4 = 50–75%; and 5 = 75–100% necrotic lesions. Whiskers denote maximum data; different letters within each site denote significant differences for the respective site (site A: lowercase, site B: uppercase; site C: Greek letters); Kruskal–Wallis test $p \leq 0.05$.

### *3.2. Field Trials*

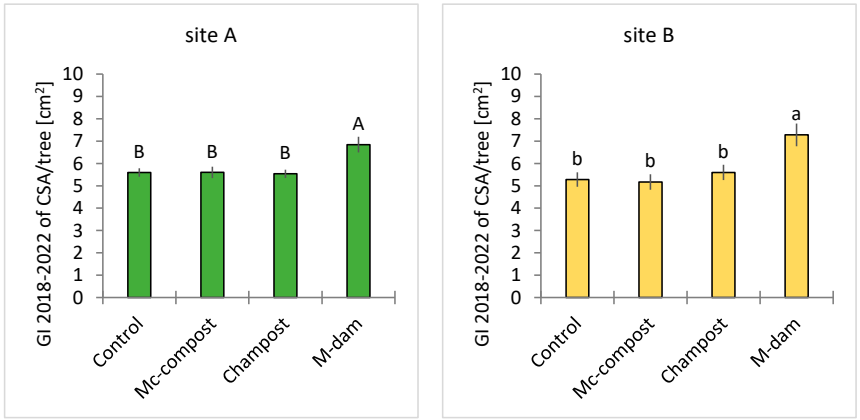
In the field trials, no interaction between treatment and rootstock/variety combinations was observed for any of the sites (Table 7). For this reason, further analysis was limited to the main effects. At sites A and B, from 2018 to 2022, the trunk CSA grew by ca. 5–7 $cm^2$, depending on which mineral or organic additive was used as a soil supplement. Trees in soils supplemented and dammed with coniferous wood shavings (M-dam) showed the most vigorous growth over the five years at sites A and B, and trunk CSA was higher than in the control (site A: 21.4%; site B: 37,7%). Champost and mc-compost had only minimally different values in the trunk CSA. (Figure 2a; Supplementary Table S4). Comparing the trunk growth of the rootstock/variety combinations at sites A and B shows that G.11 reacts differently at each site. At site A, trunk growth on M.9 was similar to that on G.11, but GalaRed had significantly stronger growth than Gala. At site B, M.9/Gala grew stronger than G.11/Gala (22.7%) (Figure 2b; Supplementary Table S4). From 2019 to 2022, the yield at site B was generally higher than at site A (Figure 3a, Supplementary Table S5). At site A, the apple yield in M-dam was 7.1% higher than in the control in 2020; and in all four years, it was higher than in the control (11.7%). There was no clear difference in yield in soil supplemented with champost or mc-compost (Figure 3a, Supplementary Table S5). The yield of the GalaRed variety on the G.11 rootstock was lower than on M.9 in 2022 (−15.0%) and over all four years (−5.4%). No differences were found between the two rootstock types in the Gala variety (Figure 3b, Supplementary Table S5). In 2020, the yield of the M-dam was higher than the control (21.3%) and mc-compost (22.7%) at site B (Figure 3a; Supplementary Table S5). Summed over the four harvest years, the yield at site B for both M-dam and champost was 6 kg per tree, or 15.7% higher than for the untreated control (Supplementary Table S5). No significant difference in yield was observed between the rootstocks, each combined with the Gala variety, at site B (Figure 3b). At site C, no difference was found

between the treatments for any of the characteristics assessed. Although not significantly different, the results show that champost and leonardite were not as effective as the control in both parameters, trunk growth and yield (Figure 4a,b, Supplementary Tables S4 and S5).

