

This is a repository copy of *The relative importance of soil moisture in predicting bacterial wilt disease occurrence*.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/172039/>

Version: Accepted Version

Article:

Jiang, Gaofei, Ningqi, Wang, Yaoyu, Zhang et al. (9 more authors) (2021) The relative importance of soil moisture in predicting bacterial wilt disease occurrence. *Soil Ecology Letters*. 356–366. ISSN 2662-2297

<https://doi.org/10.1007/s42832-021-0086-2>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



The relative importance of soil moisture in predicting bacterial wilt disease occurrence

Journal:	<i>Soil Ecology Letters</i>
Manuscript ID	SEL-2020-0105.R1
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	<p>Jiang, Gaofei; Nanjing Agricultural University, Key Laboratory of Plant immunity, Jiangsu Provincial Key Laboratory for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers</p> <p>Ningqi, Wang</p> <p>Yaoyu, Zhang</p> <p>Wang, Zhen; Nanjing Agricultural University, Key Laboratory of Plant immunity, Jiangsu Provincial Key Laboratory for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers</p> <p>Zhang, Yuling</p> <p>Yu, Jiabao</p> <p>Zhang, Yong; Southwest University, College of Resources and Environment, Key Laboratory of Efficient Utilization of Soil and Fertilizer Resources</p> <p>Wei, Zhong; Nanjing Agricultural University</p> <p>Xu, Yang-Chun; Nanjing Agricultural University, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization</p> <p>Geisen, Stefan; Wageningen Universiteit en Research</p> <p>Friman, Ville-Petri; University of York, Department of Biology</p> <p>Shen, Qirong; Nanjing Agricultural University</p>
Keywords:	Bacterial wilt disease, Soil moisture, Soil physicochemical properties, Rhizosphere bacterial communities, <i>Ralstonia solanacearum</i> , Random forest algorithm
Speciality:	Soil microbial ecology, Soil-plant interactions, Response and adaptation of soil biota to environmental changes

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



SCHOLARONE™
Manuscripts

Highlights

- Soil moisture is a key predictor of bacterial wilt disease (BWD) across China.
- Other soil properties have lesser role and are locally associated to BWD.
- Soil moisture can causally drive BWD in greenhouse experiment.
- Water management strategies could potentially be used in BWD control.

For Review Only

1
2
3 **1 Title**
4

5
6 2 The relative importance of soil moisture in predicting bacterial wilt disease occurrence
7

8 **3 Running Title**
9

10 4 Soil moisture predicts wilt disease
11
12

13 **5 Authors**
14

15 6 Gaofei Jiang^a, Ningqi Wang^a, Yaoyu Zhang^a, Zhen Wang^a, Yuling Zhang^a, Jiabao Yu^a, Yong

17 7 Zhang^b, Zhong Wei^{a,*}, Yangchun Xu^a, Stefan Geisen^c, Ville-Petri Friman^d, Qirong Shen^a
18
19

20 8 Gaofei Jiang: 0000-0002-5331-739X gjiang@njau.edu.cn
21

22 9 Ningqi Wang: 0000-0002-7788-4364 2019203023@njau.edu.cn
23

24 10 Yaoyu Zhang: 0000-0001-7562-1440 zhangyaoyu0203@126.com
25

26 11 Zhen Wang: 0000-0002-5394-9127 1475867813@qq.com
27

28 12 Yuling Zhang: 0000-0001-6352-570X l434404291@126.com
29

30 13 Jiabao Yu: 0000-0002-3685-461X yjb21123@sina.cn
31

32 14 Yong Zhang: 0000-0003-1820-0927 bioyongzhang@swu.edu.cn
33

34 15 Zhong Wei: 0000-0002-7967-4897 weizhong@njau.edu.cn
35

36 16 Yangchun Xu: 0000-0003-2740-5561 ycxu@njau.edu.cn
37

38 17 Stefan Geisen: 0000-0003-0734-727X stefan.geisen@wur.nl
39

40 18 Ville-Petri Friman: 0000-0002-1592-157X ville.friman@york.ac.uk
41

42 19 Qirong Shen: 0000-0002-5662-9620 shenqirong@njau.edu.cn
43

44 **20 Author Affiliation**
45

46 21 ^a Key Laboratory of Plant immunity, Jiangsu Provincial Key Laboratory for Organic Solid

47 22 Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource

48 23 Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing

49 24 Agricultural University
50
51
52
53
54
55
56
57
58
59
60

1
2
3 25 ^b College of Resources and Environment, Key Laboratory of Efficient Utilization of Soil and
4
5
6 26 Fertilizer Resources, Southwest University, Chongqing, China
7

8 27 ^c Laboratory of Nematology, Wageningen University, Wageningen, the Netherlands
9

10 28 ^d Department of Biology, University of York, York, UK
11
12
13
14
15

16 30 ***Corresponding Authors**

17
18 31 Email: weizhong@njau.edu.cn (Zhong Wei); Telephone number: 025-84396864
19
20
21
22

23 33 **Summary**

24
25 34 Soil-borne plant diseases cause major economic losses globally. This is partly because their
26
27 35 epidemiology is difficult to predict in agricultural fields, where multiple environmental factors
28
29 36 could determine disease outcomes. Here we used a combination of field sampling and direct
30
31 37 experimentation to identify key abiotic and biotic soil properties that can predict the
32
33 38 occurrence of bacterial wilt caused by pathogenic *Ralstonia solanacearum*. By analysing 139
34
35 39 tomato rhizosphere soils samples isolated from six provinces in China, we first show a clear
36
37 40 link between soil properties, pathogen density and plant health. Specifically, disease
38
39 41 outcomes were positively associated with soil moisture, bacterial abundance and bacterial
40
41 42 community composition. Based on soil properties alone, random forest machine learning
42
43 43 algorithm could predict disease outcomes correctly in 75% of cases, with soil moisture being
44
45 44 the most significant predictor. The importance of soil moisture was validated causally in a
46
47 45 controlled greenhouse experiment, where the highest disease incidence was observed at 60%
48
49 46 of maximum water holding capacity. Together, our results show that local soil properties can
50
51 47 predict disease occurrence across a wider agricultural landscape, and that management of
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

48 soil moisture could potentially offer a straightforward method for reducing crop losses to *R.*

49 *solanacearum*.

50

51 **Keywords**

52 Bacterial wilt disease; Soil moisture; Soil physicochemical properties; Rhizosphere bacterial

53 communities; *Ralstonia solanacearum*; Random forest algorithm

54

55

For Review Only

56 **1 Introduction**

57 Multiple physicochemical and biotic environmental factors have long been known to be
58 important for plant disease outbreaks, as suggested by the conceptual Disease Triangle model
59 (McNew, 1960). For example, soil moisture (Aung et al., 2018), pH (Rahman and Othman,
60 2020), nutrient availability (Berg and Koskella, 2018) and microbial communities (Trivedi et
61 al., 2020) all influence the severity of soil-borne diseases. Furthermore, temperature can
62 directly affect pathogen densities (Wei et al., 2015a; Pimentel and Ayres, 2018) or the
63 strength of interactions between pathogen and its competitors (Wei et al., 2017; Velásquez
64 et al., 2018) with important consequences for the disease occurrence. However, while the
65 significance of environment has been long recognised (Cheng et al., 2019), we still poorly
66 understand the relative importance of different factors, or their combinations, for the disease
67 outcomes. Furthermore, the effects of soil properties are often studied locally within one
68 specific agricultural area making it difficult to extrapolate results up to a level of a country or
69 a continent (Janvier et al., 2007; Orr and Nelson, 2018). To study this, we used China-wide
70 ~~{area of 1.3 million Km²}~~ sampling of tomato plant rhizosphere to identify key abiotic and
71 biotic soil properties associated with bacterial wilt disease occurrence, and experimentally
72 tested if one of the most important factors, soil moisture, could causally drive bacterial wilt
73 disease incidence in a greenhouse experiment.

74 *Ralstonia solanacearum* bacterium is a causative agent of notorious bacterial wilt
75 disease that leads to a systemic wilting of plants (Hayward, 1991). It can infect multiple
76 important crops belonging to the *Solanaceae* family (e.g. potato, tomato and tobacco) and
77 has a global distribution (Mansfield et al., 2012). Previous studies have identified associations
78 with multiple soil physicochemical factors and *R. solanacearum* infections both in the field
79 and greenhouse experiments (Hayward, 1991; Jiang et al., 2017; Wei et al., 2018; Siregar et

1
2
3 80 al., 2020). For example, *R. solanacearum*-infected plants have previously been associated
4
5 81 with increased soil moisture (Jiang, 2016), acidic pH (Li et al., 2017a) and high nitrogen
6
7
8 82 availability (Dalsing et al., 2015; ~~Y. Gu et al., 2020~~Gu et al., 2020a). These environmental
9
10 83 factors could affect bacterial wilt occurrence directly by favouring the growth of the pathogen,
11
12
13 84 as *R. solanacearum* needs to reach certain threshold density in the soil to express key
14
15 85 virulence factors that are triggered by quorum sensing signalling (Genin and Denny, 2012;
16
17
18 86 Peyraud et al., 2016, 2018). Alternatively, soil properties could have indirect effects on the
19
20 87 pathogen via plants or associated plant rhizosphere microbiome. Plants have evolved
21
22 88 sophisticated defence mechanisms against pathogens, and recent evidence suggests that
23
24
25 89 environmental factors can directly affect plant immunity and defence hormone pathways
26
27
28 90 (Velásquez et al., 2018). Rhizosphere microbiome also plays a crucial role in forming the first
29
30 91 line of defence against invading pathogens, often considerably shaping the disease severity
31
32
33 92 (Kwak et al., 2018; Wei et al., 2019, 2020). In general, diverse microbial communities can limit
34
35 93 pathogen growth due to intense competition for nutrients, space and other resources (Wei
36
37
38 94 et al., 2015b; ~~S. Gu et al., 2020~~Gu et al., 2020b), or because they are likely to contain highly
39
40 95 antagonistic species that can directly inhibit the pathogen for example by secreting
41
42
43 96 antimicrobial molecules (Raza et al., 2016a, 2016b). Crucially, soil properties often determine
44
45 97 the composition and diversity of rhizosphere microbiome and could hence indirectly affect
46
47 98 the likelihood of *R. solanacearum* infections.

49 99 Understanding the potential role of soil properties in *R. solanacearum* infections is
50
51
52 100 especially important because bacterial wilt dynamics often show high temporal and spatial
53
54 101 variability both between and within fields (Wei et al., 2017, 2018). Previous work has shown
55
56
57 102 that between-field variability could be driven by local fluctuations in temperature and
58
59 103 humidity (Wei et al., 2017), while within-field variation could be explained by spatial
60

1
2
3 104 differences in soil physicochemical properties or the composition of microbial communities,
4
5
6 105 which both have been associated with disease outcomes previously (Wei et al., 2018, 2019;
7
8 106 Lee et al., 2021). However, it is unclear which soil properties are relatively more important
9
10
11 107 than the others, and if the previously observed patterns hold across a wider geographical area
12
13 108 with varying local environmental conditions. To study this, we focused on six geographically
14
15 109 separated tomato fields in China (area of 1.3 million Km²) to explore the role of within- and
16
17
18 110 between-field variation in abiotic and biotic soil properties for bacterial wilt disease
19
20 111 occurrence. We first collected and analysed 139 rhizosphere soil samples originating from
21
22 112 healthy and diseased plants at every field and identified significant associations between the
23
24
25 113 disease outcome, pathogen densities and different soil properties. Second, machine learning
26
27 114 algorithm was used to identify the relatively most important soil properties associated with
28
29 115 the bacterial wilt disease, whose importance was directly tested in a greenhouse experiment.
30
31
32 116 It was found that despite considerable between-field variation, healthy and diseased plants
33
34
35 117 were consistently associated with certain soil properties, which could predict bacterial wilt
36
37 118 disease occurrence with 75% accuracy. Soil moisture, bacterial community composition and
38
39
40 119 bacterial abundances were the most important predictors of disease by incidence based on a
41
42 120 random forest model, and. Furthermore, soil moisture content treatment at 60% of maximum
43
44
45 121 water holding capacity led to the highest levels of disease incidence in a controlled
46
47 122 greenhouse experiment. Together, our findings suggest that local variation in abiotic and
48
49
50 123 biotic soil properties can reliably predict bacterial wilt disease outcomes across large
51
52 124 agricultural area.

125

126 2 Experimental Procedures

127 2.1 Sampling sites and collection of plant soil samples

128 Soil samples were collected from tomato fields at six locations in Changsha of Hunan province
129 (112°58'E, 28°11'N), Ningbo of Zhejiang province (121°67'E, 29°91'N), Nanchang of Jiangxi
130 province (115°51'E, 28°41'N), Nanjing of Jiangsu province (118°57'E, 32°03'N), Nanning of
131 Guangxi province (108°21'E, 22°49'N) and Wuhan of Hubei province (114.31'E 30.52'N) during
132 tomato bacterial wilt disease outbreaks in the summer 2015. The sampled fields in Central
133 (Hubei and Hunan), Eastern (Jiangsu, and Zhejiang) and Southern (Guangxi) China recurrently
134 experience *R. solanacearum* outbreaks (Jiang et al., 2017) and had suffered from bacterial
135 wilt disease epidemics between 3 to 15 years based on communication with the local farmers.
136 Within each sampling site, common local tomato cultivars were used: *Solanum lycopersicum*
137 cv. "Hengkang #1" in Changsha, "CTX 201" in Nignbo, "Hezuo 906" in Nanachang, "Guihong
138 #1", "Jipin" in Nanjing and "Huafan #13" in Wuhan. At each sampling site, around 12
139 symptomatic (diseased) and 12 asymptomatic (healthy) tomato plants were chosen randomly
140 based on the presence and absence of visible disease symptoms and randomly sampled at
141 the early fruiting stage ~~resulting in~~(a total of 139 rhizosphere samples). Excess root soil was
142 discarded by gently shaking and the remaining soil attached on the root surfaces was
143 collected and considered as the rhizosphere soil (Wei et al., 2011). Around 10 g of fresh
144 rhizosphere soil per plant was sampled and divided into two sealed 5 mL Eppendorf tubes to
145 retain natural soil properties. One tube was cryopreserved in 5 mL of 30% glycerol at -80 °C
146 to analyse biotic properties of rhizosphere samples (pathogen and total bacteria densities and
147 microbial community diversity and composition). Another tube was used for determining soil
148 physicochemical (abiotic) properties as described in the following section.

