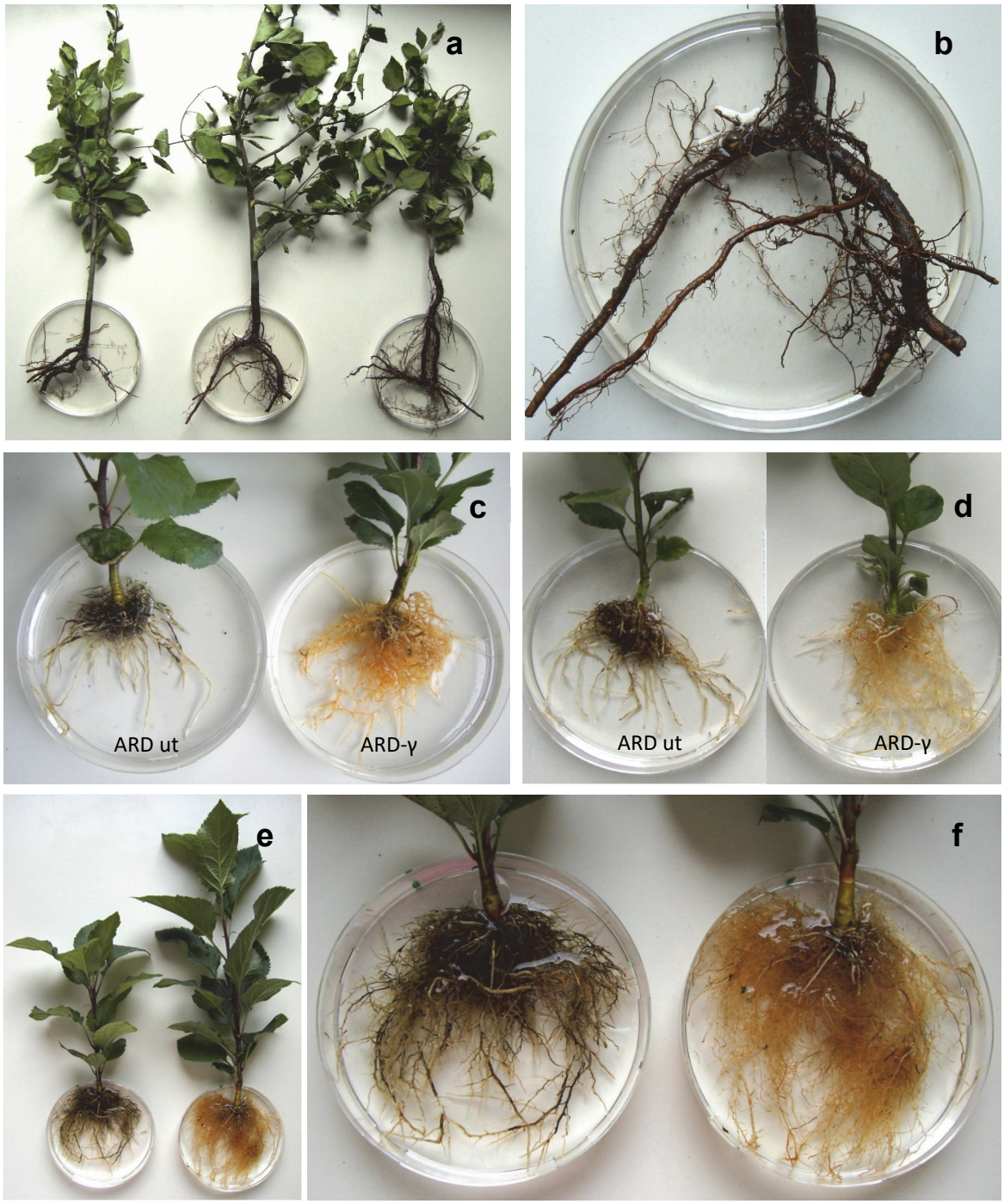
SUPPLEMENTAL INFORMATION

SUPPLEMENTAL INFORMATION – TABLE S1. Summary of histological findings on thin sections of selected root segments from M26 and Bittenfelder apple fine roots harvested after two, four and eight weeks (t2, t4, t8) after starting the greenhouse biotest in ARD soils (untreated ut, irradiated γ) from the sites Ellerhoop (E), Heidgraben (H), and Ruthe (R). Assessment data of single sections were pooled per treatment and harvest time regarding the following assessment parameters: Occurrence of mycorrhizal fungi, identified Nectriaceae (with cauliflower-like structures), other fungi, Actinobacteria, other bacteria; occurrence and location of cell necroses. Abbreviations mean: S = surface mycelia, Intr. M = mycelia within the root, Arb = arbuscules, Ves = vesicles; Rhiz = rhizodermis, Cor = cortex parenchyma, Endo = Endodermis; N = necroses, Ni = necroses with inclusion bodies; y = yellow; vbr = browning; s = symptoms present; nt = not tested. Two plants per treatment and harvest for basic selection of 15 root segments (ut) and 10 root segments (γ). Microscopic assessment of $n = 4$ selected slides per treatment and harvest time, including 12 to 20 tissue sections.