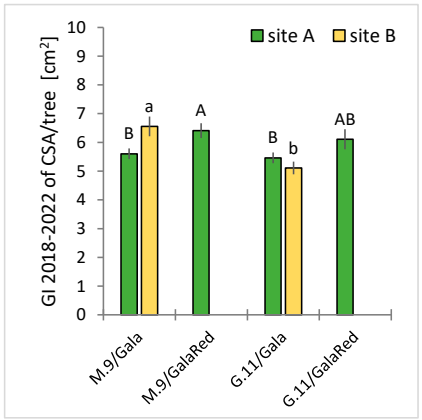
**Table 7.** *p*-values of the field trials of site C for treatment and of sites A and B for treatment, rootstock/variety combination (r/v-combi), and interaction effects of treatment and r/v-combi on growth increase in trunks (GI-TCSA), and yield per tree as calculated from the variance analysis.

| | GI-TCSA | Yield/Tree | | | |
|---|---|---|---|---|---|
| | 2018/19–2022 | 2019 | 2020 | 2021 | 2022 |
| Site A | | | | | |
| Treatment | 0.000 * | 0.341 | 0.268 | 0.126 | 0.001 * |
| R/v-combi | 0.006 * | 0.689 | 0.123 | 0.009 * | 0.012 * |
| Treatment × r/v-combi | 0.826 | 0.919 | 0.851 | 0.852 | 0.925 |
| Site B | | | | | |
| Treatment | 0.000 * | 0.236 | 0.012 * | 0.060 | 0.360 |
| R/v-combi | 0.000 * | 0.300 | 0.487 | 0.511 | 0.848 |
| Treatment × r/v-combi | 0.133 | 0.543 | 0.538 | 0.318 | 0.904 |
| Site C | | | | | |
| Treatment | 0.619 | | 0.144 | 0.443 | 0.597 |

* Significant at $p \leq 0.05$.




(**a**)




(**b**)

**Figure 2.** (**a**) Growth increase (GI) in average trunk cross-sectional areas (CSA) per tree ($cm^2$) from 2018-2022 in control and treated soils in sites A and B, and (**b**) for each rootstock/variety combination, with whiskers as standard error. Different letters within each site denote significance among the treatments for the respective site (site A: uppercase; site B: lowercase) (Tukey test; $p \leq 0.05$). Mc-compost = microbially carbonised compost; M-dam = 'Müncheberger Damm'.

