Assessment of growth suppression in apple production with replant soils

Ulrike Cavael\textsuperscript{a}, Katharina Diehl\textsuperscript{b,*,} Peter Lentzscha\textsuperscript{a}

\textsuperscript{a} Leibniz Centre of Agricultural Landscape Research (ZALF), Eberswalder Str. 84, 15374 Müncheberg, Germany
\textsuperscript{b} University of Potsdam, Institute of Environmental Sciences and Geography, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

ARTICLE INFO

Keywords:
Orchard management
Trunk cross-sectional area
Alternaria group
Apple production
Soil fatigue
Apple replant disease

ABSTRACT

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

1. Introduction

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

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

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

In the absence of in-field studies and quantification methods, orchardists are generally not aware of the extent of underperformance in established orchards. To date, comprehensive studies of quantified economic losses or yield decline have not been undertaken beyond the field level, remaining in a stage of reflection on the interaction based on practice (e.g., Brown et al., 2000; Geldart, 1994). One main hindrance orchardists face in managing ARD is that above-ground replant effects are extremely difficult to identify in the field unless trees on no-replant soil are present for direct comparison. Effects are most obvious in the first three to five years of cultivation and become almost invisible to the eye with the increasing age of the trees (Rumberger et al., 2007).

Habitat composition and crop yield are validated for agro-climatic conditions, production system and understock-cultivar combination (Lepsis and Blanke, 2004). Alterations in the morphological root structure such as the browning of roots and root tip necrosis have been observed shortly after replanting (Grunewald-Stöcker et al., 2019; Lucas et al., 2018), accompanied by inhibited root growth, reductions in growth rate and biomass, and changes in micronutrients (Simon et al., 2020). With no practical method of above-ground screening, an estimation of yield reduction is extremely difficult for orchardists and thus present a challenge to estimating the economic effects of replant for improved orchard management or risk assessment before replantation.

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

The trunk cross-sectional area (CSA) is widely used for a practical determination of growth performance in apple trees in field, as it corresponds to root and crown volume, leaf area and yield (Lo Bianco, 2019; Lepsis and Blanke, 2004; Westwood and Roberts, 1970). Since replant-related effects are expressed by a depression of vegetative performance, CSA is affected but less directly dependent on orchard management, such as crown pruning. Dendrochronological analyses have shown that the CSA reflects environmental conditions, weather and cultivation methods (Schweingruber, 2012). Therefore, the exact expression of the correlation to above-ground tree area and yield must be validated for agro-climatic conditions, production system and understock-cultivar combination (Lepsis and Blanke, 2004).

Treatments of ARD such as soil disinfection by chemical fumigation (Gur et al., 1991; Mai and Abawi, 1978; Pitcher et al., 1966; Hoestra, 1968), chloropicrin (Spith et al., 2015), disinfection (Jackson, 1979) or gamma radiation (Yim et al., 2013) provide evidence that replant disease is caused by biological agents in the soil. Changes in the abundance of multiple fungal species including root endophytic fungi, e.g., Cylindrocarpon-like fungi, Rhizoctonia and Fusarium have been found to correlate with ARD-related plant growth impediments in orchards in central Europe (Franke-Whittle et al., 2015; Manici et al., 2013). Among microflora, different genera of oomycetes (Pythium, Phytophthora), actinomycetes or bacteria (Bacillus, Pseudomonas) have been assigned a role in the ARD complex (Tweddemehlin et al., 2011). However, the relative importance of these organisms varies between study sites.

