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Soil Phosphorus Availability Determines the Invasion of Rhizospheric Microbial Networks and Peanut Infection by Ralstonia

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Research

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Title: Soil phosphorus availability determines the invasion of rhizospheric microbial networks and peanut infection by *Ralstonia*

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Abstract

Background

The soil disease-suppressiveness may depend on complex interactions between pathogens and microbiome in response to limiting nutrients. However, these interactions remain poorly understood. Thus, we investigated the significance of soil available phosphorus (AP) in modulating pathogen-microbiome interactions for the emergence or suppression of peanut wilt using receiver operating characteristic curve (ROC) analysis and structural equation modeling (SEM) approaches.

Results

We observed significant differences in the relative abundance of pathogenic and beneficial microbes, alpha- and beta-diversity indices between disease-conducive and -suppressive soils. The pathogenic (*Ralstonia)* and beneficial (*Burkholderia* and *Bacillus*) taxa dominated the rhizosphere of wilted and healthy peanut plants. The rhizosphere of healthy rather than wilted plants showed significantly higher microbial biodiversity. Moreover, co-occurrences between *Ralstonia* and microbiome species were highly positive and negative in the disease-conducive and -suppressive soil, respectively, thus predicting facilitative (*Rudaea*) and suppressive (*Burkholderia*, *Enterobacter*, *Bacillus*) role of indigenous microbes in *Ralstonia* invasion in soil. Moreover, both ROC and SEM analyses revealed that *Ralstonia* invaded rhizospheric microbial networks and caused peanut wilt, very likely by competently utilizing soil phosphorus under *copiotrophic* (high AP) than oligotrophic (low AP) conditions.

Conclusion

Our results suggest the importance of soil phosphorus availability in altering the interactions between pathobiome and beneficial microbiome. Our study concludes that feeding soil with labile nutrients could deplete microbial biodiversity and interactions while paving the way for pathogen invasion.

Keywords: bacterial wilt; network analysis; disease-suppressiveness; microbial occurrences; soil available phosphorus

Introduction

Plant health is often correlated to the level of soil microbiome biodiversity because it acts as a buffer against soil-borne pathogens and other environmental stressors [1]. There is an emerging consensus about an ongoing trench warfare between pathogens and native soil microbial communities. Plants will likely get infected if the pathogens win the battle but will remain healthy as long as the soil microbiome biodiversity keeps suppressing pathogens and improving soil conditions conduce for their growth [2]. Therefore, understanding the emergence and suppression of plant diseases as the function of microbial interactions, particularly those between soil-borne pathogens and microbiome may help us predict or control plant disease effectively.

There is an established concept of soil disease suppressiveness, which implies that natural soils can suppress the pathogens; however, this phenomenon varies and depends, among others, on cropping years, crop species, and soil types [3, 4]. Generally, the soil

66 is considered the disease-suppressive when it keeps most plants healthy, while the
67 converse is true for the disease-conducive soil [5, 6]. The adoption of some farm
68 management and soil conservation practices such as using organic fertilizers,
69 amendments, and crop rotation can enhance the soil disease suppressiveness [7, 8]. The
70 soil disease suppressiveness is attributed to several biotic and abiotic soil factors. For
71 instance, some classical studies have related soil diseases-suppressiveness to the clay
72 mineralogy that could determine the binding and interactions of soil microbes with soil
73 particles [9]. As evidenced by previous studies, the disease-conducive soils demonstrate
74 a higher enrichment of pathogenic taxa, whereas the disease-suppressive ones show a
75 higher abundance of plant-beneficial microbial taxa [10]. There is a common perception
76 that soil-borne pathogens attempt to occupy the rhizosphere's ecological niche quickly
77 to reach a sufficient number before they can infect host plant tissue. While the
78 preexisting microbes may facilitate or suppress the pathogen invasion depending on
79 their interactions with the invading microbial pathogen and prevailing soil conditions.
80 For instance, some soil-borne pathogens are excellent consumers of toxic metabolites
81 (phenolic acids) in soil under continuous cropping systems [3]. Under such conditions,
82 the invading pathogen can quickly occupy the rhizosphere niche and may cause replant
83 disease. However, in healthy soils, a higher microbial diversity could effectively occupy
84 the rhizosphere niche through efficient resource partitioning or utilization, and thus, it
85 could suppress pathogen invasion [11, 12]. Simply put, the emergence or suppression
86 of plant disease, nevertheless, depends on complex microbial interactions in the
87 rhizosphere ecosystems. However, we know little about the nature or types of

88 interactions between pathogens and non-pathogenic members in disease-conducive or
89 -suppressive soils, particularly in the context of a complex soil microbiome that
90 represents millions of microbial species.