149

1
2
3 150 2.2 Determination of abiotic and biotic soil properties
4

5
6 151 *2.2.1 Abiotic properties*
7

8 152 Abiotic physiochemical properties included soil moisture content (Moisture, %), pH, available
9
10 153 phosphorus (P, mg·kg⁻¹), available potassium (K, mg·kg⁻¹), water-soluble carbon (C, mg·kg⁻¹)
11
12
13 154 and total nitrogen (N, mg·kg⁻¹). The difference in fresh and air-dried soil sample weight was
14
15 155 used as a proxy of soil moisture for each rhizosphere sample. Soil pH was measured in a 20%
16
17 156 water (w/w) suspension (Li et al., 2017a) using a pH meter (PB-10, Sartorius, Germany).
18
19 157 Available P and K were extracted with hydrochloric acid and ammonium fluoride and
20
21 158 measured using molybdenum blue method (Pansu and Gautheyrou, 2006). The water-soluble
22
23 159 carbon and total N were determined by following a previous protocol (Pansu and Gautheyrou,
24
25 160 2006) using a multi C/N analyzer 3000 (Analytik Jena AG, Germany).
26
27
28
29

30 161

31
32 162 *2.2.2 Biotic properties*
33

34
35 163 The total DNA was extracted from ~0.25 g of cryopreserved rhizosphere soil using PowerSoil
36
37 164 DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, USA) following the manufacturer's
38
39 165 protocol. DNA quality and concentration were checked using a NanoDrop 1000
40
41 166 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Soil DNA was subjected to 16S
42
43 167 ribosomal RNA (rRNA) Illumina amplicon sequencing to determine the diversity and
44
45 168 composition of bacterial communities at Shanghai Biozeron Biological Technology Co. Ltd.
46
47 169 The V4 hypervariable region of the 16S rRNA gene was amplified with the primer pair 563F
48
49 170 (5'-AYTGGGYDTAAAGVG-3') and 802R (5'-TACNVGGGTATCTAATCC-3'). All sequences were
50
51 171 processed with QIIME (Caporaso et al., 2010). The OTU similarity cut-off was assigned at 97%
52
53 172 identity level using USEARCH (Edgar, 2010). OTUs were assigned to corresponding bacterial
54
55 173 taxa using the Ribosomal Database Project (RDP) database with the online version of the RDP
56
57
58
59
60

1
2
3 174 classifier (Cole et al., 2014). The microbial community diversity was determined as Shannon
4
5
6 175 diversity index (Shannon) and Chao1 richness index (Shannon) using the vegan R package
7
8 176 (Dixon, 2003) after removing *R. solanacearum* OTUs (Wei et al., 2018). Microbial community
9
10
11 177 composition was quantified as a dissimilarity index (Bray-Curtis) based on average Bray-Curtis
12
13 178 distance of each sample from each other at the OTU level. The pathogen and total bacterial
14
15 179 densities were examined with qPCR using *R. solanacearum*-specific primer *Rsol_fliC*
16
17
18 180 (Schönfeld et al., 2003) and general bacterial primer pair Eub338/Eub518 (Fierer et al., 2005).
19
20 181 SYBR Premix Ex Taq Kit (TaKaRa Biotech. Co, Japan) was used following the manufacturers'
21
22
23 182 protocol, and each sample was measured in triplicate using a 7500 Fast Real-Time PCR System
24
25 183 (Applied Biosystems, CA, USA).
26
27
28 184

30 185 2.3 Establishing causality between soil moisture and bacterial wilt incidence in tomato

31
32 186 A greenhouse experiment was conducted in Yixing of Jiangsu province to test whether soil
33
34
35 187 moisture can predictably drive the development of bacterial wilt disease under controlled
36
37 188 environmental conditions. Experimental soils that were free of *R. solanacearum* were
38
39
40 189 collected from a riverside of Zhangzhu town in Yixing: no *R. solanacearum* growth was
41
42 190 detected using semi-selective agar medium (Wei et al., 2018). Tomato seeds (*S. lycopersicum*
43
44
45 191 cv. "Jipin") were surface-sterilized with NaClO (3%; v:v) and germinated on moist filter paper
46
47 192 for 2 days before sowing in sterilized nursery substrate (Huaian Agricultural Technology
48
49
50 193 Development Ltd). Tomato seedlings were transplanted into plastic pots with five kg of
51
52 194 homogenized dry soils at four-leaf stage. The soil moisture content was manipulated using
53
54 195 five treatments with 40%, 50%, 60%, 70%, and 80% of maximum water holding capacity, and
55
56
57 196 twelve replicate pots were used per treatment. This moisture range covered dry (40%) and
58
59 197 flooded (80%) soils. Plants were acclimated in greenhouse conditions for three weeks before
60

1
2
3 198 pathogen inoculation and then grown in the same conditions until the end of the experiment
4
5
6 199 (constant temperature of $30\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$, relative air humidity of 80%, and 14 h of light and 10 h
7
8 200 of dark daily cycle). Water content was kept constant in each treatment by adding sterile
9
10 201 water to each pot- during acclimatization period before the infection and until the end of the
11
12 202 experiment after the infection accordingly. After three weeks of acclimatization, *R.*
13
14 203 *solanacearum* pathogen strain QL-Rs1115 (a strong virulent reference strain) was inoculated
15
16 204 to all pots using soil drenching method with resulting in final concentration of 5.0×10^6
17
18 205 $\text{CFU}\cdot\text{g}^{-1}$ soil (Wei et al., 2011). The same amount of water (10 mL) was used with all the pots,
19
20 206 which led to only momentary increase in water holding capacity in some of the low moisture
21
22 207 treatments during the drenching. The disease development was monitored on a daily basis
23
24 208 and quantified as a disease index on a scale ranging from 0 to 4 where one whole number
25
26 209 change corresponds to 25% increase in the proportion of wilted leaves per plant (Schandry,
27
28 210 2017).

211

212 2.4 Data analyses

213 2.4.1 Comparing differences in abiotic and biotic properties of healthy and diseased plant 214 rhizosphere samples

215 All measured abiotic and biotic properties were normalized between the range 0 – 1 using
216 min-max normalization before statistical analyses (Patro and Sahu, 2015). Nonparametric
217 Wilcoxon rank sum test (Wilcoxon test) was used to compare differences between healthy
218 and diseased plant rhizosphere soil samples (Cuzick, 1985). The microbial community
219 composition was ordinated by principal coordinates analysis (PCoA) using Bray-Curtis
220 distance and differences between healthy and diseased plant rhizosphere soil samples were
221 compared using the nonparametric permutational multivariate analysis of variance

1
2
3 222 (PERMANOVA, $P < 0.05$, 999 permutations) using Adonis function in R vegan package (Dixon,
4
5 223 2003). Principal component analysis (PCA), based on the Euclidean distance of the range
6
7
8 224 normalized values for overall abiotic and biotic properties, was used to visualize differences
9
10 225 between healthy and diseased plants (FactoMineR R package (Lê et al., 2008); statistical
11
12 226 significance tested using nonparametric PERMANOVA ($P < 0.05$) with 999 permutations using
13
14
15 227 Adonis function in R vegan package (Dixon, 2003)).
16
17
18 228

20 229 *2.4.2 Identifying key abiotic and biotic predictors for pathogen abundance and plant health*

21
22
23 230 To identify key abiotic and biotic predictors for pathogen abundance, we build up a model
24
25 231 using multiple linear regression function in R stats package (R Core Team, 2020) to predict *R.*
26
27 232 *solanacearum* densities based on all measured soil properties. The relative importance of
28
29
30 233 different predictors ~~were~~was estimated based on their significance for the model
31
32
33 234 performance (% of R^2) using Anova (R Core Team, 2020) and relweights functions in R
34
35 235 (Kabacoff, 2015). To understand the relationship between environmental variables and
36
37 236 disease incidence, we used Random forest approach using randomForest package in R as
38
39
40 237 follows (Cutler and Wiener, 2018). We first randomly selected 80% of the entire 139 sample
41
42 238 dataset as a training set ($n = 111$) to generate a classification model for predicting plant health
43
44
45 239 status (healthy vs. diseased) based on soil abiotic (moisture, pH, P, K, C, N) and biotic
46
47 240 properties including Chao1, Shannon and Bray-Curtis metrics of the bacterial community in
48
49
50 241 tomato rhizosphere soils. Tenfold cross-validation was performed 10 times using the rfcv
51
52 242 function to select appropriate number of predictor properties whose importance and cross-
53
54 243 validation curves were visualized by using the R ggplot2 package (Wickham et al., 2020).
55
56
57 244 Remaining 20% of samples ($n = 28$) were used as a test set to predict plant health based on
58
59 245 the abiotic and biotic rhizosphere soil properties.
60

1
2
3 246
4
5
6 247

2.4.3 Analysis of disease dynamics in a greenhouse experiment

7
8 248 The effect of soil moisture on disease dynamics was analysed based on temporal
9
10 249 changes in disease index values using a logistic growth curve (Schandry, 2017). The disease
11
12 250 dynamics curves were fitted individually for each plant using `gcFitModel` function in R `grofit-`
13
14 251 package (Kahm et al., 2010). As described previously (Wei et al., 2015b), this fit could be
15
16 252 divided into three variables describing different stages of disease development: 1) lag phase
17
18 253 referred to as the delay time of disease symptom onset after inoculation of the pathogen
19
20 254 (early infection stage); 2) disease rate referred to as the exponential increase of disease
21
22 255 progression (exponential infection stage); 3) area under progression of the disease dynamics
23
24 256 curve (AUDPC) referred to as the overall severity of wilt disease (late infection stage). Shapiro-
25
26 257 Wilk and Bartlett's tests were used to test the normality and homogeneity of the fitted
27
28 258 variables using the R `stats`-package. If the data matrix followed a normal distribution with
29
30 259 homogeneous variances, ANOVA and post hoc Tukey's HSD tests were used to compare
31
32 260 differences between different soil moisture groups ($P < 0.05$) using R `multcomp`-package
33
34 261 (Hothorn et al., 2020). Otherwise, non-parametric Kruskal-Wallis and post hoc Dunn's tests
35
36 262 were used for statistical analyses using R `agricolae` package (Mendiburu, 2020).
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

264 3 Results

265 3.1 Rhizosphere soil properties vary between healthy and diseased plants

266 We first compared the abiotic physiochemical and biotic rhizosphere soil properties (Table 1)
267 of diseased and healthy tomato plants across six sampled provinces in China (Fig. 1a, CS:
268 Changsha; NB: Ningbo; NC: Nanchang; NJ: Nanjing; NN: Nanning; WH: Wuhan). Pathogen
269 densities were on average 15.79 times higher in diseased compared to healthy plants ($P <$
270 0.0001 , Wilcoxon test, Supp. Fig. 1-2), and also the other rhizosphere soil properties differed
271 between diseased and healthy plants (Fig. 1, Supp. Fig. 1-2). Specifically, diseased plants were
272 characterized by 1.15 times higher soil moisture ($P = 0.001$), and 10.53 times higher total
273 bacterial densities ($P = 0.0002$) compared to the healthy plants (Wilcoxon test; Fig 1b and
274 Supp. Fig. 1-2). While other physiochemical soil properties, or microbial community diversity,
275 did not differ between the diseased and healthy plants ($P > 0.05$; Wilcoxon test; Fig 1b, Supp.
276 Fig. 1-2), PCoA analysis revealed that microbial community composition varied depending on
277 the plant health status ($R^2 = 0.10$, $P = 0.002$) and between provinces ($R^2 = 0.56$, $P = 0.001$,
278 PERMANOVA; Fig. 1c and Supp. Fig. 3). Moreover, differences in microbial community
279 composition between the healthy and diseased plants were location-specific: significant
280 differences were found in CS, NB, NJ and NN ($P > 0.05$) but not in NC or WH provinces ($P <$
281 0.05 , PERMANOVA; Supp. Fig. 3). Moreover, when analysed together using PCA, abiotic
282 physicochemical and biotic soil properties differed between provinces ($R^2 = 0.62$, $P = 0.001$),
283 and between healthy and diseased plants within each province ($R^2 = 0.09$, $P = 0.001$,
284 PERMANOVA; Fig. 1d and Supp. Fig. 4). Together, these results suggest that diseased and
285 healthy plants were associated with distinct soil properties despite clear between-province
286 variation in environmental conditions.

287

1
2
3 288 3.2 Soil moisture is the relatively most important factor distinguishing diseased and healthy
4
5
6 289 plant samples
7
8 290 To compare the relative importance of different soil properties, we used correlation analysis
9
10
11 291 and machine learning. We found statistically significant relationships between abiotic
12
13 292 physicochemical and biotic parameters and *R. solanacearum* pathogen densities in case of all
14
15 293 variables except for P and K availability ($P > 0.05$, Fig. 2a and Supp. Fig. 5). Specifically,
16
17 294 pathogen densities correlated negatively with N availability ($R^2 = 0.24$, $P = 0.005$) and average
18
19 295 Bray-Curtis dissimilarity (average Bray-Curtis distance of microbial community composition
20
21 296 from other 139 samples, $R^2 = 0.28$, $P = 0.001$). In contrast, pathogen densities were positively
22
23 297 associated with total bacterial densities ($R^2 = 0.60$, $P < 0.0001$), soil moisture ($R^2 = 0.55$, $P <$
24
25 298 0.0001), pH ($R^2 = 0.28$, $P = 0.001$), Shannon diversity ($R^2 = 0.20$, $P = 0.017$) and Chao1 richness
26
27 299 ($R^2 = 0.21$, $P = 0.014$; Fig. 2a and Supp. Fig. 5). Of all predictor variables, soil moisture (relative
28
29 300 weight = 40.36%), total bacterial density (relative weight = 22.77%) and soil pH (relative
30
31 301 weight = 14.59%) were the most significant predictors of pathogen densities in the tomato
32
33 302 rhizosphere (multiple regression model, AIC: 324.09; $F_{10,128} = 10.6$, $R^2 = 0.45$, $P < 0.0001$, Supp.
34
35 303 Table 1).