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

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

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

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

2. Methods

2.1. Experimental design

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

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

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

| Plot                | Year | Cultivar                  | No. of trees | Initial soil | Management |
|---------------------|------|---------------------------|--------------|--------------|------------|
| Orchard A: Commercial Plantation |
| A1.1 2009           | Evelina®/M9 | 18 | No-replant | Tall-spindle |
| A1.2 2009           | Evelina®/M9 | 18 | No-replant | cultivation |
| A1.3 2009           | Evelina®/M9 | 18 | No-replant | cultivation |
| A1.4 2009           | Evelina®/M9 | 18 | No-replant | Replant |
| A2.1 2009           | Evelina®/M9 | 18 | Replant | Tall-spindle |
| A2.2 2009           | Evelina®/M9 | 18 | Replant | cultivation |
| A2.3 2009           | Evelina®/M9 | 18 | Replant | Replant |
| A2.4 2009           | Evelina®/M9 | 18 | Replant | |
| Orchard B: Experimental and Conservation Test Station |
| B1 1984             | Various/A2  | 16 | No-replant | Oescherberg |
| B2 2009             | Various/A2  | 32 | Replant | cultivation |
| B3.1 2010           | Various/A2  | 72 | No-replant | |
| B3.2 2010           | Various/A2  | 159 | No-replant | |
| B4.1 2001           | Granny Smith /M9 | 32 | No-replant | Tall-spindle |
| B4.2 2001           | Elstar (rot) | 32 | No-replant | cultivation |
| B4.3 2001           | Michielsen®/M9 | 32 | Replant | |
| B4.4 2001           | Braeburn Schneider*/M9 | 32 | Replant | |
| B4.5 2001           | Braeburn Lochbuie* /M9 | 32 | Replant | |

2.2. Selection and measurement of plant growth-related parameters

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

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

2.2.1. Above-ground tree area

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

2.2.2. Trunk cross-sectional area (CSA)

The trunk circumference of a single tree was measured using a standard folding rule at 40.0 cm above the soil surface and a millimetre scale. For each trunk circumference, the trunk cross-sectional area (CSA<sub>40</sub>) was calculated by dividing the trunk circumference (C) by the tree age, type of top-variety and understock, and cultivation on no-replant or replant soil.

2.2.3. Abundance of soil-fungal populations

Soil cores were sampled in 10.0-cm intervals to a depth of 60.0 cm from the soil surface at 10.0 cm from the tree trunk, one for each tree across the test plots and rows (A1-2, B1-4). This was conducted using a standard Puerckhauer soil sampler 100.0 cm in length and 5.0 cm in diameter. The cores were stored at 4°C. Total DNA was extracted from 0.5-g soil using the NucleoSpin soil kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The total amount of purified DNA was assessed using a Nanodrop 1000 micro-volume spectrophotometer following the Nanodrop ND-1000 standard protocol (Kisker Biotech GmbH & Co. KG, Steinfurt, Germany).

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

For the quantification of the *Alternaria* group, standard curves were generated based on dilution series of DNA from *Alternaria tenuissima* GHS05 (efficiency > 0.91 and R² > 0.998). The fungus was stored in the culture collection of microorganisms of the working group “Fungal Interactions” at the Leibnitz Centre of Agricultural Landscape Research Müncheberg. The primers and probes used for detection of Ag were as described by Grube et al. (2015), as follows (5'-3'): AlfH (forward) TCTTTTGGTACCTTTCTGTTCCCT; AlfR (reverse) TTATCGAGGCTGA TTGAAATACA; AlHP (probe) TGGTGGTCCGACACCTAGGAGCA.

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

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

In the *Alternaria*-specific qPCR, *Fusarium* strains were used as negative control and vice versa. A set of strains was selected from A. *tenuissima* (GST09t, GHS05t, and At220) and A. *alternata* (GST37a) (Kahl et al., 2016; Müller et al., 2012) as well as *Fusarium* strains of the species *G. cingulare* (Fg23 and Fg486) and *F. culmorum* (Fc13 and Fc493) partly characterised (Fc13 and Fg23) by Korn et al. (2011). Different strains of plant-associated fungal species were used as a negative control: reference strains of *Verticillium* (CBS 130663, CBS 130339, CBS 130340, DSM 12230, and CBS 447.54), *Gibellulopsis* (CBS 747.83), *Trichoderma spp.* (St365), and *Fusarium* (Korn et al., 2011).

2.3. Statistical analysis

A tree-specific value for growth suppression was determined by dividing the tree-specific ln(Ag) by the tree-specific vegetative plant performance, as represented by CSA<sub>40</sub>, relating tree vigour suppression for the single tree: Q = ln(Ag)/CSA<sub>40</sub>.