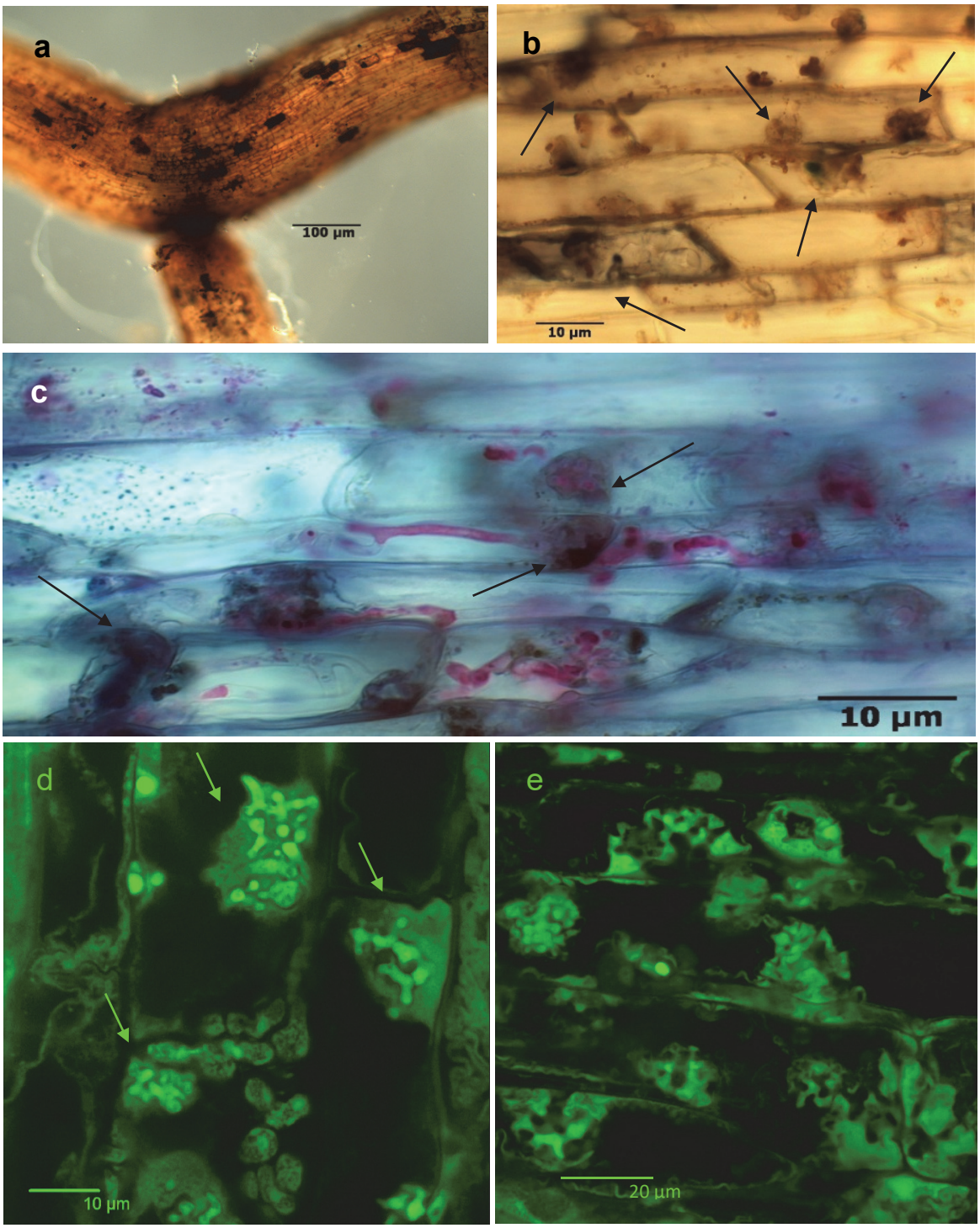
Plant and	Harvest	Sympt.	Mycorrhizal Fungi				Nectriaceae (CF)			Other Fungi			Actinobact.			Other Bact.		Cell-Necrosis			Number	Number of
Treatment			S	Intr. M	Arb	Ves	S	Rhiz	Cor	S	Rhiz	Cor	S	Cor	Stele	S	Cor	Rhiz	Cor	Endo	of slides	tiss. sect.