36
37 304 Random forest modelling was further used to analyse associations between soil
38
39 305 properties and plant health. By using all measured soil properties, we could predict bacterial
40
41 306 wilt disease outcomes with 78.6% accuracy (AUC = 0.89; Supp. Fig. 6). To eliminate the
42
43 307 obvious link between pathogen abundance and disease incidence, we re-ran the model
44
45 308 without pathogen density data (Fig. 2b). The high predictability of the model was retained,
46
47 309 and bacterial wilt disease outcomes could still be predicted with 75% accuracy (AUC = 0.75;
48
49 310 Fig. 2c). Based on ten-fold cross-validation with 10 independent model simulations (inset of
50
51 311 Fig. 2b), soil moisture was ranked as the most important individual predictor of plant health

1
2
3 312 followed by the total bacterial abundances (Fig. 2b). Together, these results suggest that
4
5 313 abiotic and biotic soil properties can reliably predict bacterial wilt disease occurrence, with
6
7
8 314 soil moisture being the relatively most important factor.
9

10 315

13 316 3.3 Variation in soil moisture can causally drive bacterial wilt disease occurrence

15 317 To directly test if soil moisture can drive variation in bacterial wilt disease incidence, we
16
17
18 318 performed a greenhouse experiment where tomato plants were exposed to *R. solanacearum*
19
20 319 type strain under different soil moisture treatments. We found that bacterial wilt disease
21
22
23 320 dynamics differed depending on soil moisture content and the stage of infection (Fig. 3). On
24
25 321 average, the highest disease incidence was observed in 60% followed by 70% soil moisture
26
27
28 322 content treatments, while no differences were observed between the other treatments (Fig.
29
30 323 3a-b). Specifically, soil moisture effects were visible during the early stages of infection in
31
32
33 324 terms of reduced lag-phase of disease onset ($F_{4,21} = 7.48$, $P < 0.0001$, ANOVA; Fig. 3b) and as
34
35 325 overall differences in area under disease progression curve (AUDPC, $\chi^2 = 13.73$, $P = 0.008$,
36
37 326 AUDPC panel), while soil moisture content had no effect on the disease rate during the
38
39
40 327 exponential phase of infection ($\chi^2 = 4.07$, $P = 0.396$, Kruskal-Wallis test; Fig. 3b). Together,
41
42 328 these results demonstrate that soil moisture alone can causally drive bacterial wilt disease
43
44
45 329 outcomes in otherwise homogenous tomato rhizosphere environments.

46
47 330

48
49
50 331

332 4 Discussion

333 Here we studied if plant-level variation in bacterial wilt disease occurrence could be explained
334 by local abiotic and biotic soil properties across six provinces in China. Our sampling data
335 shows that healthy and diseased plant rhizosphere soils were associated with distinct abiotic
336 and biotic properties which could predict bacterial wilt disease occurrence with 75% accuracy.
337 Soil moisture was identified as the most important predictor, and its causal role was tested
338 directly in a greenhouse experiment with tomato. It was found that variation in soil moisture
339 alone, could considerably change the disease dynamics resulting in different levels of disease
340 incidence. Our results are in line with previous studies that have identified a tight link
341 between moisture and plant diseases (Huber and Gillespie, 1992) with *Pseudomonas syringae*
342 (Xin et al., 2016) and *M. oryzae* pathogens in the plant phyllosphere (Li et al., 2014) and by
343 expanding this association to crops and other soil-borne bacterial diseases.

344 Moisture could affect plant pathogens in several ways (Aung et al., 2018) ranging from
345 effects on pathogen survival, movement and growth in the soil (Smilanick and Mansour, 2007;
346 Kearns, 2010) to effects on pathogen invasiveness (Li et al., 2014) or indirect effects on the
347 activation of plant defences (Panchal et al., 2016; Velásquez et al., 2018). While the
348 relationship with moisture has previously been observed with other soil-borne pathogenic
349 fungi and bacteria, including *R. solanacearum* (Chairman et al., 1981; van Elsas et al., 2000;
350 Islam and Toyama, 2004; Satou et al., 2006; Mondal et al., 2014; Jiang et al., 2018), we here
351 show that moisture was the relatively most important factor predicting bacterial wilt
352 occurrence across broad geographical scale spanning six Chinese provinces. Our sampling
353 area covered various soil types, tomato cultivars and climate conditions. While clear
354 geographical variation between locations was observed, the significance of soil moisture on
355 plant health status was significant within each field. As a result, this difference could not be

1
2
3 356 explained by local climate or agricultural practises, such as use of certain tomato cultivars. In
4
5
6 357 the future, it will be important to see if our findings can be extrapolated to other countries
7
8 358 and agricultural areas experiencing recurrent *R. solanacearum* outbreaks.
9

10 359 In addition to identifying an important country-wide link with the soil moisture, we
11
12
13 360 show that this association might not be an indirect consequence of *R. solanacearum* infection,
14
15 361 which typically leads to increased soil moisture via reduced water uptake and transpiration in
16
17
18 362 the infected plants (Jiang, 2016). Instead, by using controlled greenhouse experiment, we
19
20 363 demonstrate that soil moisture alone can causally drive bacterial wilt disease outcomes in
21
22
23 364 otherwise identical soil environmental conditions. Highest levels of disease incidence were
24
25 365 observed at 60% and 70% maximum water holding capacity soil moisture treatments, and
26
27
28 366 there are several potential reasons for this. First, it is possible that this moisture content level
29
30 367 was optimal for the plant growth (Kramer, 1983) leading to more efficient root exudation
31
32
33 368 (Larson and Funk, 2016) and improved growth and colonisation of the plant by the pathogen
34
35 369 (van Elsas et al., 2000; Islam and Toyama, 2004). Moreover, non-optimal soil moisture levels
36
37
38 370 have previously been shown to lead overexpression of plant resistance genes (Sinha et al.,
39
40 371 2016; Jiang et al., 2018), which could have also affected the observed differences in disease
41
42
43 372 occurrence, as reported before (Mondal et al., 2014). Alternatively, it is possible that certain
44
45 373 moisture levels were directly beneficial to the pathogen, allowing more efficient growth,
46
47
48 374 movement and colonisation of the plant (Beattie, 2011; Aung et al., 2018; Velásquez et al.,
49
50 375 2018). Finally, soil moisture is known to affect the availability of oxygen (Mainiero and Kazda,
51
52 376 2005) and nutrients (Cavagnaro, 2016), which could have affected the *R. solanacearum*
53
54
55 377 growth (Dalsing et al., 2015) or the strength of microbiome-mediated pathogen suppression
56
57 378 (Chen et al., 2007; Brockett et al., 2012) in the rhizosphere. Further experiments are **hence**
58
59 379 **however** needed to **directly** test these explanations-**directly**.
60

1
2
3 380 In addition to soil moisture, also some soil physiochemical properties, such as
4
5
6 381 microbiome composition, pH and nitrogen availability, differed between healthy and diseased
7
8 382 plants depending on the sampling sites. This is in line with previous findings showing a clear
9
10
11 383 link between bacterial community composition and bacterial wilt disease outcomes (Wei et
12
13 384 al., 2018, 2019), highlighting also the importance of microbial interactions for *R.*
14
15 385 *solanacearum* infections (Wei et al., 2019; Wen et al., 2020; Lee et al., 2021). While
16
17 386 differences in bacterial community richness and diversity of healthy and diseased plants were
18
19
20 387 only significant in Nanjing, bacterial community composition was more consistently
21
22 388 associated with plant health status indicative of its importance in predicting bacterial wilt
23
24 389 disease occurrence (Wei et al., 2019). In the future, it would be interesting to test if the
25
26 390 abundance and activity of certain *R. solanacearum*-suppressing bacteria, such Firmicutes and
27
28 391 Actinobacteria (Lee et al., 2021), were positively or negatively affected by the soil moisture
29
30 392 content. Furthermore, it has previously been shown that bacterial wilt disease is aggravated
31
32 393 in acidic soils (Li et al., 2017a, 2017b; Wang et al., 2017) and by high nitrogen availability (Y.
33
34 394 ~~Gu et al., 2020~~Gu et al., 2020a), while high C, N, P and K availabilities have been linked ~~with~~
35
36 395 to healthy plant rhizosphere (Wang et al., 2017; Wei et al., 2018; Wu et al., 2020). However,
37
38 396 we found that the physicochemical soil properties did not consistently differ between healthy
39
40 397 and diseased plants ~~machine learning algorithm~~. It is also possible that some of the healthy
41
42 398 plants were latently infected by *R. solanacearum*, and hence, did not show visible disease
43
44 399 symptoms despite being infected (Hayward, 1991; Genin and Denny, 2012). While certain
45
46 400 healthy plants overlapped in their soil properties with the diseased plants, we did not see
47
48 401 clear clustering of healthy plant samples at the field level. This suggests that the proportion
49
50 402 of latently infected plants was low, or that their microbiome properties were more similar to
51
52 403 healthy plants. In the future, it would be interesting to test if our algorithm can predict
53
54
55
56
57
58
59
60

1
2
3 404 bacterial wilt disease occurrence using other unrelated datasets, and if its performance can
4
5
6 405 be improved by taking latent infections into account. Furthermore, model predictions could
7
8 406 potentially be improved by including temporally, locally and globally varying some other
9
10 407 abiotic and biotic variables that vary temporally, such as temperature, humidity and
11
12 408 humidityprecipitation. - Furthermore,As it is likely that certain-these environmental factors
13
14
15 409 will have interactive effects, which shouldtheir effects be further explored experimentally.
16
17
18 410

411 **5 Conclusions**

412 We conclude that soil properties can be used as reliable predictors of bacterial wilt disease
413 occurrence, with soil moisture being one of the most import single factors that consistently
414 differed between healthy and diseased plants across all sampling locations. Moreover, while
415 other soil properties played important roles, their effects were often sampling location-
416 specific, indicative of their potential importance at the local scale. The causal role of soil
417 moisture was directly validated in a greenhouse house experiments, which highlights the
418 value of direct experimentation in separating causes from consequences in plant pathology
419 studies. The obtained information will be helpful for developing predictive modelling to
420 better understand the epidemiology of bacterial wilt disease outbreaks in spatially and
421 temporally varying agricultural environments and should be validated in the future with
422 unrelated datasets from other countries and agricultural areas. Finally, the importance of soil
423 moisture suggest that relatively simple water management practises could potentially be
424 effective way to control bacterial wilt disease occurrence.

425

1
2
3 426 **Acknowledgments**
4

5
6 427 We thank Dr. Alexandre Jousset and Dr. Zhipeng Liu for helpful discussions. This research was
7
8 428 financially supported by the National Natural Science Foundation of China (41922053,
9
10 429 42090060, 31972504 and 42007038 to Z.W., G.J. and Y.X. respectively), the Natural Science
11
12
13 430 Foundation of Jiangsu Province (SBK20180527, SBK2020042856 and SBK2019040382), the
14
15 431 National Key Research and Development Program of China (2017YFD0200206 to Y.Z.), the
16
17 432 China Postdoctoral Science Foundation (2019M651848 to G.J.), and the Fundamental
18
19 433 Research Funds for the Central Universities (KYXK202009-KYXK202012). S.G. is funded by the
20
21
22 434 NWO-Veni grant (016.Veni.181.078 to S.G.). V.F. is funded by the Royal Society
23
24 435 (RSG\R1\180213 and CHL\R1\180031) and jointly by a grant from UKRI, Defra, and the
25
26 436 Scottish Government, under the Strategic Priorities Fund Plant Bacterial Diseases programme
27
28 437 (BB/T010606/1) at the University of York.
29
30
31
32
33
34

35

36 439 **Conflict of Interest**

37 440 The authors declare that there are no relevant conflicts of interest.
38
39
40 441
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

- Aung, K., Jiang, Y., He, S.Y., 2018. The role of water in plant-microbe interactions. *The Plant Journal* 93, 771–780. doi:10.1111/tpj.13795
- Beattie, G.A., 2011. Water relations in the interaction of foliar bacterial pathogens with plants. *Annual Review of Phytopathology* 49, 533–555. doi:10.1146/annurev-phyto-073009-114436
- Berg, M., Koskella, B., 2018. Nutrient- and dose-dependent microbiome-mediated protection against a plant pathogen. *Current Biology* 28, 2487–2492.e3. doi:10.1016/j.cub.2018.05.085
- Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry* 44, 9–20. doi:10.1016/j.soilbio.2011.09.003
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunencko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7, 335–336. doi:10.1038/nmeth.f.303
- Cavagnaro, T.R., 2016. Soil moisture legacy effects: Impacts on soil nutrients, plants and mycorrhizal responsiveness. *Soil Biology and Biochemistry* 95, 173–179. doi:10.1016/j.soilbio.2015.12.016
- Chairman, J.F.P., Gardner, W.R., Elliott, L.F. (Eds.), 1981. *Water potential relations in soil microbiology*, SSSA Special Publications. John Wiley & Sons, Ltd.
- Chen, M.M., Zhu, Y.G., Su, Y.H., Chen, B.D., Fu, B.J., Marschner, P., 2007. Effects of soil moisture and plant interactions on the soil microbial community structure. *European Journal of Soil Biology* 43, 31–38. doi:10.1016/j.ejsobi.2006.05.001
- Cheng, Y.T., Zhang, L., He, S.Y., 2019. Plant-microbe interactions facing environmental challenge. *Cell Host & Microbe* 26, 183–192. doi:10.1016/j.chom.2019.07.009
- Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research* 42, D633–642. doi:10.1093/nar/gkt1244
- Cutler, F. original by L.B. and A., Wiener, R. port by A.L. and M., 2018. randomForest: Breiman and cutler's random forests for classification and regression.
- Cuzick, J., 1985. A Wilcoxon-type test for trend. *Statistics in Medicine* 4, 87–90. doi:10.1002/sim.4780040112
- Dalsing, B.L., Truchon, A.N., Gonzalez-Orta, E.T., Milling, A.S., Allen, C., 2015. *Ralstonia solanacearum* uses inorganic nitrogen metabolism for virulence, ATP production, and detoxification in the oxygen-limited host xylem environment. *MBio* 6, e02471. doi:10.1128/mBio.02471-14
- Dixon, P., 2003. VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14, 927–930. doi:10.1111/j.1654-1103.2003.tb02228.x
- Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26, 2460–2461. doi:10.1093/bioinformatics/btq461