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

As the data did not follow normal distribution, Spearman’s rank
Correlation coefficients (\(\rho\)) were calculated for the total data set (\(n = 375\)). Significant correlations were accepted at \(p < 0.05\). The analyses were conducted using IBM SPSS Statistics 22.

3. Results

3.1. Correlation between plant growth related parameters

The CSA measured 40 cm above the soil surface was employed as a sensitive plant physiological parameter based on significant high correlations between \(\text{CSA}_{40}\) and the above-ground tree area. The correlation between \(\text{CSA}_{40}\) and the above-ground tree area across all trees of orchard B (B1, B2, B3 and B4) showed a logarithmic progression between both plant growth parameters, with an increase in the above-ground tree area for each increase in \(\text{CSA}_{40}\) (\(\rho = 0.892\)) (Fig. 1). The increase in \(\text{CSA}_{40}\) plateaued towards a maximum at a relationship of 200 cm\(^2\) to 200 thousand pixels/unit area. The differences in tree age had little effect on the correlation between \(\text{CSA}_{40}\) and the above-ground tree area. Although the planting years differed by 26 years, the vegetative plant performance of single trees overlapped within a range of \(\text{CSA}_{40}\) from 150 to 220 cm\(^2\) and the above-ground tree area from 140 thousand to 200 thousand pixels/tree area.

The cultivation of trees had no effect on the correlation coefficient between \(\text{CSA}_{40}\) and the above-ground tree area. Trees cultivated on no-replant soil and replant soil showed a similar correlation coefficient (\(\rho_s = 0.613\), respectively \(\rho_s = 0.676\)) (Fig. 2). There was a high correlation between both plant growth parameters across all top-varieties (\(\rho_s = 0.894\)).

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

The mean \(\text{CSA}_{40}\) of the trees cultivated on no-replant soil was significantly higher compared to the trees cultivated on replant soil (\(p \leq 0.001\)). With a mean \(\text{CSA}_{40}\) for no-replant soil of 24.1 cm\(^2\) compared to 14.5 cm\(^2\) for replant soil, the difference was 50%. A shift to a lower mean \(\text{CSA}_{40}\) was observed, which was linked to a 50% reduction in the range of variation; this shifted from 40.1 cm\(^2\) on no-replant soil to 19.3 cm\(^2\) on replant soil. Similarly, the \(\text{CSA}_{40}\) for no-replant soil showed a coefficient of variation of CV = 37.4% compared to the much less distributed CV = 27.4% for replant soil. For no-replant soil, as well as for replant soil, the distribution of \(\text{CSA}_{40}\) was based on the strong heterogeneity of \(\text{CSA}_{40}\) for trees within direct vicinity. Thus, a thick tree could stand beside a very weak tree without a trend of decreasing or increasing \(\text{CSA}_{40}\) along rows in an orchard. A stable mean of \(\text{CSA}_{40}\) was given by 18 trees. The frequency distribution of trees sorted according to \(\text{CSA}_{40}\) for no-replant and replant soils is shown below for orchard A (Fig. 3).

3.3. Abundances of soil-fungal populations on no-replant and replant soil

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

Increased abundances of the total fungal DNA were found on replant soils (Table 2), with the mean abundance of total fungal DNA being significantly increased (\(p \leq 0.01\)) and twice as high as that for no-replant soil on orchard A. The increase in abundance was less strongly pronounced for orchard B. The abundance became more heterogeneous in replant soil with a high CV in orchards A and B (respectively, 79.6%, 94%) compared to no-replant soil with a CV of approximately 35%. Conversely, total extractable soil DNA was significantly decreased (\(p \leq 0.05\)) on replant soils, with a mean soil DNA of 95.8 ng/µl (SD = 31.4 ng/µl). Replant soils also showed stronger heterogeneity, CV = 32.8%, compared to no-replant soil, with a mean of 121.9 ng/µl (SD = 10.1 ng/µl) and CV = 8.3%.