M26 CE2_2016																						
E ARD ut	t2	s	0	0	0	0	0	1	1	1	0	1	0	1	0	1	1	0	N	0	4	16
	t4	s	0	1	1	0	1	1	1	0	0	0	0	1	0	1	1	N	N	0	4	16
	t8	s	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	N	N	0	4	16
E ARD-γ	t2	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	3	12
	t4	nt	nt																			
	t8	no	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	20
H ARD ut	t2	s	0	0	0	0	1	1	1	0	0	0	1	1	0	1	1	N	N	0	3	12
	t4	s	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	N	N	0	4	16
	t8	s	1	1	1	1	0	0	0	0	0	0	1	1	0	1	1	N	N	0	4	16
H ARD-γ	t2	no	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	16
	t4	no	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	0	4	16
	t8	no	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	N	0	0	4	16
R ARD ut	t2	s	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	N	N	y	4	16
	t4	s	0	1	1	0	1	1	1	0	0	0	1	0	0	1	0	N	0	0	4	16
	t8	s	0	0	1	0	0	1	1	0	0	0	1	1	0	1	0	N	N	y	3	15
R ARD-γ	t2	no	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	16
	t4	no	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	12
	t8	no	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	12
M26 CE2_2017																						
E ARD	t2	s	0	0	0	0	1	1	0	0	1	0	0	1	0	0	1	N	N	0	4	20
	t4	s	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	N	N	0	4	20
	t8	s	0	1	1	0	0	1	1	0	0	0	1	1	0	0	1	Ni	N	0	4	20
E ARD-γ	t2	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	20
	t4	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	20
	t8	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	20
H ARD ut	t2	s	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	N	N	0	4	20
	t4	s	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	N	N	0	4	20
	t8	s	0	1	1	0	0	1	1	0	0	0	1	1	0	1	1	Ni	N	y	4	20
H ARD-γ	t2	no	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	4	20
	t4	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	N	0	0	4	20
	t8	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	20
R ARD ut	t2	s	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	N	N	0	4	20
	t4	s	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	N	N	0	4	16
	t8	s	1	1	1	0	0	1	1	0	0	0	0	0	0	1	0	N	N	y	4	16
R ARD-γ	t2	s	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	N	N	0	4	16
	t4	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	20
	t8	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	16
Bit CE2_2017																						
R ARD ut	t2	s	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	0	0	4	20
	t4	s	1	1	1	0	0	1	1	0	1	0	1	1	1	1	1	N	N	0	4	20
	t8	s	0	1	1	0	0	1	1	0	0	0	0	0	0	1	0	N	N	y	4	20
R ARD-γ	t2	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	20
	t4	no	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	4	20
	t8	s	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	vbr	0	y	4	20

SUPPLEMENTAL INFORMATION – TABLE S2. Pearson's correlation coefficients for frequencies of Actinobacteria, other bacteria, cauliflower like structures (CF) of Nectriaceae and cell necroses in root thin sections and corresponding p-values. Significant correlation coefficients are highlighted in bold. Data were obtained for two plants each from three harvest times (two, four, and eight weeks of cultivation in ARD soils) from three locations (Ellerhoop E, Heidgraben H, Ruthe R) in two greenhouse experiments: CE2 2016 and 2017 (A), CE2 2016 only (B), and CE2 2017 only (C).