- 1
2
3 Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial
4 community structure by use of taxon-specific quantitative PCR assays. *Applied and*
5 *Environmental Microbiology* 71, 4117–4120. doi:10.1128/AEM.71.7.4117-4120.2005
6
7 Genin, S., Denny, T.P., 2012. Pathogenomics of the *Ralstonia solanacearum* species complex.
8 *Annual Review of Phytopathology* 50, 67–89. doi:10.1146/annurev-phyto-081211-
9 173000
10
11 Gu, Y., Wang, X., Yang, T., Friman, V.P., Geisen, S., Wei, Z., Xu, Y., Jousset, A., Shen, Q.,
12 2020a. Chemical structure predicts the effect of plant-derived low-molecular weight
13 compounds on soil microbiome structure and pathogen suppression. *Functional*
14 *Ecology* 34, 2158–2169. doi:<https://doi.org/10.1111/1365-2435.13624>
15
16 Gu, S., Wei, Z., Shao, Z., Friman, V.P., Cao, K., Yang, T., Kramer, J., Wang, X., Li, M., Mei, X.,
17 Xu, Y., Shen, Q., Kümmerli, R., Jousset, A., 2020b. Competition for iron drives
18 phytopathogen control by natural rhizosphere microbiomes. *Nature Microbiology* 5,
19 1002–1010. doi:10.1038/s41564-020-0719-8
20
21 Hayward, A.C., 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas*
22 *solanacearum*. *Annual Review of Phytopathology* 29, 65–87.
23 doi:10.1146/annurev.py.29.090191.000433
24
25 Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., Schuetzenmeister, A., Scheibe, S., 2020.
26 multcomp: Simultaneous inference in general parametric models.
27
28 Huber, L., Gillespie, T.J., 1992. Modeling leaf wetness in relation to plant disease
29 epidemiology. *Annual Review of Phytopathology* 30, 553–577.
30 doi:10.1146/annurev.py.30.090192.003005
31
32 Islam, T., Toyama, K., 2004. Effect of moisture conditions and pre-incubation at low
33 temperature on bacterial wilt of tomato caused by *Ralstonia solanacearum*.
34 *Microbes and Environments* 19, 244–247. doi:10.1264/jsme2.19.244
35
36 Janvier, C., Villeneuve, F., Alabouvette, C., Edel-Hermann, V., Mateille, T., Steinberg, C.,
37 2007. Soil health through soil disease suppression: Which strategy from descriptors
38 to indicators? *Soil Biology and Biochemistry* 39, 1–23.
39 doi:10.1016/j.soilbio.2006.07.001
40
41 Jiang, G., 2016. Pathogenesis and modelling of infection dynamics in *Ralstonia*
42 *solanacearum* (thesis). [Http://www.Theses.Fr](http://www.Theses.Fr). Toulouse 3.
43
44 Jiang, G., Wei, Z., Xu, J., Chen, H., Zhang, Y., She, X., Macho, A.P., Ding, W., Liao, B., 2017.
45 Bacterial wilt in China: History, current status, and future perspectives. *Frontiers in*
46 *Plant Science* 8, 1549. doi:10.3389/fpls.2017.01549
47
48 Jiang, Y., Huang, M., Zhang, M., Lan, J., Wang, W., Tao, X., Liu, Y., 2018. Transcriptome
49 analysis provides novel insights into high-soil-moisture-elevated susceptibility to
50 *Ralstonia solanacearum* infection in ginger (*Zingiber officinale* Roscoe cv.
51 Southwest). *Plant Physiology and Biochemistry* 132, 547–556.
52 doi:10.1016/j.plaphy.2018.10.005
53
54 Kabacoff, R. (Ed.), 2015. *R in Action*, 2nd Edition. ed. Manning Publications Co., CTUnited
55 States.
56
57 Kahm, M., Hasenbrink, G., Lichtenberg-Fraté, H., Ludwig, J., Kschischo, M., 2010. grofit:
58 Fitting biological growth curves with R. *Journal of Statistical Software* 33, 1–21.
59 doi:10.18637/jss.v033.i07
60
61 Kearns, D.B., 2010. A field guide to bacterial swarming motility. *Nature Reviews*.
62 *Microbiology* 8, 634–644. doi:10.1038/nrmicro2405
63
64 Kramer, P.J., 1983. *Water relations of plants*. Elsevier Science, Oxford.

- 1
2
3 Kwak, M.J., Kong, H.G., Choi, K., Kwon, S.K., Song, J.Y., Lee, J., Lee, P.A., Choi, S.Y., Seo, M.,
4 Lee, H.J., Jung, E.J., Park, H., Roy, N., Kim, H., Lee, M.M., Rubin, E.M., Lee, S.-W., Kim,
5 J.F., 2018. Rhizosphere microbiome structure alters to enable wilt resistance in
6 tomato. *Nature Biotechnology* 36, 1100–1109. doi:10.1038/nbt.4232
- 7
8 Larson, J.E., Funk, J.L., 2016. Seedling root responses to soil moisture and the identification
9 of a belowground trait spectrum across three growth forms. *New Phytologist* 210,
10 827–838. doi:10.1111/nph.13829
- 11
12 Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. *Journal*
13 *of Statistical Software* 25, 1–18.
- 14
15 Lee, S.M., Kong, H.G., Song, G.C., Ryu, C.M., 2021. Disruption of Firmicutes and
16 Actinobacteria abundance in tomato rhizosphere causes the incidence of bacterial
17 wilt disease. *The ISME Journal* 15, 330–347. doi:10.1038/s41396-020-00785-x
- 18
19 Li, S., Liu, Y., Wang, J., Yang, L., Zhang, S., Xu, C., Ding, W., 2017a. Soil acidification
20 aggravates the occurrence of bacterial wilt in south China. *Frontiers in Microbiology*
21 8, 703. doi:10.3389/fmicb.2017.00703
- 22
23 Li, S., Xu, C., Wang, J., Guo, B., Yang, L., Chen, J., Ding, W., 2017b. Cinnamic, myristic and
24 fumaric acids in tobacco root exudates induce the infection of plants by *Ralstonia*
25 *solanacearum*. *Plant and Soil* 412, 381–395. doi:10.1007/s11104-016-3060-5
- 26
27 Li, Y., Uddin, W., Kaminski, J.E., 2014. Effects of relative humidity on infection, colonization
28 and conidiation of *Magnaporthe oryzae* on perennial ryegrass. *Plant Pathology* 63,
29 590–597. doi:10.1111/ppa.12127
- 30
31 Mainiero, R., Kazda, M., 2005. Effects of *Carex rostrata* on soil oxygen in relation to soil
32 moisture. *Plant and Soil* 270, 311–320. doi:10.1007/s11104-004-1724-z
- 33
34 Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M.,
35 Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G., Foster, G.D., 2012. Top
36 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant*
37 *Pathology* 13, 614–629. doi:10.1111/j.1364-3703.2012.00804.x
- 38
39 Mendiburu, F. de, 2020. agricolae: Statistical procedures for agricultural research.
- 40
41 Mondal, B., Bhattacharya, I., Khatua, D.C., 2014. Incidence of bacterial wilt disease in West
42 Bengal, India. *Academia Journal of Agricultural Research* 2, 139–146.
- 43
44 Orr, R., Nelson, P.N., 2018. Impacts of soil abiotic attributes on *Fusarium* wilt, focusing on
45 bananas. *Applied Soil Ecology* 132, 20–33. doi:10.1016/j.apsoil.2018.06.019
- 46
47 Panchal, S., Chitrakar, R., Thompson, B.K., Obulareddy, N., Roy, D., Hambright, W.S.,
48 Melotto, M., 2016. Regulation of stomatal defense by air relative humidity. *Plant*
49 *Physiology* 172, 2021–2032. doi:10.1104/pp.16.00696
- 50
51 Pansu, M., Gautheyrou, J. (Eds.), 2006. Handbook of soil analysis: Mineralogical, organic and
52 inorganic methods. Springer, Berlin, Heidelberg. doi:10.1007/978-3-540-31211-6_29
- 53
54 Patro, S.G.K., Sahu, K.K., 2015. Normalization: [Aa](#) preprocessing stage. ArXiv 1503.06462.
- 55
56 Peyraud, R., Cottret, L., Marmiesse, L., Genin, S., 2018. Control of primary metabolism by a
57 virulence regulatory network promotes robustness in a plant pathogen. *Nature*
58 *Communications* 9, 418. doi:10.1038/s41467-017-02660-4
- 59
60 Peyraud, R., Cottret, L., Marmiesse, L., Gouzy, J., Genin, S., 2016. A resource allocation
trade-off between virulence and proliferation drives metabolic versatility in the plant
pathogen *Ralstonia solanacearum*. *PLoS Pathogens* 12, e1005939.
doi:10.1371/journal.ppat.1005939

- 1
2
3 Pimentel, C.S., Ayres, M.P., 2018. Latitudinal patterns in temperature-dependent growth
4 rates of a forest pathogen. *Journal of Thermal Biology* 72, 39–43.
5 doi:10.1016/j.jtherbio.2017.11.018
6
7 R Core Team, 2020. The R Stats Package.
8 Rahman, K.A., Othman, R., 2020. Influence of pH levels on disease development in oil palm
9 seedling roots infected with *Ganoderma boninensis*. *Rhizosphere* 13, 100181.
10 doi:10.1016/j.rhisph.2019.100181
11
12 Raza, W., Wang, J., Wu, Y., Ling, N., Wei, Z., Huang, Q., Shen, Q., 2016a. Effects of volatile
13 organic compounds produced by *Bacillus amyloliquefaciens* on the growth and
14 virulence traits of tomato bacterial wilt pathogen *Ralstonia solanacearum*. *Applied*
15 *Microbiology and Biotechnology* 100, 7639–7650. doi:10.1007/s00253-016-7584-7
16
17 Raza, W., Ling, N., Liu, D., Wei, Z., Huang, Q., Shen, Q., 2016b. Volatile organic compounds
18 produced by *Pseudomonas fluorescens* WR-1 restrict the growth and virulence traits
19 of *Ralstonia solanacearum*. *Microbiological Research* 192, 103–113.
20 doi:10.1016/j.micres.2016.05.014
21
22 Satou, M., Kubota, M., Nishi, K., 2006. Measurement of horizontal and vertical movement of
23 *Ralstonia solanacearum* in soil. *Journal of Phytopathology* 154, 592–597.
24 doi:10.1111/j.1439-0434.2006.01152.x
25
26 Schandry, N., 2017. A practical guide to visualization and statistical analysis of *R.*
27 *solanacearum* infection data using R. *Frontiers in Plant Science* 8, 623.
28 doi:10.3389/fpls.2017.00623
29
30 Schönfeld, J., Heuer, H., van Elsas, J.D., Smalla, K., 2003. Specific and sensitive detection of
31 *Ralstonia solanacearum* in soil on the basis of PCR amplification of *fliC* fragments.
32 *Applied and Environmental Microbiology* 69, 7248–7256.
33 doi:10.1128/AEM.69.12.7248-7256.2003
34
35 Sinha, R., Gupta, A., Senthil-Kumar, M., 2016. Understanding the impact of drought on foliar
36 and xylem invading bacterial pathogen stress in chickpea. *Frontiers in Plant Science*
37 7, 902. doi:10.3389/fpls.2016.00902
38
39 Siregar, B.A., Giyanto, Hidayat, S.H., Siregar, I.Z., Tjahjono, B., 2020. Epidemiology of
40 bacterial wilt disease on *Eucalyptus pellita* F. Muell. in Indonesia. *IOP Conference*
41 *Series: Earth and Environmental Science* 468, 012033. doi:10.1088/1755-
42 1315/468/1/012033
43
44 Smilanick, J.L., Mansour, M.F., 2007. Influence of temperature and humidity on survival of
45 *Penicillium digitatum* and *Geotrichum citri-aurantii*. *Plant Disease* 91, 990–996.
46 doi:10.1094/PDIS-91-8-0990
47
48 Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant-microbiome interactions:
49 from community assembly to plant health. *Nature Reviews Microbiology* 18, 607–
50 621. doi:10.1038/s41579-020-0412-1
51
52 van Elsas, J.D., Kastelein, P., van Bekkum, P., van der Wolf, J.M., de Vries, P.M., van
53 Overbeek, L.S., 2000. Survival of *Ralstonia solanacearum* Biovar 2, the causative
54 agent of potato brown rot, in field and microcosm soils in temperate climates.
55 *Phytopathology* 90, 1358–1366. doi:10.1094/PHYTO.2000.90.12.1358
56
57 Velásquez, A.C., Castroverde, C.D.M., He, S.Y., 2018. Plant-pathogen warfare under changing
58 climate conditions. *Current Biology* 28, R619–R634. doi:10.1016/j.cub.2018.03.054
59
60 Wang, R., Zhang, H., Sun, L., Qi, G., Chen, S., Zhao, X., 2017. Microbial community
composition is related to soil biological and chemical properties and bacterial wilt
outbreak. *Scientific Reports* 7, 343. doi:10.1038/s41598-017-00472-6