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

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

Additionally, abundances of Ag were quantified in intervals of 10 cm to a depth of 60 cm from the soil surface for no-replant and replant soil (Fig. 4). A main proportion of total Ag to a soil depth of 60 cm was amassed at soil depths 0–10 cm and 10–20 cm. For orchard B, an Ag proportion of 97% in no-replant soil and 87% in replant soil was observed at a soil depth of 0–20 cm. At the same soil depth, a proportion of 93% in replant soil was found for orchard A. An exception was a small Ag proportion of only 27% at a soil depth of 0–20 cm in the no-replant soil of orchard A. In addition, a tenfold increase in Ag abundance to 60 cm soil depth in replant soil was found to be mainly induced at soil depths of 0–20 cm; thus, soil depths of 20–60 cm were subsequently disregarded in ensuing analyses.

### 3.4. Growth suppression at the single-tree level

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

In combining contrasting Ag abundance with CSA<sub>40</sub>, we formed the quotient Q = ln(Ag)/CSA<sub>40</sub>. Thus, the tree-specific Ag abundance in soil was normalised for tree-specific vigour. This resulted in a tree-specific relation between Ag and CSA<sub>40</sub> represented by the algorithm (Q). As Q increases, the CSA<sub>40</sub> decreases, with reduced variation. ln(Ag) showed an inverse progression, and the linear adjustment of CSA<sub>40</sub> and ln(Ag) exhibited a moderate goodness of fit (R<sup>2</sup> = 0.69 R<sup>2</sup> = 0.56). Both data sets tended towards an indirect correlation (R<sup>2</sup> = −0.36) (Fig. 5).

A k-means cluster analysis was performed based on the data set of Q for all trees, resulting in five clusters with significant differences in ln(Ag) and CSA<sub>40</sub> (Fig. 6, Table 3).

Tree vigour suppression for each cluster was well separated. Cluster 1 represented trees that were not affected by replant and were considered vital (1). Tree-specific tree vigour suppression can be derived from ln(Ag) and (CSA<sub>40</sub>) in relation to cluster 1. The greatest difference between clusters was found between no tree vigour suppression (vital) (1) and escalating tree vigour suppression (2), with a reduction in tree vigour of almost 38%. The higher clusters of tree vigour suppression were followed by progressively lower additional reductions in CSA<sub>40</sub> (Table 4).

#### 3.5. Validation of ARD impact levels in orchard A

The sampling of CSA<sub>40</sub> was repeated in November 2016 for a subset of trees in orchard A (A1.2, A1.3, A2.3). CSA<sub>40</sub> was increased for each cluster within the vegetation period. A moderate good logarithmic correlation was shown compared to the tree-specific cluster value measured in April 2016 (R<sup>2</sup> = 0.79). After one vegetation period, the mean CSA<sub>40</sub> still differed between clusters and tree vigour, with

---

**Table 2**

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

| Orchard | Soil-variant | total fungal DNA (genome/g soil) | CV (%) | Ag (genome/g soil) | CV (%) | Ag/total fungal DNA (%) |
|---------|--------------|---------------------------------|--------|-------------------|--------|------------------------|
| A       | no-replant   | 5.6 × 10<sup>5</sup> (SD = 2.1 × 10<sup>5</sup>) | 37.8   | 67<sup>a</sup> (SD = 73) | 109.0  | 0.01<sup>a</sup> (SD = 0.01) |
| A       | replant      | 10.7 × 10<sup>5</sup> (SD = 8.5 × 10<sup>5</sup>) | 79.6   | 2706<sup>b</sup> (SD = 3507) | 129.6  | 0.35<sup>b</sup> (SD = 0.49) |
| B       | no-replant   | 7.8 × 10<sup>5</sup> (SD = 2.7 × 10<sup>5</sup>) | 35.0   | 322<sup>a</sup> (SD = 269) | 83.5   | 0.04<sup>a</sup> (SD = 0.04) |
| B       | replant      | 9.1 × 10<sup>5</sup> (SD = 8.6 × 10<sup>5</sup>) | 94.0   | 3025<sup>b</sup> (SD = 3960) | 130.9  | 0.33<sup>b</sup> (SD = 0.37) |

Indicated significances (p ≤ 0.05) refer to the comparison within (not between) orchards.

---

![Fig. 4. Distribution of Ag abundance in 10-cm intervals from the soil surface to 60 cm soil depth.](Image)
decreasing tree vigour accompanying an increasing level of replant impact. Thus, the trees remained within their specific cluster of tree vigour suppression (Table 5).