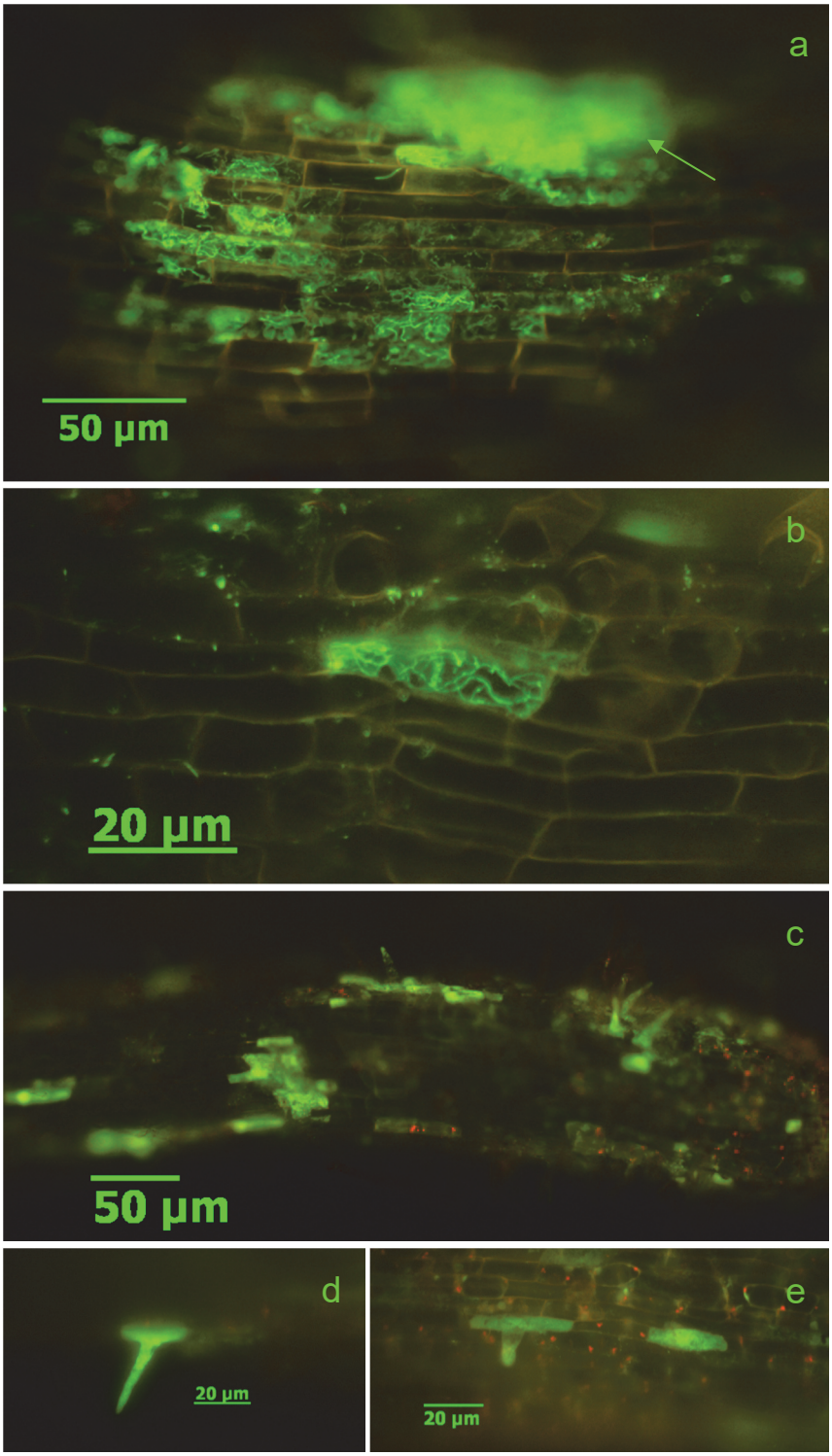
A: *CE2 2016 and 2017*				
	CF (Nectriaceae)	Actinobacteria	Other bacteria	Cell necroses
CF (Nectriaceae)		-0.082 p = 0.745	-0.11 p = 0.655	**0.57** p = 0.0132
Actinobacteria			**0.88** p = 0.0000013	0.097 p = 0.702
Other bacteria				0.1 p = 0.68
B: *CE2 2016*				
	CF (Nectriaceae)	Actinobacteria	Other bacteria	Cell necroses
CF (Nectriaceae)		0 p = 1	-0.11 p = 0.772	0.5 p = 0.174
Actinobacteria			**0.79** p = 0.012*	0.42 p = 0.259
Other bacteria				0.35 p = 0.353
C: *CE2 2017*				
	CF (Nectriaceae)	Actinobacteria	Other bacteria	Cell necroses
CF (Nectriaceae)		0.14 p = 0.711	0.12 p = 0.749	**1** p = 0.0000***
Actinobacteria			**0.89** p = 0.0011**	0.14 p = 0.711
Other bacteria				0.12 p = 0.749



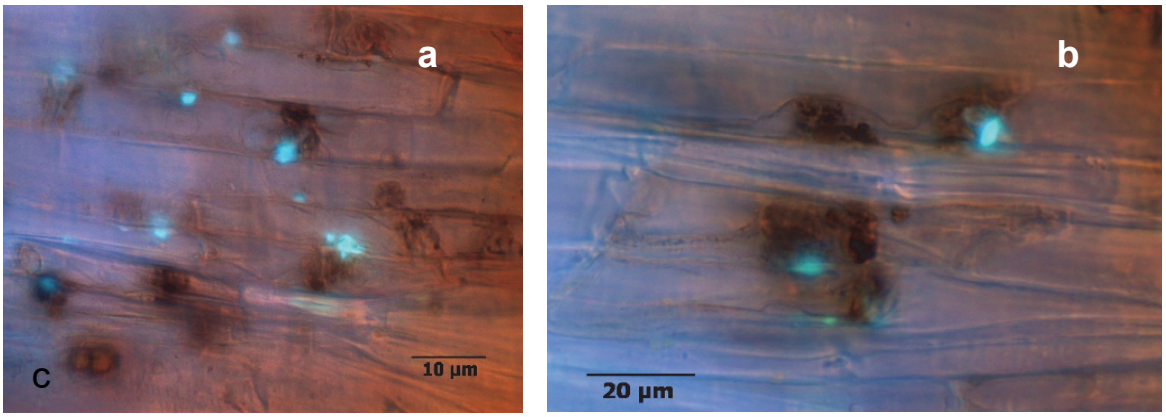
SUPPLEMENTAL INFORMATION – FIGURE S1. Examples of tested root systems: a) Bittenfelder plants from an ARD field plot in Ellerhoop (harvest June 2016), in the second year of growth in the 4th replant generation; and b) washed root system in detail, with fine roots (arrows) in a Petri-dish with 14 cm in diameter (experiment CE1 according to Mahnkopp et al., 2018). c) M26 plants after four weeks of cultivation (c) and Bittenfelder plants after two weeks of cultivation (d) in soils from the reference site Ellerhoop with the variants