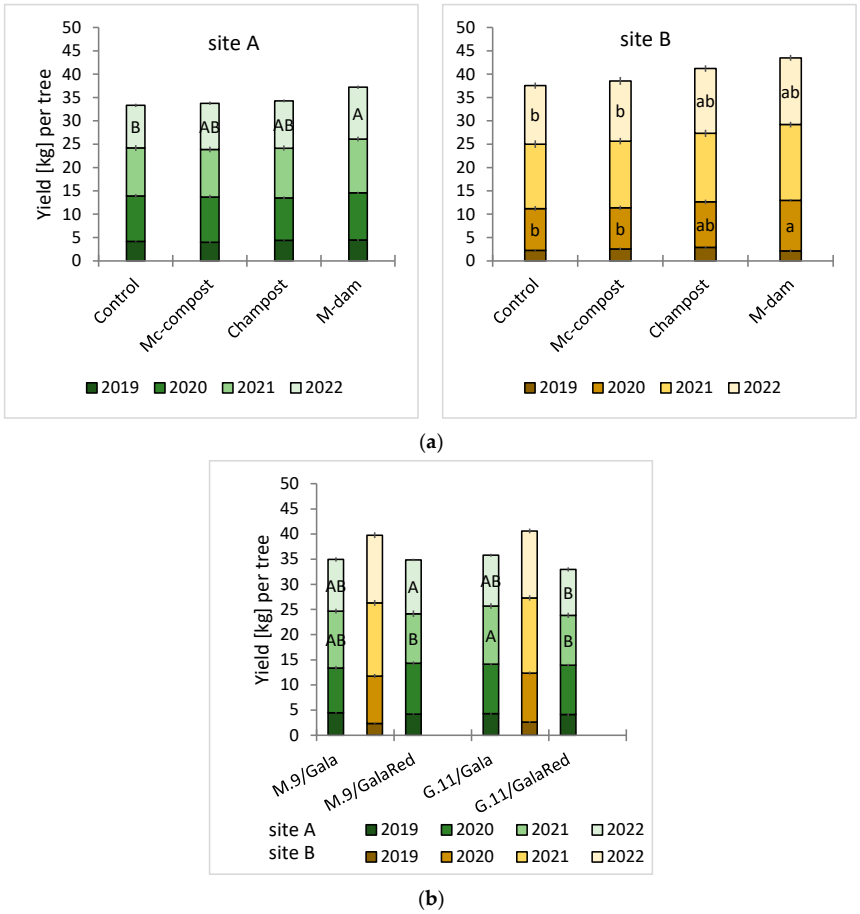



**Figure 3.** (**a**) Yield (kg) of harvested apples per tree in control and supplemented soils in sites A and B accumulated over the years 2019–2022; (**b**) and for each rootstock/variety combination, with whiskers as standard error for each harvest per site and year. Different letters within each site denote significance among the treatments for each site (site A: uppercase; site B: lowercase) and year separately; treatments without letters are not significantly different (Tukey test; $p \leq 0.05$). Mc-compost = microbially carbonised compost; M-dam = 'Müncheberger Damm'.

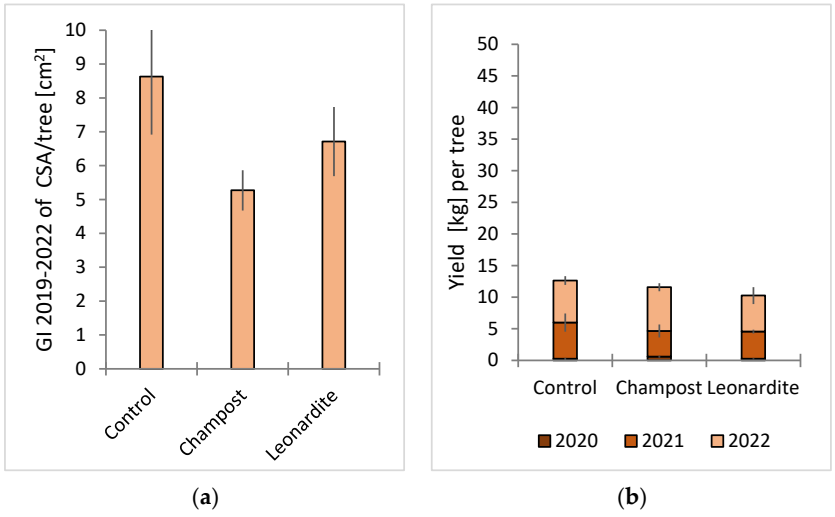



**Figure 4.** (**a**) Growth increase (GI) in average trunk cross-sectional areas (CSA) per tree ($cm^2$) from 2019–2022; (**b**) and yield (kg) of harvested apples per tree from 2020–2022 in control and treated soils in site C, with whiskers as standard error.

## 4. Discussion

In the bioassays, steam sterilisation had a positive effect on the growth increase and root growth of the plants compared to nonsterilised soils but did not yield the same results for every site. Mixing different volumes of sterilised (100 °C) and nonsterilised soils did not help to find soil sickness classification as determined by Otto and Winkler [29]; only in one soil mixture of site C (with a volume fraction of one nonsterilised and two sterilised) did we detect similar plant growth as in fully sterilised soils, thus we conclude that ARD is less severe at this site. We also could not detect any difference between nonsterilised and 50 °C sterilised soils, as Yim et al. [12] did. Steam sterilisation is commonly recommended for its effectiveness against pathogenic micro-organisms in the soil and is also known to alter the soil abiotic conditions and thus the availability of nutrients, resulting in specific plant-soil interactions [31,32]. After 12 weeks, plants grown in sterilised soils at 100 °C showed the lowest degree of root necroses, contrary to 50 °C sterilisation. Probably 50 °C was not high enough to eliminate all organisms and influence positively nutrient availability in the soil. DNA multiscans of nonsterilised and sterilised soils (100 °C) confirmed the suppressive effect on soil fungi, since mostly all organisms that appeared in the nonsterilised samples were missing in the steam-sterilised soils of the three sites (Supplementary Table S3).