3.6. Growth and yield suppression at the field level

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

Table 3
Mean Q per cluster and significant differences in CSA40 and ln(Ag) between clusters.

| Cluster | Q   | Q 95% CI | CSA40 (cm²) | ln(Ag) (genome/g soil) | 95% CI |
|---------|-----|----------|-------------|------------------------|--------|
| (1)     | 0.16| [0.15, 0.17] | 29.1a       | 4.7a                    | [4.0, 5.3] |
| (2)     | 0.36| [0.38, 0.35] | 13.8        | 18.1b                   | [11.1, 16.5] |
| (3)     | 0.53| [0.59, 0.48] | 11.0d       | 3.7b                    | [6.5, 8.0] |
| (4)     | 0.78| [1.07, 0.64] | 7.5d        | 8.6c                    | [7.5, 9.6] |
| (5)     | 1.11| [2.54, 0.79] | 7.5d        | 8.3bc                   | [7.1, 9.5] |

a to d indicate significant differences (p ≤ 0.05).
the replant field, with no spatial concentration of clusters across trees or rows.

The total effect of replanting was calculated by adding the weighted reduction in tree vigour for all clusters. The result showed a replant impact of ~46% in the reduction of tree vigour compared to no-replant soil (Table 6). Based on the calculation of replant impact at the field level, the mean yields produced in our case study were calculated to be approximately 40 t/ha when replanting.

### Discussion

The potential yield for this case study location, as stated by the experience of the orchardist, is approximately 80 t/ha (pers. comm. Günzel, 2017). The calculated strong suppression of tree vigour according to our results, ~46%, halves this potential yield, thereby supporting the ARD impact of 50% stated in a previous study (Brown et al., 2008). Overall, the regional mean yields under optimal weather conditions in Brandenburg are approximately on par with the calculated mean yield under replant conditions in our study (35–40 t/ha) (Amt für Statistik Berlin-Brandenburg, 2018). This unexpectedly low mean yield across production sites in the region may be related to differences in orchard management but also in part to already widespread growth suppression due to replanting at a number of sites. With a large share of apple production sites in Brandenburg under more or less intensive use over many years, comprehensive monitoring research would be required to address the articulated concern of farmers in the region regarding replant impact in the larger production area.

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

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

Fungal abundances of the class Dothideomycetes have previously been associated with former apple tree stations on commercial and intensively managed orchards (Doeksin et al., 2018; Tilston et al., 2018; Mazza et al., 2015). Increased fungal abundances of the order Pleosporales are related to phenotypical expression of replant effects (Tilston et al., 2018). Abundances of the Alternaria group (Ag) (class Dothideomycetes, order Pleosporales, family Pleosporaceae) were strongly increased in replant soil in this study, with proven differences in the Alternaria group (Ag) abundance on a logarithmic scale between no-replant and replant soil. Abundances of Ag are here related to the phenotypical expression of replant effects on the single-tree level due to an inverse progression between Ag abundance and $CSA_{40}$. The percentage increase in Ag abundance was more strongly pronounced than the percentage increase in total fungal DNA. This results in an increased proportion of Ag in total fungal DNA in the replanting soil. Therefore, the Ag was derived as a replant-sensitive responding population. The possible function of the Ag within the system requires further investigation.

As long as individual members of the community in replant soil, as well as potential interactions, are still unknown, replant-sensitivity of the Ag can be attributed to neither primary nor secondary processes and functions (saprophytic/necrotrophic/pathogenic) in replant soil. The increase in the total fungal DNA was not compensated due to increased Ag abundance, suggesting that further populations are involved in replant-related effects in soil in a responsive manner. The methodological approach can, in principle, be adapted to other site-specific replant-sensitive soil-fungal populations.

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

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

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

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

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

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

Acknowledgements

The research for this paper was financed by the German Federal Ministry of Education and Research (BMBF) within the frame of the BonaRes Initiative (FKZ: 031B0025D), www.ordiamur.de. The authors gratefully acknowledge the work of our team for their assistance, namely, Ilona Bartelt, Petra Lange and Dr. Hilmar Świerczewski. We also thank Lutz Günzel and his team for providing access and insight into his plantation during our research cooperation.