ARD untreated (ARD ut) and ARD irradiated (ARD-γ). e) M26 test plants after eight weeks of cultivation in Heidgraben soil in untreated ARD soil (e, left) and in ARD-γ soil (e, right), and f) close-up of the root systems with black and brown segments (ARD ut; left) and light yellow roots (ARD-γ; right) in Petri-dishes with 9 cm in diameter (biotest experiment CE2 2017 according to Mahnkopp et al., 2018).



Supplemental Information – Figure S2. Whole mounted samples of replant diseased M26 fine roots harvested from the experiment CE2 2016. a) Black cell clusters scattered over a fine root grown in ARD soil from the site Ruthe, two weeks after the start of the experiment; b) necrotic cells with Nectriaceae infections and intracellular cauliflower-like structures (arrows) after eight weeks, unstained; c) toluidine blue stained necrotic tissue with dark cauliflower-like structures (arrows) connected with violet stained fungal mycelium; d) and e) details of Nectriaceae infections in fine roots grown for two weeks in ARD soil from the Heidgraben site, acid fuchsine-lactophenol stained, Confocal Laser Scanning Microscopy. d) Bright stained fungal hyphae within the dense cauliflower like structures (arrows) adhering to the host cell walls; and e) severely damaged cells with several fungal nodule structures.