- 1
2
3 Wei, Z., Friman, V.P., Pommier, T., Geisen, S., Jousset, A., Shen, Q., 2020. Rhizosphere
4 immunity: targeting the underground for sustainable plant health management.
5 *Frontiers of Agricultural Science and Engineering* 7, 317–328.
6
7 Wei, Z., Gu, Y., Friman, V.P., Kowalchuk, G.A., Xu, Y., Shen, Q., Jousset, A., 2019. Initial soil
8 microbiome composition and functioning predetermine future plant health. *Science*
9 *Advances* 5, eaaw0759. doi:10.1126/sciadv.aaw0759
10
11 Wei, Z., Hu, J., Gu, Y., Yin, S., Xu, Y., Jousset, A., Shen, Q., Friman, V.P., 2018. *Ralstonia*
12 *solanacearum* pathogen disrupts bacterial rhizosphere microbiome during an
13 invasion. *Soil Biology and Biochemistry* 118, 8–17. doi:10.1016/j.soilbio.2017.11.012
14
15 Wei, Z., Huang, J., Yang, T., Jousset, A., Xu, Y., Shen, Q., Friman, V.P., 2017. Seasonal
16 variation in the biocontrol efficiency of bacterial wilt is driven by temperature-
17 mediated changes in bacterial competitive interactions. *Journal of Applied Ecology* 5,
18 1440–1448. doi:10.1111/1365-2664.12873
19
20 Wei, Z., Huang, J.F., Hu, J., Gu, Y.A., Yang, C.L., Mei, X.L., Shen, Q.R., Xu, Y.C., Friman, V.P.,
21 2015a. Altering transplantation time to avoid periods of high temperature can
22 efficiently reduce bacterial wilt disease incidence with tomato. *PloS One* 10,
23 e0139313. doi:10.1371/journal.pone.0139313
24
25 Wei, Z., Yang, T., Friman, V.P., Xu, Y., Shen, Q., Jousset, A., 2015b. Trophic network
26 architecture of root-associated bacterial communities determines pathogen invasion
27 and plant health. *Nature Communications* 6, 8413. doi:10.1038/ncomms9413
28
29 Wei, Z., Yang, X., Yin, S., Shen, Q., Ran, W., Xu, Y., 2011. Efficacy of *Bacillus*-fortified organic
30 fertiliser in controlling bacterial wilt of tomato in the field. *Applied Soil Ecology* 48,
31 152–159. doi:10.1016/j.apsoil.2011.03.013
32
33 Wen, T., Zhao, M., Liu, T., Huang, Q., Yuan, J., Shen, Q., 2020. High abundance of *Ralstonia*
34 *solanacearum* changed tomato rhizosphere microbiome and metabolome. *BMC*
35 *Plant Biology* 20, 166. doi:10.1186/s12870-020-02365-9
36
37 Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani,
38 H., Dunnington, D., RStudio, 2020. ggplot2: Create elegant data visualisations using
39 the grammar of graphics.
40
41 Wu, X., Li, H., Wang, Y., Zhang, X., 2020. Effects of bio-organic fertiliser fortified by *Bacillus*
42 *cereus* QJ-1 on tobacco bacterial wilt control and soil quality improvement.
43 *Biocontrol Science and Technology* 30, 351–369.
44 doi:10.1080/09583157.2020.1711870
45
46 Xin, X.F., Nomura, K., Aung, K., Velásquez, A.C., Yao, J., Boutrot, F., Chang, J.H., Zipfel, C., He,
47 S.Y., 2016. Bacteria establish an aqueous living space in plants crucial for virulence.
48 *Nature* 539, 524–529. doi:10.1038/nature20166
49
50
51
52
53
54
55
56
57
58
59
60

Table 1: Differences in abiotic physicochemical and biotic soil properties between healthy and diseased plants

Factor	Name (Units)	Statistical method	Diseased vs healthy plants (<i>P</i> -values)*					
			CS	NB	NC	NJ	NN	WH
Moisture	Soil moisture content (%)	Wilcoxon test	<0.001	0.026	0.115	0.009	0.006	0.922
pH	Soil pH value	Wilcoxon test	0.312	0.729	0.025	0.016	0.954	0.431
Phosphorus	Available phosphorus (mg·kg ⁻¹)	Wilcoxon test	0.514	0.63	0.606	0.079	0.862	0.224
Potassium	Available potassium (mg·kg ⁻¹)	Wilcoxon test	0.114	0.319	0.001	0.928	0.012	0.699
Carbon	Water-soluble carbon (mg·kg ⁻¹)	Wilcoxon test	0.799	0.378	0.599	0.009	0.008	0.047
Nitrogen	Water-soluble nitrogen (mg·kg ⁻¹)	Wilcoxon test	0.887	0.143	0.028	0.211	0.419	0.401
Pathogen density	<i>R. solanacearum</i> density (log ₁₀ <i>fliC</i> gene copies g ⁻¹ soil)	Wilcoxon test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Bacterial density	Total bacterial density (log ₁₀ <i>16S rRNA</i> gene copies g ⁻¹ soil)	Wilcoxon test	0.002	0.04	0.645	0.005	0.014	0.081
Shannon	Shannon index for bacterial community diversity (OUT level)	Wilcoxon test	>0.999	0.198	0.519	0.002	0.291	0.133
Chao1	Chao1 index for bacterial community richness (OTU level)	Wilcoxon test	0.755	0.977	0.133	0.002	0.198	0.401
Bray-Curtis	Bray-Curtis dissimilarity index for bacterial community composition	PERMANOVA test	0.034	0.023	0.298	0.001	0.003	0.067

1
2
3 *Sampling locations are abbreviated as follows: CS = Changsha, NB = Ningbo, NC = Nanchang, NJ = Nanjing, NN = Nanning and WH = Wuhan. P-
4 values less than 0.05 are shown in red colour. Details of the analysis are listed in Supplementary Figures 2 – 4.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

For Review Only

1
2
3 442 **Figure legends**
4

5 443 **Figure 1. Differences in abiotic physicochemical and biotic soil properties between healthy**
6
7 **and diseased plants. (a)** Map of China showing sampling locations and provinces (CS =
8
9 Changsha, NB = Ningbo, NC = Nanchang, NJ = Nanjing, NN = Nanning and WH = Wuhan;
10
11 445 numbers in parentheses show the number of samples included in each location). **(b)**
12
13 446 Comparison of the normalised physicochemical (blue) and biotic (black) parameters between
14
15 447 healthy (green) and diseased (red) tomato plant rhizosphere samples (ns denote for non-
16
17 448 significant correlation ($P > 0.05$) and stars (**, ***, ****) denote significant correlation at
18
19 449 levels $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively). Pathogen and total bacterial
20
21 450 abundances are abbreviated as 'Pathogen' and 'Bacteria', respectively. **(c)** Comparison of
22
23 451 microbial community composition (PCoA) between healthy and diseased tomato plant
24
25 452 rhizosphere samples (*status*) at each sampling location (*site*). **(d)** Comparison of abiotic soil
26
27 453 physicochemical properties and biotic soil properties (PCA) between healthy and diseased
28
29 454 tomato plant rhizosphere samples (*status*) at each sampling location (*site*).
30
31 455
32
33
34
35
36
37
38
39

40 457 **Figure 2. The relative importance of abiotic physicochemical and biotic soil properties in**
41
42 458 **predicting bacterial wilt disease occurrence. (a)** Correlation coefficients (ranging from
43
44 459 negative (purple) to positive (cyan)) between *R. solanacearum* pathogen densities and abiotic
45
46 460 physicochemical (blue) and biotic (black) soil properties across all tomato rhizosphere
47
48 461 samples (ns denote for non-significant correlation ($P > 0.05$) and stars (**, ***, ****) denote
49
50 462 significant correlation at levels $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively). **(b)** Relative
51
52 463 importance rank of abiotic physicochemical (blue) and biotic (black) soil properties and ten-
53
54 464 fold cross-validation of random forest model (inset in **b**) based on the training set (80% of
55
56 465 randomly selected rhizosphere samples). Total bacterial abundances are abbreviated as
57
58
59
60

1
2
3 466 'Bacteria'. (c) Validation of random-forest model with a test set (20% of remaining samples)
4
5
6 467 predicting plant disease outcomes based on soil properties: green and red filled cells denote
7
8 468 for correct predictions and filled cells with white crosses denote for false predictions.
9

10 469

11
12
13 470 **Figure 3. Causal validation of the role of soil moisture driving bacterial wilt disease dynamics**

14
15 471 **in a greenhouse experiment. (a)** Mean disease progression curves in different soil moisture

16
17
18 472 treatments based on logistic curve fitting (left inset shows goodness-of-fit and significance for

19
20 473 each treatment). **(b)** Comparison of disease dynamics between different treatments in terms

21
22
23 474 of lag-phase before disease onset (early stage), disease rate (exponential stage) and area

24
25 475 under progression of disease curve (AUDPC, late stage). Different small letters above violin

26
27
28 476 plots denote for significant differences between treatment groups ($P < 0.05$).
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Dear Dr. Jiang:

4 Manuscript ID SEL-2020-0105 entitled "The relative importance of soil moisture in
5 predicting bacterial wilt disease occurrence" which you submitted to the Soil Ecology
6 Letters, has been reviewed. The comments of the reviewer(s) are included at the bottom of
7 this letter.
8
9

10 The reviewer(s) have recommended publication, but also suggest some minor revisions to
11 your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise
12 your manuscript.
13
14

15 To revise your manuscript, log into <https://mc.manuscriptcentral.com/selett> and enter your
16 Author Center, where you will find your manuscript title listed under "Manuscripts with
17 Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been
18 appended to denote a revision.
19
20

21 You may also click the below link to start the revision process (or continue the process if you
22 have already started your revision) for your manuscript. If you use the below link you will
23 not be required to login to ScholarOne Manuscripts.
24
25

26 *** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed
27 to a webpage to confirm. ***
28
29

30 [https://mc.manuscriptcentral.com/selett?URL_MASK=ac36874ccbfa4e449d36f64e151a619](https://mc.manuscriptcentral.com/selett?URL_MASK=ac36874ccbfa4e449d36f64e151a619b)
31 [b](https://mc.manuscriptcentral.com/selett?URL_MASK=ac36874ccbfa4e449d36f64e151a619b)
32
33

34
35 You will be unable to make your revisions on the originally submitted version of the
36 manuscript. Instead, revise your manuscript using a word processing program and save it on
37 your computer. Please also highlight the changes to your manuscript within the document
38 by using the track changes mode in MS Word or by using bold or colored text.
39
40

41 Once the revised manuscript is prepared, you can upload it and submit it through your
42 Author Center.
43
44

45 When submitting your revised manuscript, you will be able to respond to the comments
46 made by the reviewer(s) in the space provided. You can use this space to document any
47 changes you make to the original manuscript. In order to expedite the processing of the
48 revised manuscript, please be as specific as possible in your response to the reviewer(s).
49
50

51 **IMPORTANT:** Your original files are available to you when you upload your revised
52 manuscript. Please delete any redundant files before completing the submission.
53
54

55 Because we are trying to facilitate timely publication of manuscripts submitted to the Soil
56 Ecology Letters, your revised manuscript should be uploaded in the next two weeks. If it is
57 not possible for you to submit your revision in this time, you should contact with us as soon
58 as possible, or we may have to consider your paper as a new submission.
59
60

1
2
3 Once again, thank you for submitting your manuscript to the Soil Ecology Letters and I look
4 forward to receiving your revision.
5

6
7 Sincerely,
8 Editorial Office, Soil Ecology Letters
9

10 **Response to Editor:** Thanks for handling our manuscript and your encouraging decision of
11 'minor revision'. We have carefully revised the manuscript following the constructive
12 comments and suggestions from reviewers, and our point by point answers can be found
13 below.
14
15

16
17 **Reviewer(s)' Comments to Author:**
18

19 Reviewer: 1
20

21 Comments to the Author

22 This paper deals with defining the soil properties to predict bacterial wilt occurrence in
23 fields. Based on field evaluation of bacterial wilt occurrence in tomato, soil properties from
24 various fields in China, microbiome/statistical analysis, and validation experiments in green
25 house, authors propose that soil moisture is the most important predictor for bacterial wilt
26 in field-growing tomato. Both biotic and abiotic factors differed among sites and between
27 diseased and healthy plants, and this was the same in soil microbial composition. Among
28 the soil properties, soil moisture was the most important predictor of pathogen density and
29 disease outcome in fields. Contribution of soil moisture content to bacterial wilt was further
30 validated in a greenhouse.
31
32
33

34
35 The main conclusion of this paper is acceptable based on the analysis and interpretation of
36 the result, however, I found a couple of points to be clarified in detail in methods or to be
37 discussed in discussion.
38
39

40 **Response 1 to first reviewer:** Thank you very much for your time to revise our manuscript.
41 Please find our responses to your comments below.
42

43
44 1) Selection of diseased or healthy plants from fields: what was the exact criteria to
45 differentiate the plants "diseased" or "healthy"? Was it solely based on wilting symptoms?
46 If this is the case, authors should consider latent infection status of bacterial wilt in fields.
47 Please discuss this.
48
49

50 **Response 2 to first reviewer:** Yes, the diseased and healthy plants were chosen solely based
51 on visible wilting symptoms as described on lines 138-139. We fully agree that some of the
52 healthy plants could have been latently infected by *Ralstonia* and now consider this
53 possibility in the text. It is possible that some of the healthy plants were latently infected by
54 *R. solanacearum*, and hence, did not show visible disease symptoms despite being infected.
55 While certain healthy plants overlapped in their soil properties with the diseased plants, we
56 did not see clear clustering of healthy plant samples at the field level. This suggests that the
57 proportion of latently infected plants was low, or that their microbiome properties were
58 more similar to healthy plants. This is now discussed on lines 394-399.
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

2) Methodology to validate disease severity depending on soil moisture; the *Ralstonia* challenge to tomato plants was done by a soil drenching method which will change the soil moisture content. Authors described a way to maintain the constant soil moisture before pathogen inoculation. But with soil drenching inoculation, how did they control the soil moisture? Did authors only focus on the water content during acclimatization period?