References

Amt für Statistik Berlin-Brandenburg. 2018. Statistischer Bericht. C I 6 / j-18.

Ernteberechnungserstattung über Obst im Marktobstbau im Land Brandenburg. 2018. https://www.statistik-berlin-brandenburg.de/publikationen/statistische-recherchen/2018/SB_CO2-06-09_20180113_R.pdf (Accessed 09 April 2019).

Amt für Statistik Berlin-Brandenburg. 2017. Statistischer Bericht. C I 6 / j-17.

Ernteberechnungserstattung über Obst im Marktobstbau im Land Brandenburg. 2017. https://mlul.brandenburg.de/media_fast/4055/Marktobstbau_SB_C02-06-00_20170111_R.pdf (Accessed 13 December 2018).

Brown, P.S., Fuller, K.D., McRae, K., Fillmore, S.A.E. 2010. Response of 'Honeycrisp’ apple trees to combinations of pre-plant fumigation, deep ripping, and hog manure compost incorporation in a soil with replant disease. HortScience 45 (11), 1702-1707. https://doi.org/10.21273/HORTICS.45.11.1702.

Brown, G.S., Smith, S.B., Gibson, L., Kitchener, A., Potter, K., Jennings, D., Schimanski, L., 2014. J. Clean. Prod. 73, 125–135.https://doi.org/10.1016/j.jclepro.2013.09.017.

Cerutti, A.K., Beccaro, G.L., Braun, S., Bosco, S., Donno, D., Notarnicola, B., Bounous, G., 2014. J. Clean. Prod. 73, 125–135. https://doi.org/10.1016/j.jclepro.2013.09.017.

Cerutti, A.K., Beccaro, G.L., Baglioni, M., Donno, D., Bounous, G., 2013. Multifunctional ecological footprint analysis for assessing eco-efficiency: a case study of fruit production systems in Northern Italy. J. Clean. Prod. 40, 108–117. https://doi.org/10.1016/j.jclepro.2012.09.029.

Cesaranò, G., Zotti, M., Antignani, V., Marra, R., Scala, F., Bonanomi, G., 2017. Soil sickness and negative plant-soil feedback: a reappraisal of Hypotheses. J. Plant Pathol. 99 (3), 545–570. https://doi.org/10.4454/jpp.9903.5460.

Deakin, G., Tilston, E.L., Bennett, J., Passey, T., Harrison, N., Fernández, F., Xu, X., 2019. Spatial structuring of soil microbial communities in commercial apple orchards. Appl. Soil Ecol. 130, 1–12. https://doi.org/10.1016/j.apsoil.2018.05.015.

Ferrer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71 (7), 4117–4120. https://doi.org/10.1128/AEM.71.7.4117-4120.2005.

Franke-Whittle, I.H., Manici, L.M., Insam, H., Stres, B., 2015. Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards. Plant Soil 395 (1–2), 317–333. https://doi.org/10.1007/s11104-015-2562-x.

Fraser, C., Alm, E.J., Polz, M.F., Spratt, B.G., Hanage, W.P., 2009. The bacterial species challenge: making sense of genetic and ecological diversity. Science 323 (5915), 741–746.https://doi.org/10.1126/science.1159388.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb01858.x.

Greene, P., Byard, R., Robinson, T.L., Carpenter, S., Hoying, S.A., Iungerman, K.A., Merwin, I.A., Seidenfeld, J., 2012. Phylogenetic classification of mycorrhizae and rusts. Mol. Ecol. 21 (5), 1188–1189. https://doi.org/10.1111/j.1365-294X.2011.05017.x.

Korn, U., Müller, T., Ulrich, A., Müller, M.E., 2018. Impact of aggressiveness of Fusarium graminearum and F. culmorum isolates on yield parameters and mycotoxin production in wheat. Mycotoxin Res. 27, 3, 195–206. https://doi.org/10.1007/s12550-016-0096-5.