91 Microbial interactions are hard to measure at the community level; however, in
92 recent years, their visualization by networks has become a commonly used and
93 powerful tool to unveil their complexity and to discern the co-occurrence patterns
94 among microbial species in a community [13]. The microbial network visualization can
95 provide deep insights into the complex microbial inter-species relationships, and some
96 recent studies have attempted to link the microbial co-occurrence patterns to pathogen
97 invasion [14]. In some studies, researchers have used network analyses as a tool to
98 identify pathogens and or key microbial players that may act as defenders of plants [15,
99 16]. Therefore, we anticipate that an in-deep network analysis would substantially
100 broaden our knowledge about the interactions between pathogens and other microbial
101 species, thus helping us develop a thorough understanding of disease -suppressive/-
102 conducive soils.

103 Bacterial wilt, an economically important disease of several plant species, is
104 mainly caused by a soil-borne pathogen *Ralstonia solanacearum* (hereafter *Ralstonia*),
105 and annually it substantially reduces crop yield worldwide [17]. The soil-borne
106 *Ralstonia* is known to infect more than 200 plant species belonging to 54 families, for
107 instance, *Solanaceae* crop species such as tobacco (*Nicotiana tabacum*) and tomato
108 (*Lycopersicon esculentum*), and *Leguminous* crop species such as peanut (*Arachis*
109 *hypogaea*), and *Musaceae* crop species such as banana (*Musa paradisiaca*) [18, 19].

110 *Ralstonia* infects and invades plants through colonizing the vascular system of the roots,
111 thus leading to the wilting symptoms and eventually causing the plant death [20]. Till
112 today, the discovery and introduction of effective control measures for the bacterial wilt
113 still represent a significant challenge to researchers. For instance, the use of chemicals
114 (pesticides, fumigants) and biological control agents such as rhizobacteria have shown
115 promising results. However, there are significant environmental concerns about the
116 chemical use and efficiency of biological control agents in natural soil systems [21, 22].
117 Thus, there is a pressing demand to understand the complexity of *Ralstonia* interactions
118 with the different members of the soil microbiome to develop a mechanistic
119 understanding of the emergence and suppression of bacterial wilt.

120 The pathogenic invasion of soil and plant systems depends on the soil conditions
121 and the pathogen's ability to occupy a niche in the rhizosphere ecosystem; however, we
122 know a little about the underlying ecological reasons for the *Ralstonia* invasion.
123 Nevertheless, there are some plausible explanations regarding *Ralstonia* and other
124 pathogens invasion. First, soil conditions, for instance, the availability of nutrient and
125 organic acids may promote soil-borne pathogens [5]. Second, some pathogens
126 like *Ralstonia* express higher tolerance to soil and environmental stresses due to their
127 genetic and physiological properties, which make them very persistent and potent for
128 an extended time-period [23]. Third, pathogens can competently colonize and occupy
129 the rhizosphere niche by different means. They can competitively exploit the available
130 soil resources or inhibit competing microbes by producing antibiotics and or get support
131 from other microbes via cross-feeding/cheating [24, 25]. Moreover, some evidence

suggests that the availability of essential nutrients such as phosphorus (P) and nitrogen (N) may correlate or contribute to *Ralstonia* abundance in soil and root tissues [26, 27]. Despite these explanations, we do not know mechanistically whether, and how, soil properties or nutrient availability determine *Ralstonia* abundance and interactions with other members of soil microbial communities. Thus, understanding how the soil environment mediates the interactions between soil-borne pathogens and microbiome may, therefore, help to develop environmental-friendly disease management practices to reduce the incidence of *Ralstonia* invasion and peanut wilt.

Here we study peanut-cultivated fields as a model system to investigate the potential relevance of the incidence of bacterial wilt and the relationship between *Ralstonia* and soil microbiome. We hypothesize that interactions between *Ralstonia* and different members of the soil microbiome, based on co-occurrence networks, will be relatively more negative in the disease-suppressive than disease-conducive soils. Moreover, we attempt to identify the interactions and significance of key members of soil microbiome that negatively or positively impact *Ralstonia* invasion in the context of soil beneficial- and patho-microbiome. Then, we hypothesize that differences between properties of disease suppressive and conducive soils, for instance, nutrient contents, will also predict the interaction of *Ralstonia* with different members of the soil microbiome and their cumulative effects on plant growth.