In apple orchards in Brandenburg, individual solutions such as influencing the soil with additives and the choice of Malling rootstocks are already integrated into crop management. Geneva rootstocks are still not widely used in Germany. However, so far, their interaction with organic supplements has not yet been investigated under experimental conditions. This is why we focused in this five-year study on the increase in the trunk cross-sectional area (CSA) over the years as well as on the apple yield in soils supplemented with champost, mc-compost, leonardite, and coniferous wood shavings in the form of M-dam in interaction with selected rootstock/variety combinations. All four organic supplements were used with the aim of improving the organic matter and suppressive properties of the soils. With regard to the varying nutrient contents of the supplements, we did not expect any effects, as the nutrients in the supplements are present in bound form and the mineral fertilisation at the three sites was optimised according to the soil contents. Due to the major differences in cultivation and the use of soil additives, the data for conventional (sites A and B) and organic farming (site C) are compared separately. The increase in trunk CSA and yield in 2019–2022 was site-dependent, with site B generally performing better. At both sites, trunks grew bigger in M-dam. This may be due to the irrigation of the plants and the water storage capacity in the dam, which also has a positive effect on the absorption of nutrients from the soil. During repeated application of the M-dam on trees that were planted in replant soils, Diehl et al. [28] also observed a significant increase in trunk CSA when compared to the control. Based on their observations, this phenomenon is caused by the formation of adventitious roots in the additional layer (dam). In addition, the plants growing in the M-dam are able to maintain their growth phases in the summer months, unlike ARD-exposed apple trees, which have to fight the disease in the summer and shift their growth to the winter period. Van Schoor et al. [4] showed in pot trials and a 2-year field trial that compost and compost extracts used in soils associated with ARD increased seedling growth in pots and shoot growth in fields. The yield is dependent on many other abiotic influences, such as temperature, especially early frosts, and sunburn, so that the positive effects of M-dam are not as evident for yield as for trunk growth. Additionally, we assume that the impact of the stronger growth in M-dam will be more evident and bear more fruit in the following cultivation years. In all four years, yield at site A was higher in M-dam and tendentially also in champost, although not significantly. At site B, yields in M-dam and champost were similarly high and ca. 15.7% higher than the untreated control. Due to the variability of the data, the significance could not be recognised at site B. At site C, neither champost nor leonardite had any positive effect on CSA and yield, but the trunk growth in the control was more vigorous compared to the two conventionally farmed sites. No significance was observed due to the very large variation between the replicates. The initial conditions on the farm were also not optimal in terms of irrigation,

and additionally, in 2021, a canker outbreak caused by *Nectria galligena* was recorded in the field. This apparently had a negative impact on the low harvests, which were striking. Therefore, the results of this site should be seen critically.

M.9 has been the most widely used apple rootstock for years, characterised by its compact growth. GENEVA® G.11 is said to be particularly suitable for replanting soils and is popular because of its vigour and great yield efficacy [17,24,33]. This could not be confirmed in our field trials. The most popular apples for cultivation in this region are the varieties Gala and GalaRed in conventional orchards and Topaz on organic farms. In further research, the combination of rootstocks and other varieties should be tested in more detail.

It is known that organic additives have, in addition to their ability to supply nutrients and improve soil water holding capacity, disease-suppressive effects [23]. Via DNA multi-scan at the beginning (2017) and the end of the field trial (2022) (Supplementary Table S2A; Figure S1), we spotted various fungal genera like Alternaria, Fusarium, Pythium, Phytophthora, and Verticillium. These genera contain saprophytic as well as beneficial, but also plant-pathogenic species. Since we did not perform in-depth studies on the pathogenicity of the species (requiring time-consuming reisolations and pathogenicity tests), no statement can be made about their aggressiveness. We can only show that the DNA hybridisation signal levels for detected micro-organisms increased from 2017 to 2022. The soil properties of all three sites examined in 2017 showed remarkable differences in organic substances and other elementary nutrients. Although site B had the lowest clay content as compared to sites A and C, the organic substances, total nitrogen, and total carbon were comparatively higher than in sites A and C and may have been supportive in the early growth phase of planted trees. Further investigations are needed to find the interaction between plant growth, yield, pathogen occurrence, and the soil properties of the sites.