Lanza, G., Rebensburg, P., Kern, J., Lentsch, P., Wirth, S., 2016. Impact of chars and明晰he soil microbial community composition in a dynamic incubation experiment. Soil Tillage Res. 164, 18–24. https://doi.org/10.1016/j.still.2016.01.005.

Mazzola, M., Manici, L.M., Kelderer, M., Caputo, F., Thalheimer, M., 2012. Planting in the ‘inter-row’ to overcome replant disease in apple orchards: a study on the effectiveness of the practice based on microbial indicators. Plant Soil 357 (1–2), 381–393. https://doi.org/10.1007/s11104-011-1172-6.

Persson, P., Friberg, H., 2016. Genus-Specific Primers for Study of Fusarium Communities in Field Samples. Appl. Environ. Microbiol. 82 (2), 491–501. https://doi.org/10.1128/AEM.02748-15.

Judith, C., Alm, E.J., Polz, M.F., Spratt, B.G., Hanage, W.P., 2009. The bacterial species challenge: making sense of genetic and ecological diversity. Science 323 (5915), 741–746.https://doi.org/10.1126/science.1159388.
potential of fluorescent pseudomonads colonizing wheat heads against mycotoxin producing alternaria and fusaria. Front. Microbiol. 9, 2124. https://doi.org/10.3389/fmicb.2018.02124.

Nicola, L., Insam, H., Pertot, I., Stres, B., 2018. Reanalysis of microbiomes in soils affected by apple replant disease (ARD): Old foes and novel suspects lead to the proposal of extended model of disease development. Appl. Soil Ecol. 129, 24–33. https://doi.org/10.1016/j.apsoil.2018.04.010.

Philippot, L., Andersson, S.G.E., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nat. Rev. Microbiol. 8, 523–529. https://doi.org/10.1038/nrmicro2367.

Pitcher, R.S., Way, D.W., Savory, B.M., 1966. Specific replant diseases of apple and cherry and their control by soil fumigation. J. Hortic. Sci. 44, 379–396. https://doi.org/10.1080/00221589.1966.11514184.

Rumberger, A., Merwin, I.A., Thies, J.E., 2007. Microbial community development in the rhizosphere of apple trees at a replant disease site. Soil Biol. Biochem. 39 (7), 1645–1654. https://doi.org/10.1016/j.soilbio.2007.01.023.

Russo, N.L., Robinson, T.L., Fazio, G., Aldwinckle, H.S., 2007. Field evaluation of 64 apple rootstocks for orchard performance and fire blight resistance. HortScience 42, 1517–1525.

Schweingruber, F.H., 2012. Trees and wood in dendrochronology: Morphological, Anatomical, and Tree-Ring Analytical Characteristics of Trees Frequently Used in Dendrochronology. Springer Verlag, New York. 10.1007/978-3-642-77157-6.

Simon, M., Lehn dorff, E., Wrede, A., A melan, W., 2020. In-field heterogeneity of apple replant disease: relations to abiotic soil properties. Sci. Hortic. 259, 108809. https://doi.org/10.1016/j.scienta.2019.108809.

Spåth, M., Insam, H., Peintner, U., K elderer, M., Kuhnert, R., Franke-Whittle, I.H., 2015. Linking soil biotic and abiotic factors to apple replant disease: a greenhouse approach. J. Phytopathol. 163 (4), 267–299. https://doi.org/10.1111/jph.12518.

Tewoldemedhin, Y.T., Mazzola, M., Labuschagne, I., McLeod, A., 2011. A multi-phasic approach reveals that apple replant disease is caused by multiple biological agents, with some agents acting synergistically. Soil Biol. Biochem. 43 (9), 1917–1927.

Wolińska, A., Kuźniara, A., Zielenkiewicz, U., Banach, A., Błaszczyszk, M., 2018. Indicators of arable soils fatigue—bacterial families and genera: a metagenomic approach. Ecol. Ind. 93, 490–500. https://doi.org/10.1016/j.ecolind.2018.05.031.

Yim, B., Smalla, K., Winkelmann, T., 2013. Evaluation of apple replant problems based on different soil disinfection treatments—links to soil microbial community structure? Plant Soil 366 (1–2), 617–631. https://doi.org/10.1007/s11104-012-1454-6.