Response 2 to first reviewer: We agree that soil drenching infections will increase the soil moisture content levels. However, this effect was kept consistent to all moisture treatments and limited to soil drenching application period (one day): soil moisture contents were otherwise carefully manipulated between treatments before and after pathogen inoculation. We have now described this in the manuscript on lines 233-239.

3) Some of the references in text were not indicated with the proper citation format. Authors need to double check this.

Response 2 to first reviewer: We have now corrected the format of referencing throughout the manuscript (on lines 81, 93, 95 and 391 and in the 'References' list).

Reviewer: 2

Comments to the Author

Considering the poor understanding of the relative importance of different factors, or their combinations, for plant disease, this study is timing to show a clear link between soil properties, pathogen density and plant health, by analysing 139 tomato rhizosphere soils samples isolated from six provinces in China. The authors found disease outcomes were positively associated with soil moisture that determined bacterial community, especially abundance of some bacterial groups. The exquisite experimental design together with advanced algorithm allows quantify the relative importance of soil properties in predicting bacterial wilt disease occurrence. The study certainly has sufficient novelty and updated our knowledge of what edaphic variables promote pathogenic *Ralstonia solanacearum* thus caused occurrence of bacterial wilt. I like the overall story and don't have much criticism with the research. Before considering acceptance, I have a few concerns as below.

Response 1 to second reviewer: We thank reviewer for positive comments. Please, find our detailed responses to your comments below.

1. The valid experiment was conducted to test the causality though, I have one major question about the collected 139 samples based on which "water" was attributed to the biggest predictor to wilt. As water content depend on the weather of the sampling day (rain or not), the local climatic information, i.e. average precipitation per year or during growth, thus, might be better used as variable.

Response 2 to second reviewer: This is a very good point, and we fully agree that local weather data could potentially be very useful for predicting bacterial wilt disease incidence

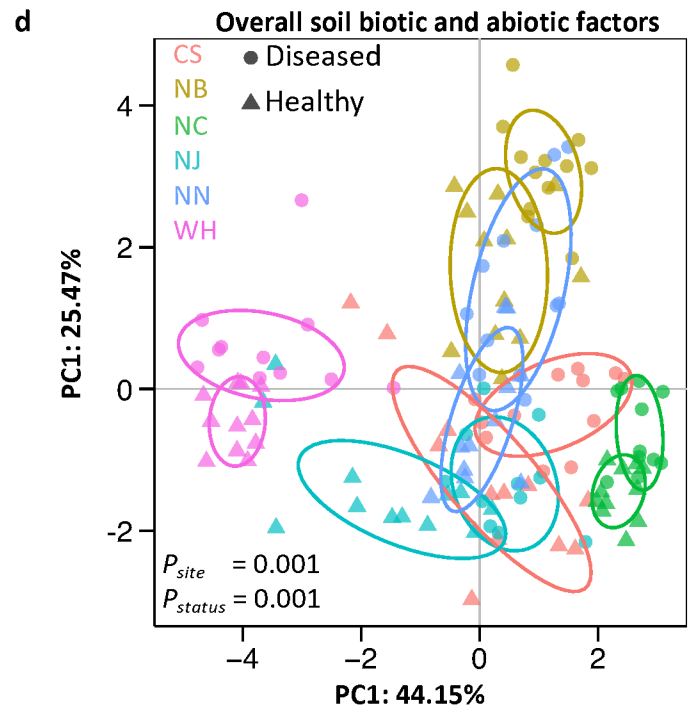
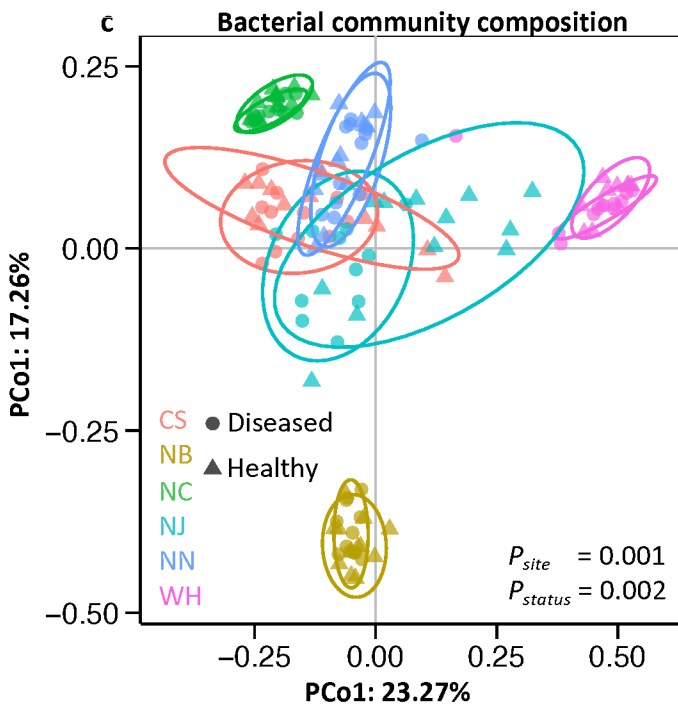
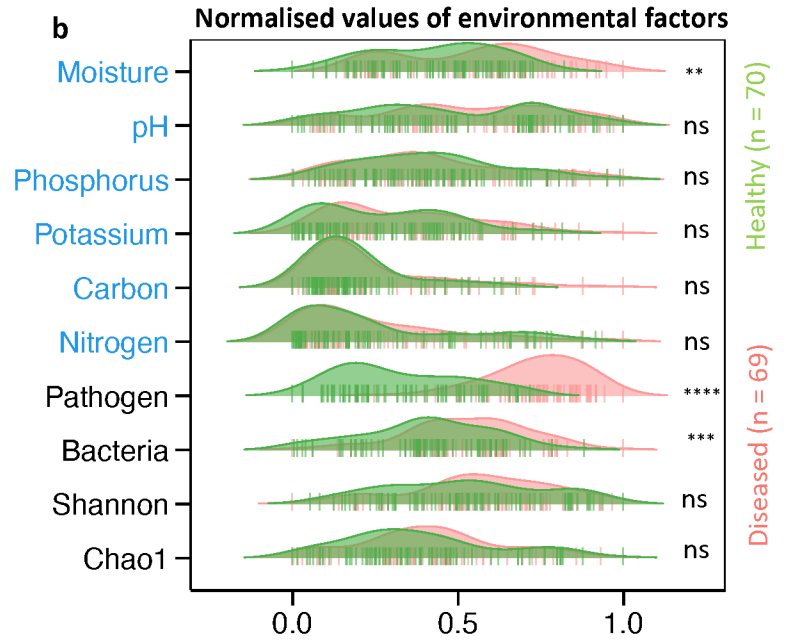
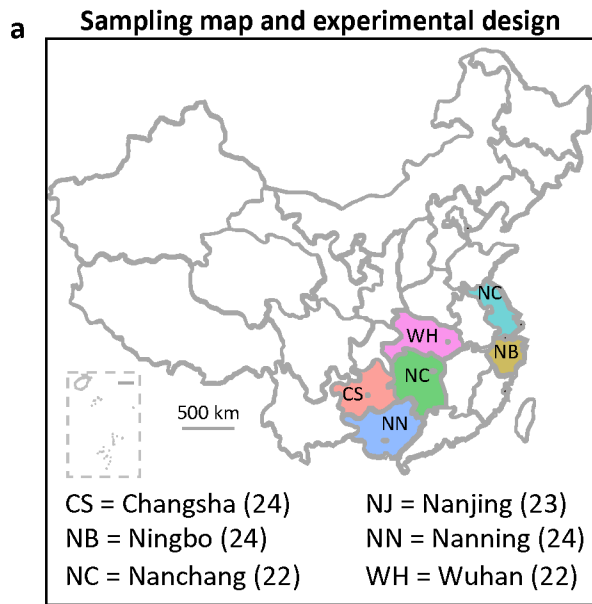
1
2
3 across wider geographical areas. This would be especially useful in the face of global climate
4 change. We now briefly discuss about this on lines 402-404.
5
6

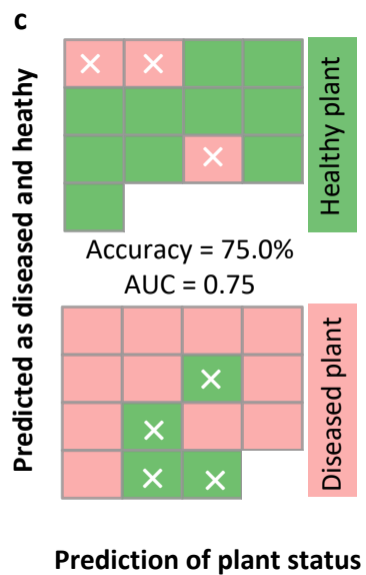
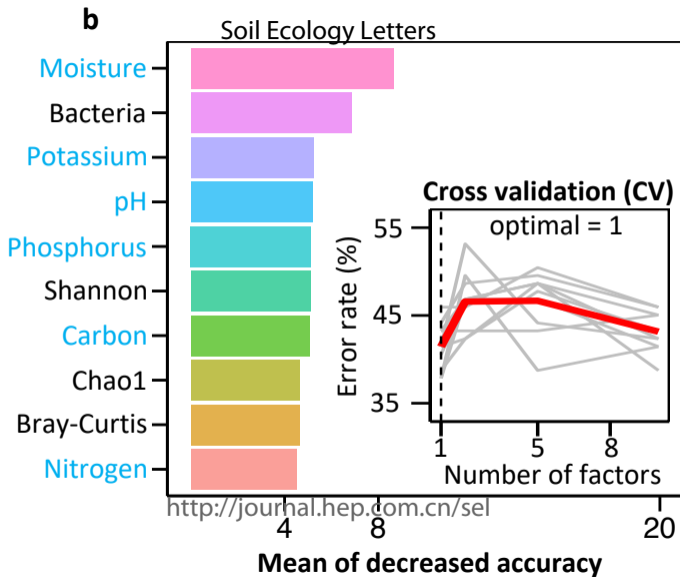
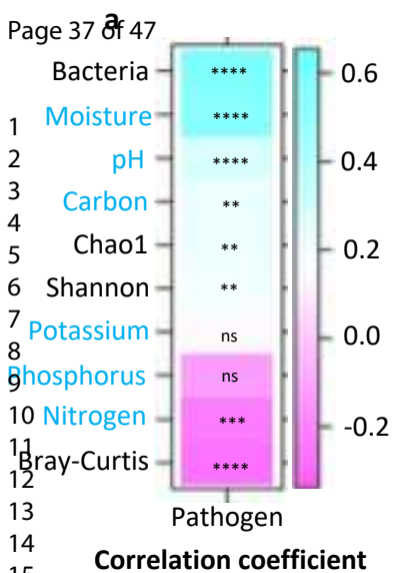
7 2. Line 68, maybe move this sentence to the end of introduction
8

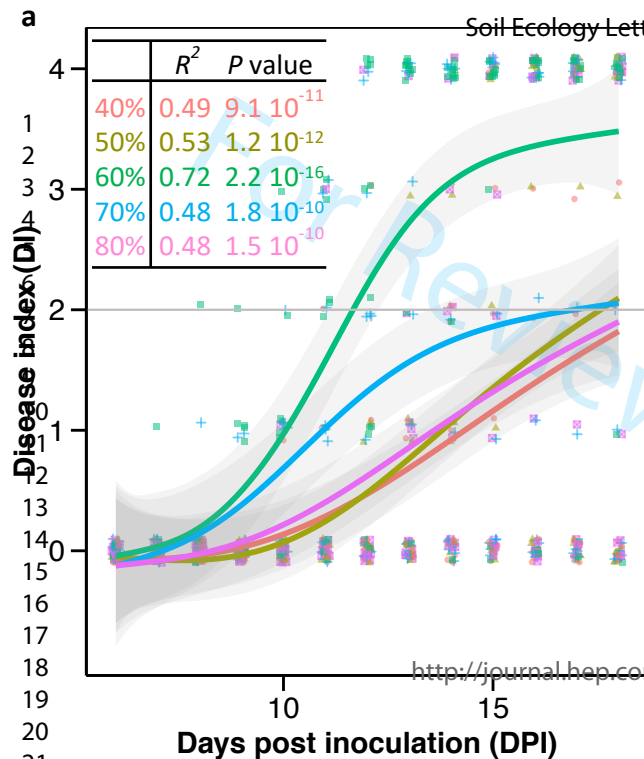
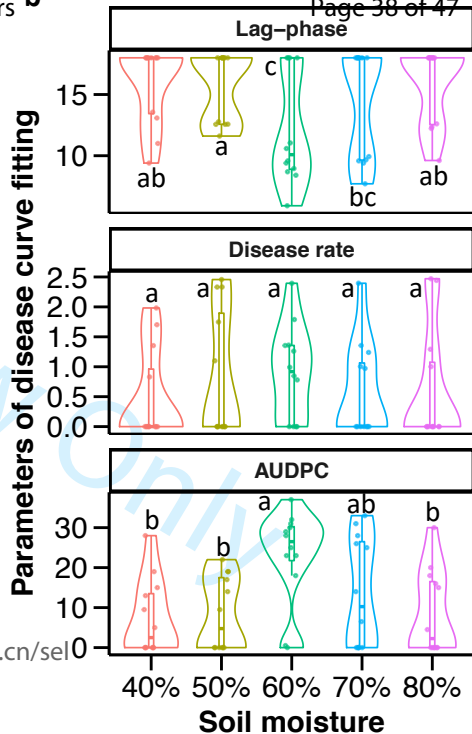
9 **Response 3 to second reviewer:** Thank you for your suggestion. We would prefer to give
10 readers an overview of our research question already at the beginning of the introduction.
11 We believe this is helpful for communicating our research questions clearly and setting
12 everything in the relevant context.
13
14