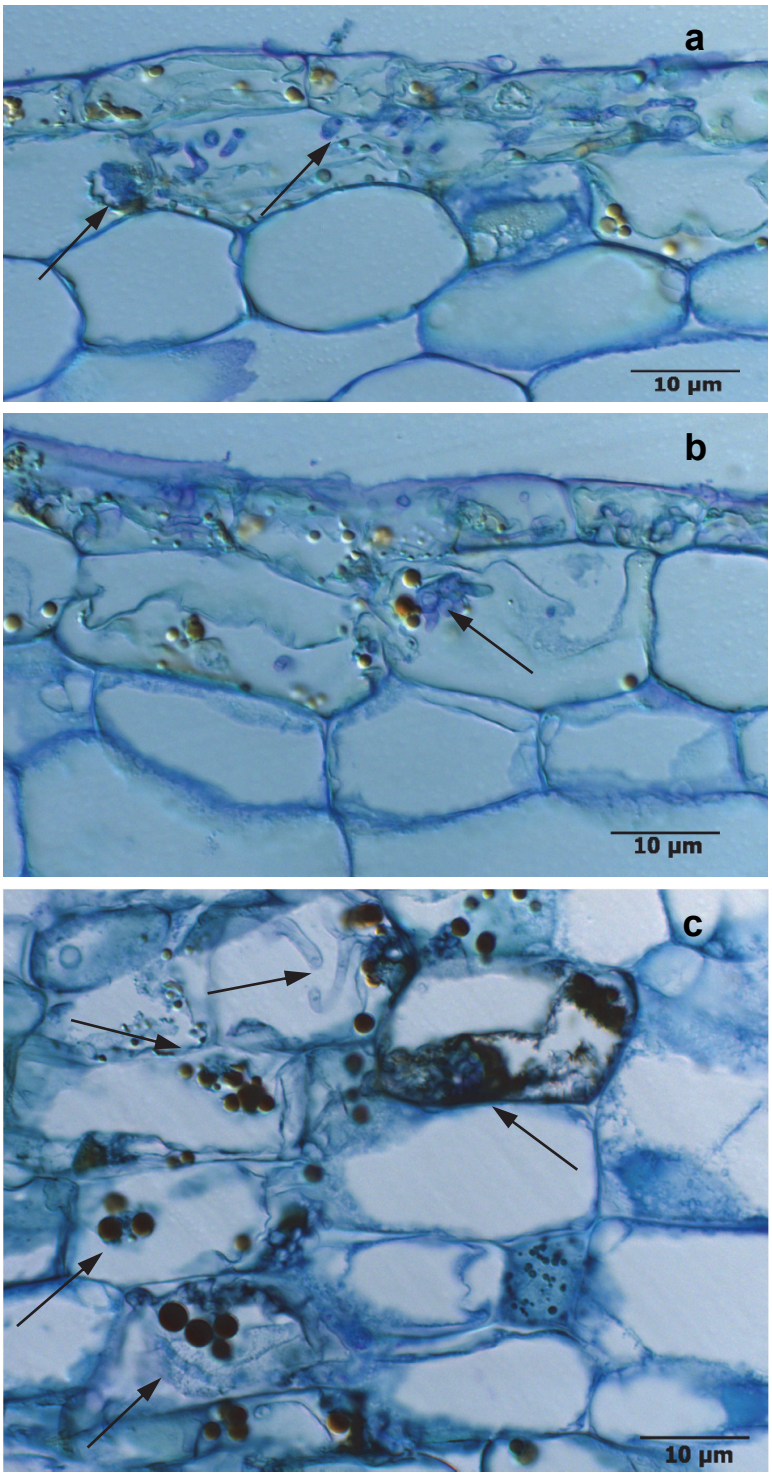
Supplemental Information – Figure S3. Actinobacteria, a) on the root surface (arrow) and intracellularly in non-vital root tissue of Bittenfelder plants cultivated in ARD soil from Ruthe (R ARD ut, experiment CE2 2017); b) after two weeks of cultivation; c)–e) even in root hairs, after four weeks of cultivation. Fluorescence microscopy after staining with FUN®1 cell stain according to Grunewaldt-Stöcker et al. (2019).