To test these hypotheses, we investigated the fungal and bacterial communities of bulk and rhizosphere soils and their physicochemical properties. In particular, we aim at comparing the structure and diversity of microbial communities and then discerning

and comparing the co-occurrence patterns between the pathogen (*Ralstonia*) and prominent microbial players in the disease-suppressive and -conducive soils. Finally, we aim to link soil properties with microbial interactions to comprehend the role of plant-microbiome-soil interactions in the emergence or suppression of plant disease caused by the *Ralstonia*. The results of this study may provide new insights into the role of soil microbiome and edaphic properties in the management and control of soil-borne pathogens.

Results

Species composition and diversity of microbial communities

Generally, based on the relative abundance, the bacterial phyla such as *Proteobacteria* (47.35%), *Acidobacteria* (17.89%), *Actinobacteria* (8.97%), *Chloroflexi* (6.04%), *Firmicutes* (5.34%), and *Bacteroidetes* (5.22%) dominated all soil samples (Fig. 1A). Specifically, the *Acidobacteria, Chloroflexi, Gemmatimonadetes*, and *Planctomycetes* showed a higher abundance in the bulk soil, whilst *Proteobacteria, Actinobacteria, Firmicutes*, and *Bacteroidetes* were the dominant taxa in rhizosphere soil (Fig. 1A). The fungal communities were dominated by the classes such as *Sordariomycetes, Dothideomycetes, Eurotiomycetes*, and *Mortierellomycetes* (Additional file 1: Figure S1).

At genus level, the bacterial genera *Ralstonia* (7.47%), *Burkholderia* (7.23%), *Ktedonobacter* (3.50%), *Enterobacter* (3.46%), and *Bacillus* (3.09%) were the dominant taxa (Fig. 1B). The relative abundance of bacterial genera *Ralstonia*, which

was represented by many pathogenic species possibly responsible for the peanut bacterial wilt, was significantly higher in IR (26.95%), followed by HR (2.63%), IB (0.16%) and HB (0.13%) treatments (Fig. 1B). Interestingly, compared to the HB, the relative abundance of *Ralstonia* was not significantly higher in the IB ($P > 0.05$) treatment. However, some other well-known plant-beneficial genera such as *Burkholderia* and *Bacillus* were significantly higher in the HR (Fig. 1B) treatment. But similar to *Ralstonia*, they showed no significant difference in HB vs IB ($P > 0.05$) treatments. Although we could not confirm whether all sequences assigned to the *Ralstonia* and plant beneficial genera (*Burkholderia*, *Bacillus*) represented pathogenic or beneficial species based solely on 16S amplicon sequencing, still, our results indicated a faster and slower proliferation of potential pathogenic and beneficial taxa, respectively, in the rhizosphere of infected peanut plants. However, not all plant-beneficial bacteria were enriched in the HR treatment. For example, some bacteria such as *Enterobacter* that may be of benefit to peanut growth was significantly enriched in the IR treatment (Fig. 1B).

The alpha-diversity of soil bacterial communities was highest in HB and IB, followed by HR treatments, while IR had lowest alpha-diversity (Fig. 1C). The fungal alpha-diversity was lowest in IR, though it did not differ among other treatments (Additional file 1: Figure S1). The Principal coordinate analysis (PCoA) combined with one-way permANOVA indicated that both bacterial and fungal community composition significantly different between bulk and rhizosphere soil, and between HR and IR treatments; however it did not differ between HB and IB treatments (Fig. 1D,

198 Additional file 1: Figure S1, Table S1, and Table S2).

199

200 **Co-occurrence pattern of microbial communities**

201 Network analyses were conducted to reveal the potential co-occurrence pattern of
202 bacterial communities (Additional file 1: Figure S2), and we mainly focused the co-
203 occurrence pattern among potential pathogens and other members of microbiome.
204 Therefore, the nodes directly related to the potential causal agents of peanut wilt. The
205 *Ralstonia*-related nodes were filtered from each whole network to construct the
206 *Ralstonia*-related subnetwork (Fig. 2).

207 There were 208, 154, 9, and 94 nodes directly relating to the *Ralstonia* in the HB,
208 HR, IB, and IR (Fig. 2ABCD) subnetworks, respectively. The proportion of negative
209 co-occurrences between *Ralstonia* and other microorganisms (**PNCRM**) were 83.17,
210 52.60, 11.11, and 70.21% in the HB, HR, IB, and IR subnetworks, respectively (Fig. 2).
211 The HB and HR subnetworks always had higher degree, betweenness centrality and
212 closeness centrality (Fig. 2E-F). These results suggested that healthy soil (HB and HR)
213 had tighter co-occurrences between pathogen and other microorganisms, which turned
214 to be very loose in the disease-conducive soils.