15 3. Fig. 1c and d, any chance to give the legend of abiotic variables, I might miss it.
16
17

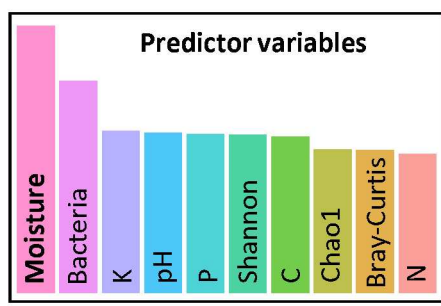
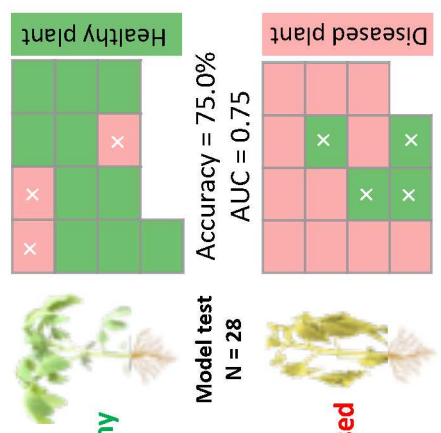
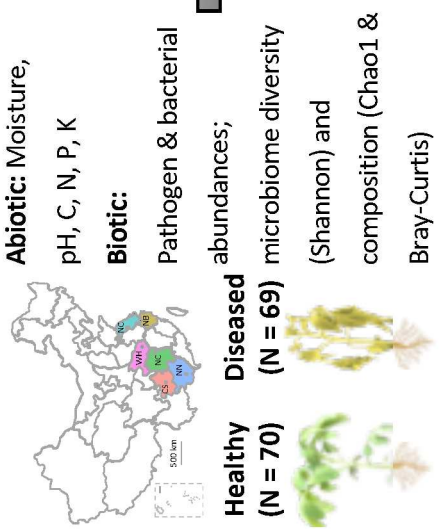
18 **Response 4 to second reviewer:** Panels C and D describe overall differences in bacterial
19 community composition and abiotic soil properties using multivariate analysis (Principal
20 component analysis, *i.e.*, PCA). Individual samples are further separated along with the
21 health status of the plant (healthy vs. diseased) and field of isolation in both panels. As a
22 result, each observation (individual dots) represents an overall value based on multiple
23 variables.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60





**b**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Importance rank

Predicting plant health status

Random forest classification

Sampling and characterising soil properties

Supplementary files

The relative importance of soil moisture in predicting bacterial wilt disease occurrence

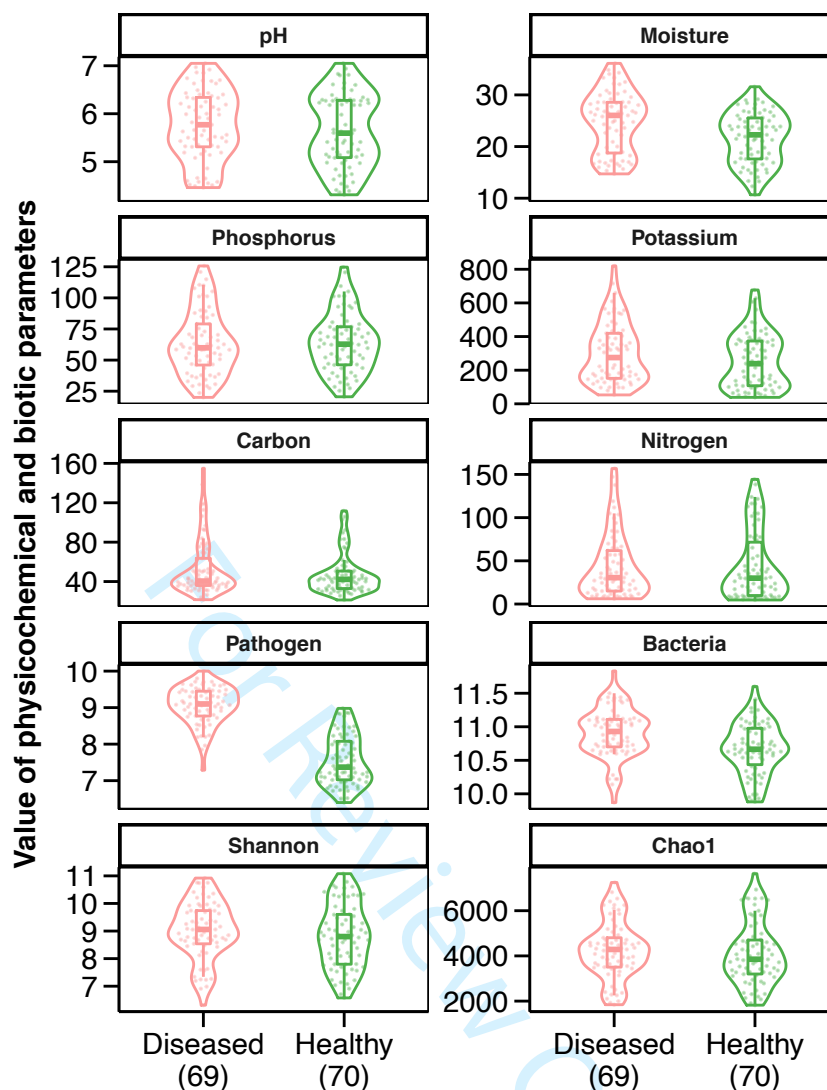
Gaofei Jiang, Ningqi Wang, Yaoyu Zhang, Zhen Wang, Yuling Zhang, Jiabao Yu, Yong Zhang, Zhong Wei, Yangchun Xu, Stefan Geisen, Ville-Petri Friman, Qirong Shen

The supplementary information contains six files. The table file includes statistical information about multiple regression analysis, and figure files provide further detail on the variation of physicochemical properties and bacterial communities in tomato rhizosphere and how it was linked to pathogen density and plant healthy in tomato rhizosphere microbiomes.

1
2
3 **13 Supplementary Table 1. ANOVA table summarizing the relative importance of abiotic**
4 **14 physicochemical and biotic soil properties in predicting pathogen densities in tomato**
5 **15 rhizosphere samples.**
6
7

Predictor		Sum	Mean			Relative
variable	Df	Square	Square	<i>F</i> -value	<i>P</i> -value	weight
pH	1	10.29	10.29	18.68	<0.0001↓	14.59%
Moisture	1	28.45	28.45	51.66	<0.0001↑	40.36%
Phosphorus	1	0.14	0.14	0.25	0.6170↑	0.20%
Potassium	1	0.14	0.14	0.26	0.6141↑	0.20%
Carbon	1	0.07	0.07	0.12	0.7310↓	0.09%
Nitrogen	1	0.03	0.03	0.05	0.8271↓	0.04%
Bacterial						
abundance	1	16.05	16.05	29.15	<0.0001↑	22.77%
Shannon	1	1.78	1.78	3.24	0.0744↑	2.53%
Chao1	1	1.33	1.33	2.42	0.1226↓	1.89%
Bray-Curtis	1	0.11	0.11	0.21	0.6499↑	0.16%
Residuals	128	70.50	0.55			
Model Summary			AIC: 324.09; $F_{10,128} = 10.6$, $R^2 = 0.45$, $P < 0.0001$			

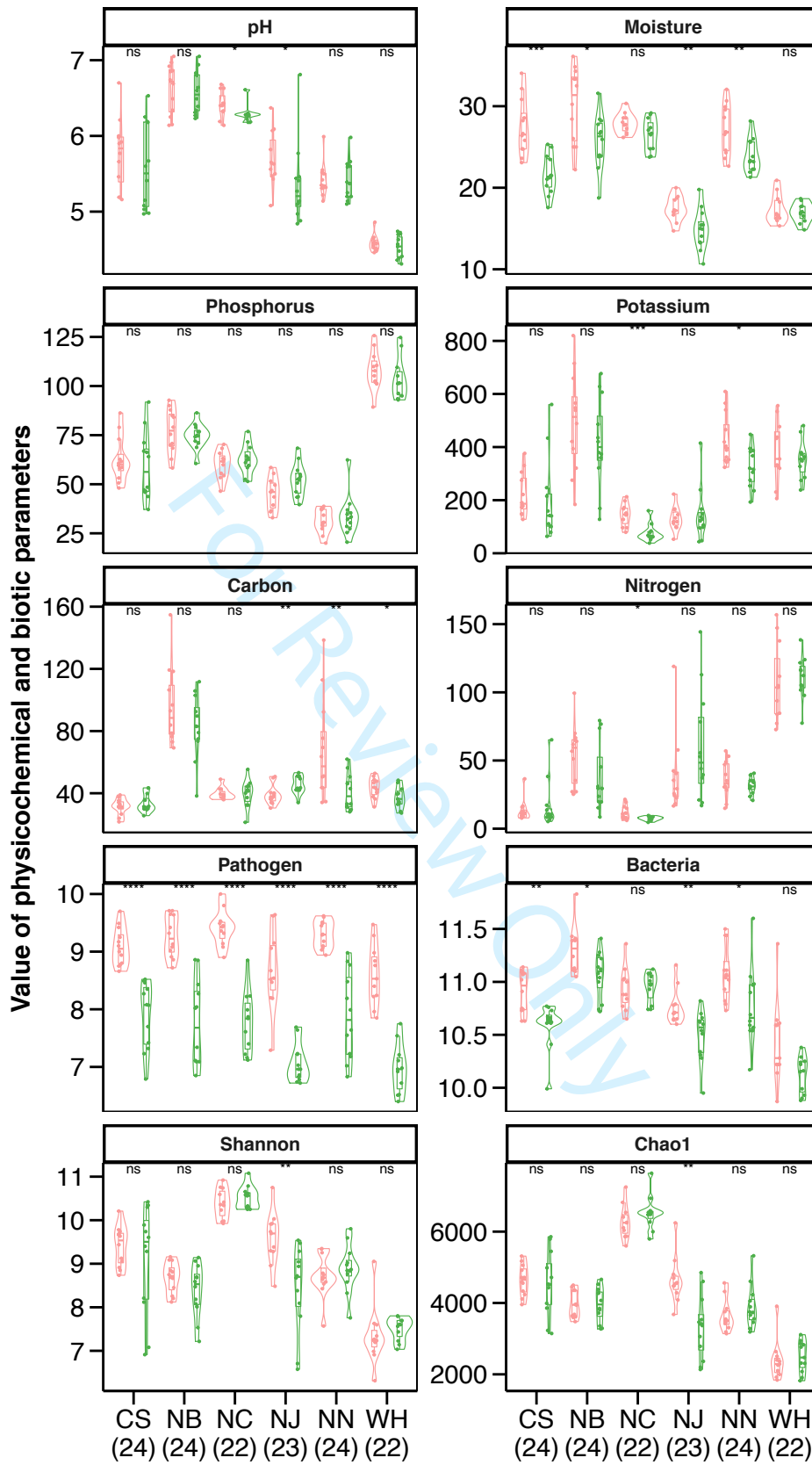
16 Note: The significant effects ($P < 0.05$) are shown in red colour and the 'up' and 'down' arrows
17 denote for positive and negative effects, respectively, based on multiple regression model.
18



19

20 **Supplementary Figure 1. Differences in abiotic physicochemical and biotic soil**
 21 **properties between diseased and healthy plants.**