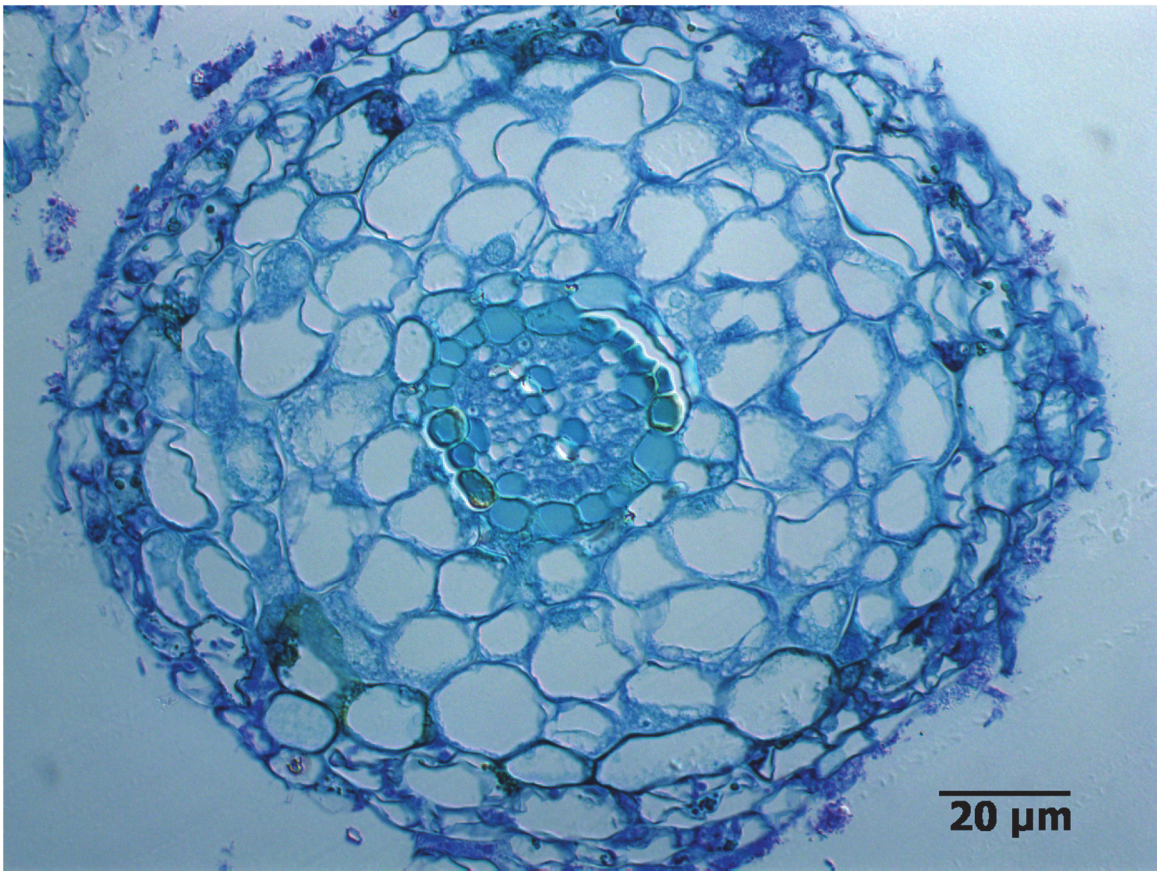
Supplemental Information – Figure S4. Nectriaceae with brownish cauliflower-like structures and chlamydospores (c) in cortex cells of complete necrotic root fine roots of M26 grown in untreated ARD soil from the Ruthe site; a) and b) callose deposits fluorescing in light blue at the fungal penetration sites after aniline blue staining.