215 Some well-known anti-*Ralstonia* species such as *Haliangium* were negatively
216 correlated with *Ralstonia* in the HB treatment. Moreover, *Burkholderia*, *Actinospica*
217 and *Streptacidiphilus* also negatively correlated with *Ralstonia* in HR treatment.
218 Similarly, *Enterobacter* and *Acinetobacter* negatively correlated with the *Ralstonia* in
219 the IR treatment. However, some potentially phytopathogenic bacterial species, for

instance, *Rudaea* positively correlated with the *Ralstonia* in IR treatment.

Potential predictors and determinants of peanut bacterial wilt

The ROC analyses revealed that available phosphorus in buck soils was the only investigated soil property that could well predict the incidence of peanut bacterial wilt (AUC = 0.901) (Fig. 3). All other soil properties, including soil pH, SOM, TN, AN, TK and AK, were poor predictors of peanut wilt due to the low AUC values (Fig. 3). The relative abundance of *Ralstonia* in bulk soil was also a poor predictor of the peanut bacterial wilt (AUC = 0.427). The relative abundance of *Ralstonia* in rhizosphere soil appeared to be a strong predictor and determinant of the peanut bacterial wilt (AUC = 1) due to their successful invasion of soil and plant systems. Moreover, the bacterial rather than fungal diversity was the strongest predictor of the peanut bacterial wilt (Fig. 3).

Based on the ROC analyses, the SEM was subsequently conducted to investigate the potential linkages among soil physicochemical properties, microbial community parameters, and the incidence of peanut bacterial wilt (Fig. 4). The SEM model showed that neither available P nor *Ralstonia* in bulk soil alone directly influenced the peanut bacterial wilt. Instead, available P only significantly influenced the interactions between pathogen and soil microbial communities. The negative correlation between available P and PNCRM in bulk soil suggested that higher P content may suppress the negative co-occurrences between pathogen and other microorganisms. Moreover, the PNCRM in bulk soil significantly negatively correlated with the relative abundance of

242 rhizospheric *Ralstonia* and the incidence of peanut bacterial wilt (Fig. 4). Similarly, the
243 rhizospheric *Ralstonia* significantly positively correlated to the incidence of peanut
244 bacterial wilt. While, the results obtained from microcosm experiment showed that the
245 average relative abundance of *Ralstonia* in high available P soils was significantly
246 higher than that in low AP soils (Fig. 5), thus confirming that higher soil AP would help
247 the enrichment of *Ralstonia* in peanut rhizosphere.
248

249 # Discussion

250 **Observed differences in microbial communities between wilt-conducive and**
251 **disease-suppressive fields**

252 As observed in our and other studies, bacterial wilt significantly reduced peanut
253 biomass and yield [28]. Some *Ralstonia* species especially *R. solanacearum* are direct
254 determinants of peanut wilt because of their higher ability to invade peanut plants, and
255 cause plant wilting and necrosis [19]. After observing differences between healthy and
256 diseased peanut fields, we attempt to discern differences in microbial communities in
257 the corresponding soils. The *Ralstonia* species showed a higher abundance in the
258 rhizosphere than bulk soil. Considering the *copiotrophic* strategy of *Ralstonia*, our
259 results suggested that the rhizosphere promoted its growth by providing different
260 nutrients and host plant labile-derived resources [29]. According to Koch's postulates
261 [30], of course, we could not clarify if all sequences assigned to *Ralstonia* were
262 pathogenic solely by 16S amplicon sequencing. Nevertheless, the relative abundance
263 of *Ralstonia* was highest in the rhizosphere soil of infected plants. Although *Ralstonia*

also showed substantially higher abundance in the rhizosphere soil of healthy plants, it did not cause peanut wilt. These findings suggested that pathogenic *Ralstonia* species can only invade peanut plants if it attains high abundance, which is necessary for successful colonization of the host system by overcoming biotic and abiotic barriers. Moreover, the second most abundant genus, *Burkholderia*, was highly enriched in the rhizosphere soil of healthy peanut. The *Burkholderia* species are well-known plant-beneficial bacteria, because they not only promote peanut nodulation [31], but also directly suppress the development of *R. solanacearum* [32]. High enrichment of plant-beneficial bacteria such as *Burkholderia* in rhizosphere soil should be a logical explanation as to why peanut plants were healthy in *Ralstonia*'s presence in a healthy peanut field. However, the relative abundance of both potential pathogenic *Ralstonia* species and plant-beneficial bacteria such as *Burkholderia*, *Enterobacter, Bacillus* and other abundant genera showed no difference between bulk soils in HB and IB treatments (Fig. 1B), thus indicating that the incidence of peanut wilt may not be a direct consequence of presence of pathogenic and plant-beneficial microbes in the bulk soil.