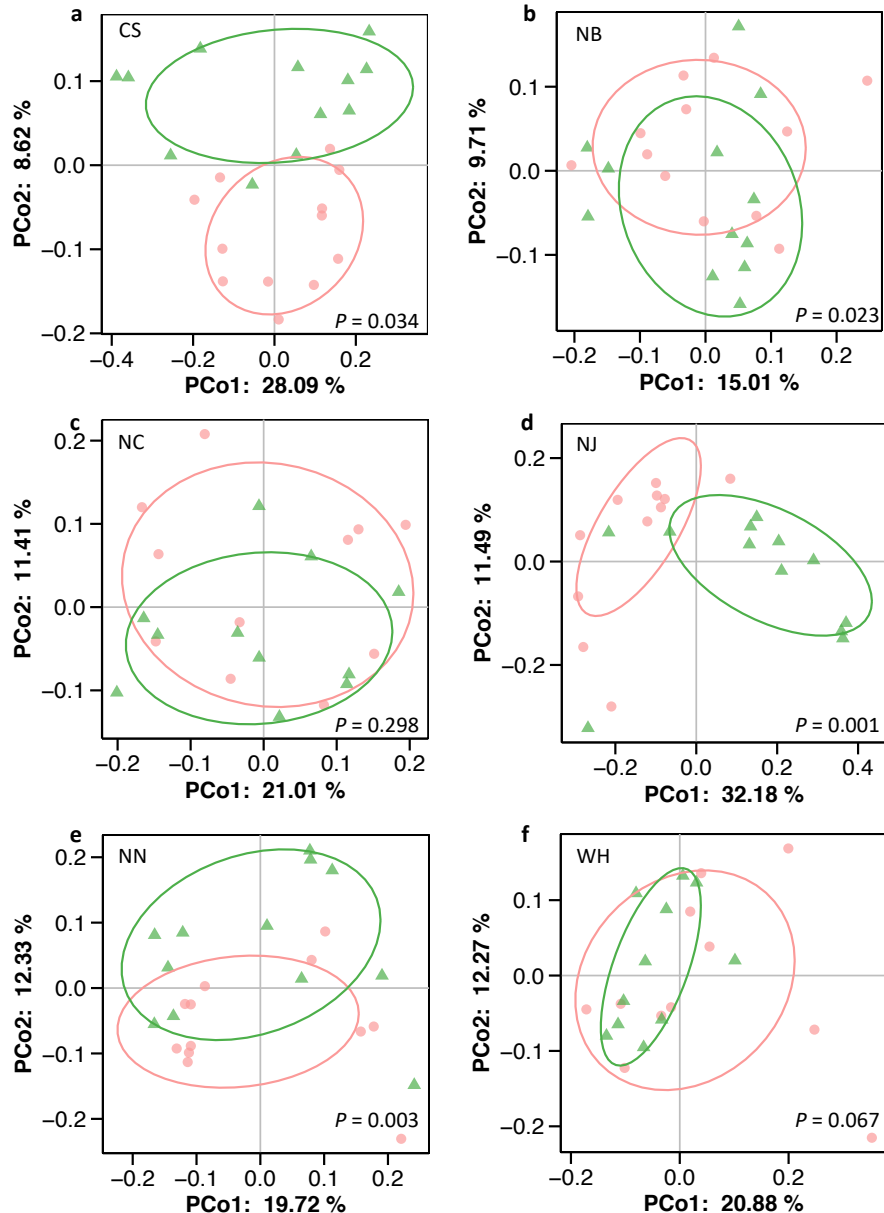
22 All data is pooled over sampling locations and the numbers in the parentheses denote the
 23 sample size in each group. The 'ns' denotes for non-significant difference ($P > 0.05$) and stars
 24 (*, **, *** and ****) show significant differences at levels $P < 0.05$, $P < 0.01$, $P < 0.001$ and
 25 $P < 0.0001$, respectively. Pathogen and total bacterial abundances are abbreviated as 'Pathogen'
 26 and 'Bacteria', respectively. Each violin plot shows the distribution of 69 and 70 rhizosphere
 27 soil samples in diseased and healthy plants, respectively.



1
2
3 29 **Supplementary Figure 2. Differences in abiotic physicochemical and biotic soil**
4 **properties of diseased and healthy plants in different sampling locations (provinces).**
5
6

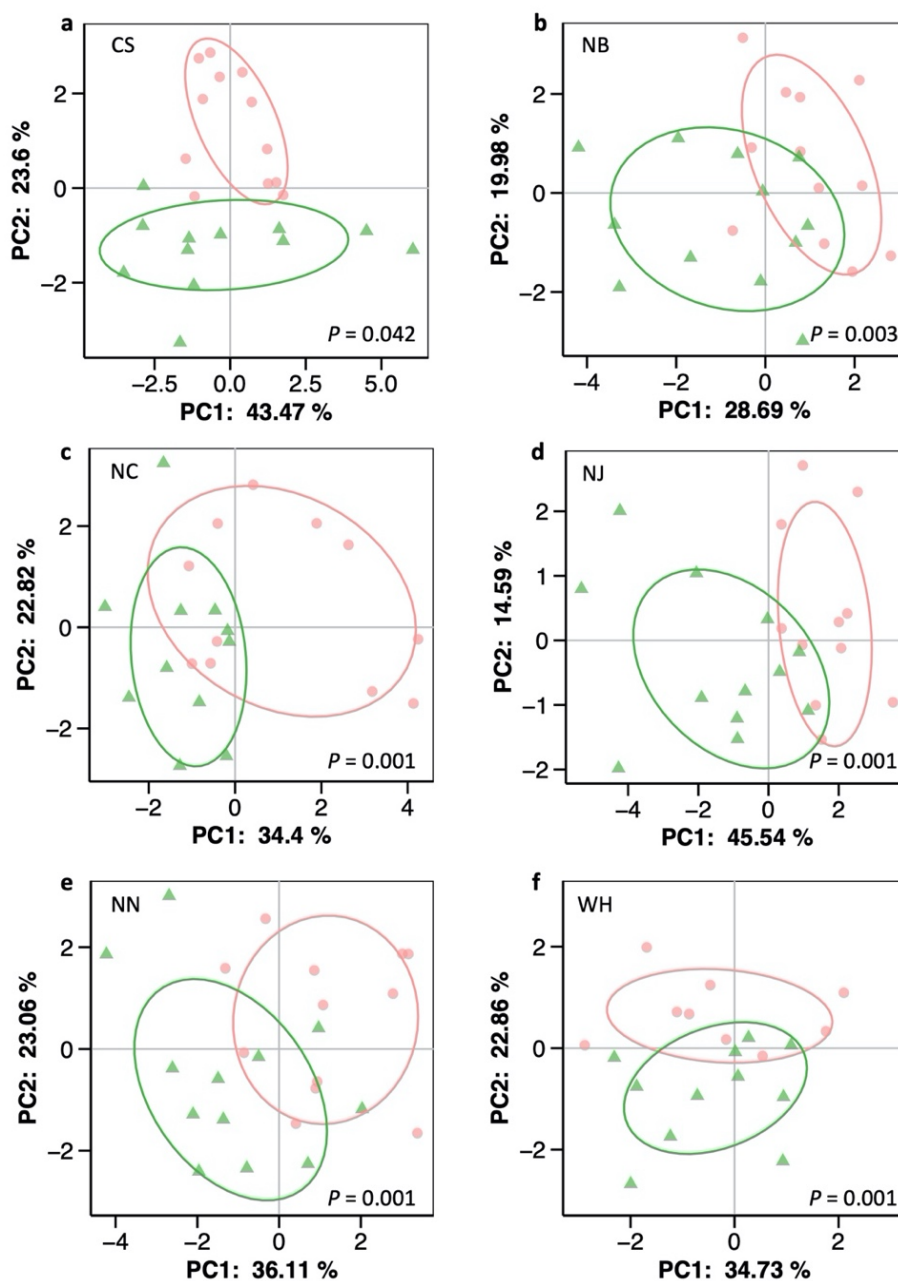
7 31 Numbers in the parentheses show sample size in each group. The 'ns' denotes for non-
8
9 32 significant difference ($P > 0.05$) and stars (*, **, *** and ****) show significant differences
10
11 33 at levels $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively. Pathogen and total bacterial
12
13 34 abundances are abbreviated as 'Pathogen' and 'Bacteria', respectively. Each violin plot shows
14
15 35 the distribution of rhizosphere soils in each province. Sampling locations are abbreviated as
16
17 36 follows: CS = Changsha, NB = Ningbo, NC = Nanchang, NJ = Nanjing, NN = Nanning and
18
19 37 WH = Wuhan.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only



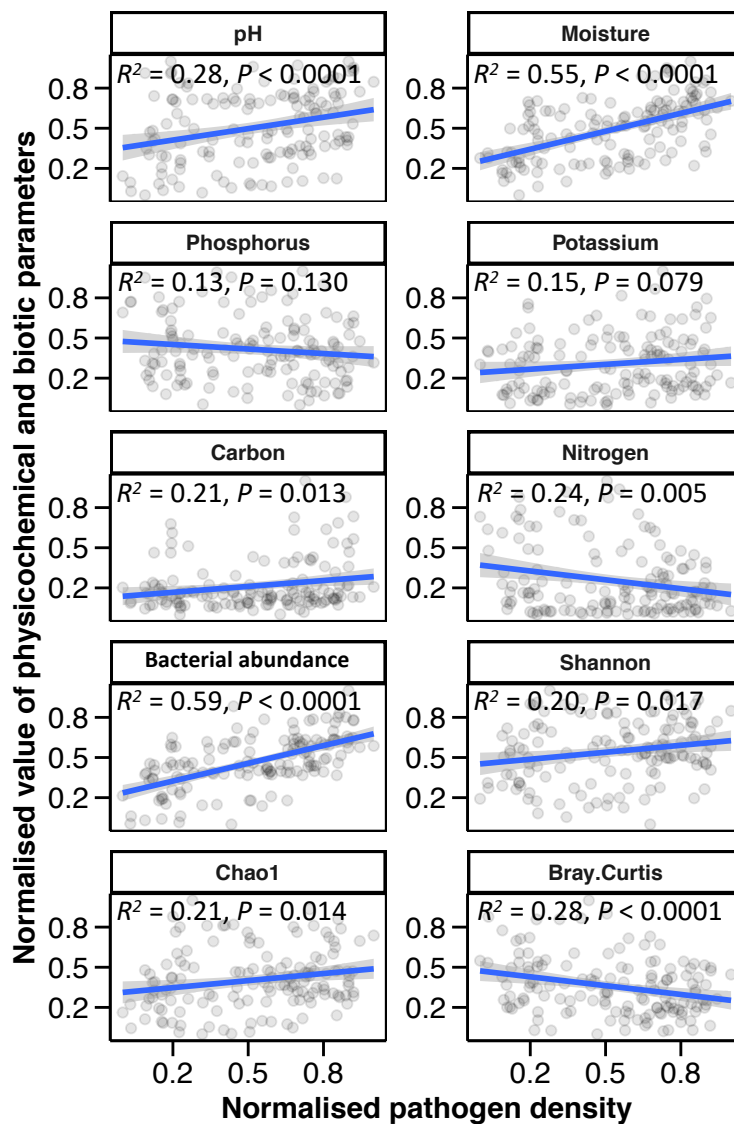
Supplementary Figure 3. Differences in bacterial community composition (PCoA) between healthy (green) and diseased (red) rhizosphere soil samples in different sampling locations (a-f).

PERMANOVA was used to identify microbial composition difference between the rhizosphere soil of diseased and healthy plants in each location based on Bray–Curtis distance matrices. P -values are indicated in each panel. Green triangles and red circles denote healthy and diseased plants, respectively. Sampling locations are abbreviated as follows: CS = Changsha, NB = Ningbo, NC = Nanchang, NJ = Nanjing, NN = Nanning and WH = Wuhan.



47
48 **Supplementary Figure 4. Differences in physicochemical and biotic parameters (PCA)**
49 **between healthy (green) and diseased (red) rhizosphere soil samples in different**
50 **sampling locations (a-f).**

51 PERMANOVA was used to identify the environmental difference between the rhizosphere
52 soil of diseased and healthy plants in each location based on Euclidean distance matrices. *P*-
53 values are indicated in each panel. Green triangles and red circles denote healthy and diseased
54 plants, respectively. Sampling locations are abbreviated as follows: CS = Changsha, NB =
55 Ningbo, NC = Nanchang, NJ = Nanjing, NN = Nanning and WH = Wuhan.

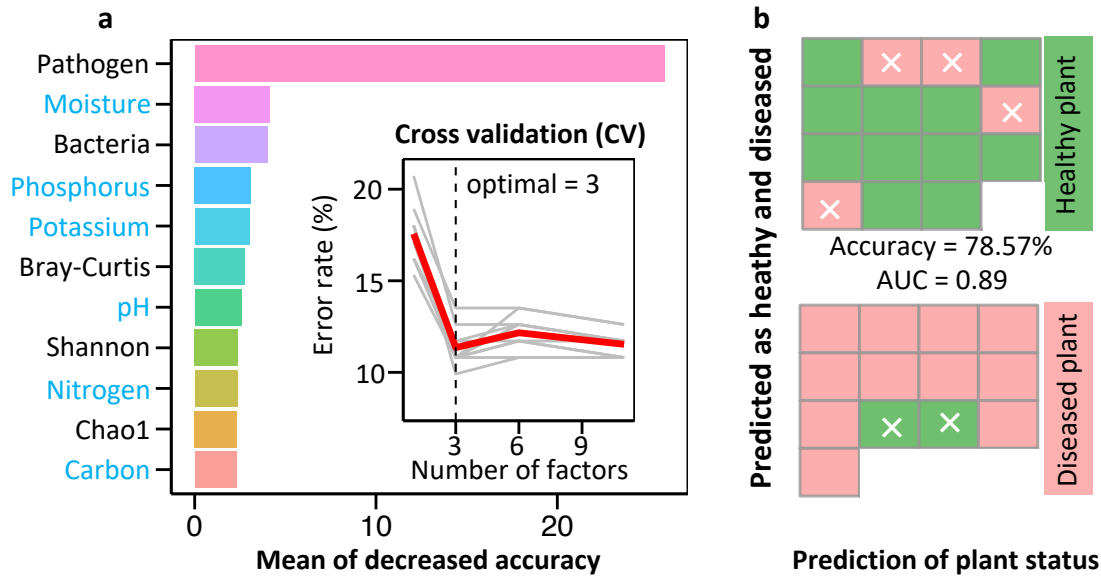


56

57 **Supplementary Figure 5. Linear correlations between the normalised pathogen**
 58 **densities and abiotic physicochemical and biotic soil properties (averaged over healthy**
 59 **and diseased plant samples).**

60 Blue lines indices the linear fitting of curves, while R^2 indicates the Spearman correlation
 61 coefficient of the linear regression and P -values the significance of each correlation.

62



63

64 **Supplementary Figure 6. Comparing the relative importance of all soil parameters in**
 65 **predicting plant health status.**

66 (a) Relative importance rank of overall abiotic physicochemical (blue) and biotic (black) soil
 67 properties and ten-fold cross-validation of random forest model (inset in a) based on the
 68 training set (80% of randomly selected rhizosphere samples). Pathogen and total bacterial
 69 abundances are abbreviated as 'Pathogen' and 'Bacteria', respectively. (b) Validation of
 70 random-forest model with a test set (20% of remaining samples) predicting plant disease
 71 outcomes based on soil properties: green and red filled cells denote for correct predictions,
 72 while filled cells with white crosses denote for false predictions. The overall model gained an
 73 average accuracy (78.6%) in classifying plant status, with 84.6% accuracy for diseased and
 74 73.3% accuracy for health plants (AUC = 0.89).

75