Sep[3 BK4 avi.avi

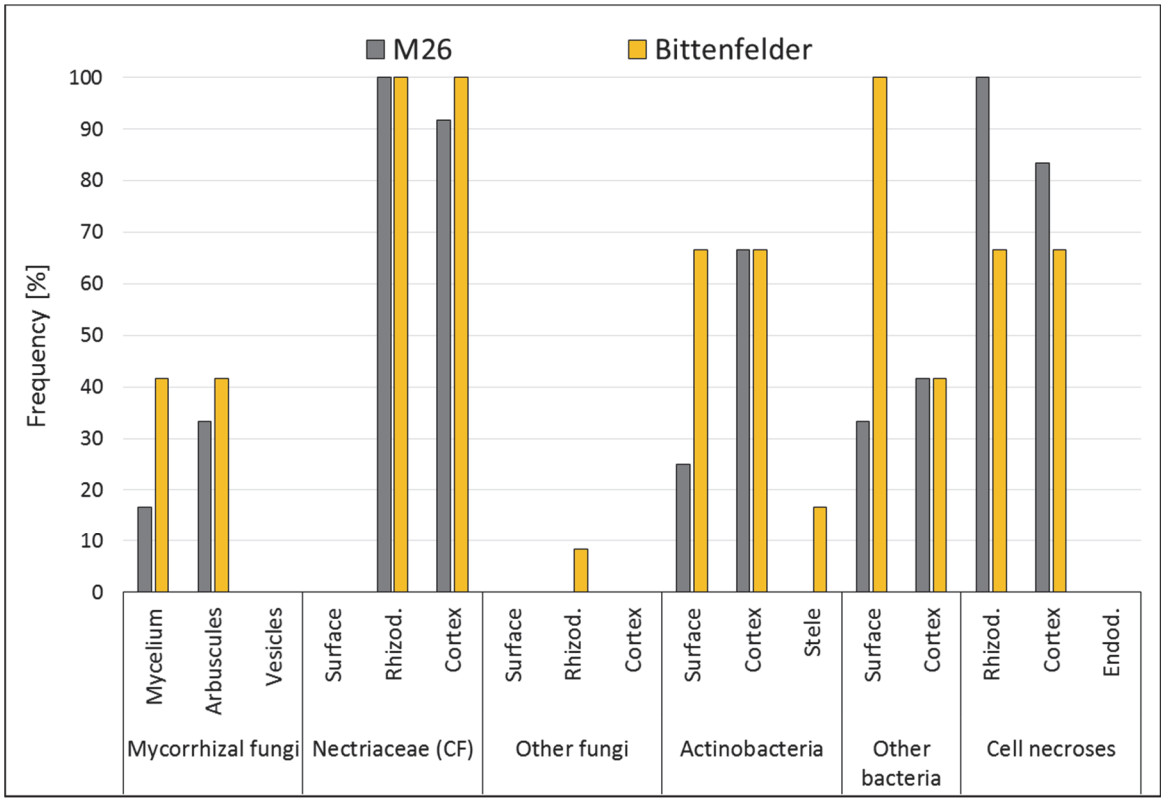
Supplemental Information – Figure S5. Avi File: Confocal Laser Scanning series of the cauliflower like (CF) fungal structures in fine root tissue of M26, grown for two weeks in ARD soil from the Heidgraben site.



Supplemental Information – Figure S6. Toluidine blue stained thin sections of M26 apple fine roots from ARD soils of the Ellerhoop and Heidgraben sites (t8, experiment CE2 2017) with early Nectriaceae infections. a) and b) Intracellular fungal hyphae and developing CF structures (arrows), and brown inclusion bodies in rhizodermal and cortical cells; c) black-brown inclusion bodies in the apoplast and in infected damaged cells with hyphae and CF structure (arrows).



SUPPLEMENTAL INFORMATION – FIGURE S7. Cross section of a fine root of M26 after two weeks in ARD soil from the Ruthe site, CE2 2016. Fungal infections in the rhizodermal and outer cortical cell layers, whereas the endodermis and the stele are free of Nectriaceae infections, experiment CE2 2016.



SUPPLEMENTAL INFORMATION – FIGURE S8. Occurrence and localization of microorganisms and cell necroses in fine root tissue sections of replant diseased apple plants of two genotypes (M26 and Bittenfelder) in experiment CE2 2017. Microscopic ratings were pooled from three harvest times at two, four, and eight weeks of cultivation in ARD soil from Ruthe. Given are % frequencies, $n = 12$ slides per treatment (4 slides per harvest time, each slide with 5 tissue sections).