## 5. Conclusions

The approaches in this work served to ameliorate apple plant growth and yield in Brandenburg soils and highlight the importance that apple replant disease (ARD) in orchards has to be seen in interaction with soil infestation and soil parameters including organic substance and C/N ratio in soil.

The results of the pot trials showed that steam sterilisation (100 °C) had a positive effect on the root/shoot mass and an increase in shoot growth as compared to soils that were not sterilised. Therefore, it can be concluded that the soils of all three sites were affected by ARD.

In this five-year practical study, the focus of our work was set on the impact of the organic supplements, champost, microbially carbonised (mc)-compost, and 'Müncheberger Damm' (M-dam) on apple trunk growth and yield at two conventionally farmed sites. M-dam, with plots being dammed with coniferous wood shavings spread at a height of 20 cm and a width of 50 cm in a row, had the highest impact on trunk growth and apple yield at both conventionally farmed sites. Trunk growth in M-dam was ca. 21–38% higher at the two sites. Over the four yield years again, M-dam scored the highest yields (15.7%), but also, the application of champost showed a positive trend as compared to the untreated control. In one year (2022), champost and mc-compost also rendered a similar high yield, like in M-dam. With the current results, the cultivation method M-dam, although very cost-intensive, or the application of other organic materials like champost are recommended to strengthen apple growth and yield.

Malling (M.9) and Geneva rootstocks (G.11) were used in combination with the apple varieties Gala and GalaRed. Their impact was also site- and year-dependent. G.11 could not meet the high hopes as compared to M.9, which may be due to the selected varieties and their growth characteristics. In 2021, Gala on G.11 at site A rendered the highest yield, but Gala on M.9 showed a similar tendency. In 2022, GalaRed on M.9 rendered the highest yields, but Gala on G.11 brought similarly high yields. GalaRed on G.11 had in that year the lowest yields. Due to the changing significance of the yields per year and site, no clear

conclusion regarding yield and rootstock/variety combination can be drawn from this five-year study; hence, we recommend long-term practical studies (longer than five years) with more variety combinations be carried out in order to find significant results.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14040678/s1, Table S1. Determination of soil parameters and nutrients and supplements performed according to DIN-standards inhouse and by Eurofins Agricultural Analytics Germany (Jena); Table S2. (A) Analysis of pathogenic organisms in the soil samples of sites A, B, and C before the experiment (2017) and (B) Number of nematodes per $cm^2$ soil in samples of soils of sites A, B and C; Table S3. Analysis of pathogenic organisms (DNA-multiscan) in non-sterilised and sterilised (at 100 °C) soils of sites A, B, and C 12 weeks after plant growth (pot trial 1), median over three singly analysed pots; Table S4. Growth increase (GI) of average trunk cross-sectional areas (CSA) per tree ($cm^2$) from 2018–2022 in control and treated soils in sites A, B, and C; Table S5. Yield (kg) of apples harvested per tree from 2019 to 2022 in terms of treatments and rootstock/variety combination at sites A and B and from 2020 to 2022 at site C; Figure S1. DNA Multiscan hybridisation signal intensities for predominantly fungal pathogenic micro-organisms, detected in soil samples from sites A, B, and C in 2022, 5 years after the soil treatments. The intensity of the DNA hybridisation signals (displayed in the respective bars) is proportional to the amount of DNA in the soil samples: 0 (not detected) to 6 (strongest).

**Author Contributions:** Conceptualisation, C.F.; C.F., D.S. and R.D.F.-K. performed the experiments and analysed the data; writing—original draft preparation, R.D.F.-K.; writing—review and editing, R.D.F.-K., D.S. and C.F.; project administration, C.F. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data presented in this study are available upon request from the corresponding author.

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**Conflicts of Interest:** Author Daniel Schneider is employed by the company Markendorf Obst e.G. He was hired to cultivate the apple orchards. Neither he nor Markendorf Obst e.G. were involved in selecting the supplements or the rootstock/variety combinations, therefore no conflict of interest is declared for him. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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