The alpha-diversity indices were significantly lower in the rhizosphere soil of infected than that of healthy plants (Fig. 1C), thus clearly suggesting a 'rhizosphere effect' of diseased plants on soil microbial communities [33]. The low alpha-diversity also predicted that the rhizosphere immunity of infected plants was compromised because of pathogen invasion. Recently some empirical and theoretical studies have suggested that high microbial biodiversity is an indicator of microbial buffer and

immunity against environmental stressors such as soil-borne pathogens [34]. Thus, our results from healthy and diseased peanut field suggests that high soil microbial diversity is an indicator of soil and plant health [2, 3]. Meanwhile, our results are similar to some previous studies showing differences between microbial diversity indices in bulk and rhizosphere soils [3]. This is because plants can create completely different and required microbiota in the rhizosphere than bulk soil. Moreover, we also anticipate that this phenomenon may be a consequence of the discrepant co-occurrence pattern among *Ralstonia* and other microbial members in rhizosphere and bulk soil, which would in return potentially alter the proliferation of *Ralstonia* species and structure of microbial communities in these environments.

Co-occurrence pattern among *Ralstonia* and other microbial members determines the incidence of bacterial wilt of peanut

In statistical terms, the positive and negative linkage between species represents facilitative and antagonistic interactions, respectively [10, 35]. In this study, we observed a higher PNCRM in the disease-suppressive (HB, 83.17%, Fig. 2A) than -conducive (IB, 11.11%, Fig. 2C) soils, thus potentially indicating that *Ralstonia* in former soil (i) may be more directly suppressed by other microbial members, and/or (ii) may face greater competition from indigenous soil microbes for nutrients and resources. Indeed, some microorganisms that negatively correlated with *Ralstonia* species in HB such as *Haliangium* species have been reported as anti-*Ralstonia* bacteria [36]. Given that bulk soil is the main reservoir of rhizosphere-colonizing microorganisms, we

anticipate that anti-*Ralstonia* microorganisms may suppress its transmission, transfer, colonization and proliferation in the disease-suppressive soil after plantation, thus limiting its invasion and the incidence of peanut wilt. Overall, negative links in HB may stabilize co-oscillation in communities, and thus may promote microbial functioning and community stability, which is essential for soil and plant health [37].

Contrary to HB, the PNCRM was low in the HR soil (52.60%, Fig. 2B). This might be due to the fact that HR soil had low level of pathogen, thus suggesting that pathogen densities were not high enough to activate the rhizosphere defense system. Moreover, we also found more documented plant-beneficial microorganisms that showed negative correlation with *Ralstonia* such as *Burkholderia* [32], *Actinospica*, *and Streptacidiphilus* [36].

Moreover, the low NPCRM in IB soil implied that some other bacteria such as *Rudaea (a pathogenic species)* might had promoted the *Ralstonia* species in the disease-conducive soil (IB, 11.11%, Fig. 2C). Our data suggested that the invasion of *Ralstonia* species in IB soil was aided by some non-detrimental microorganisms. Recently some studies have predicted a supportive role of indigenous microbial species for pathogens, and have termed this component of microbiome as pathobiome [10, 38]. Therefore, we also anticipate that *Ralstonia* species in IB soil can survive and proliferate with and/or without help of indigenous microorganisms, and thus may attain higher densities for colonization and virulence after plantation to cause peanut wilt. Furthermore, de Vries et al. (2018) suggested that microbial communities in which a dominant proportion of microbial taxa are connected through positive links could ultimately experience

instability. Because in these communities, different members of microbial communities may respond in tandem to varying environmental conditions, which could pave the way for pathogenic invasions of microbial networks and interactions. Therefore, our experimental results confirmed the previous theoretical predictions that low PNCRM destabilized the microbial communities and reduced microbial diversity in the disease-conduce soil, which ultimately led to the proliferation of *Ralstonia* after plant cultivation to cause peanut wilt.

The PNCRM was high in IR than HR soil. According to systemic induced resistance hypothesis, plants have the ability to attract diverse microbial microbes by releasing diverse metabolites under pathogen attack. Our results are in line with previous theoretical predictions that plants may foster antagonistic microbial interactions to suppress pathogens [39, 40]. For example, *Arabidopsis* selectively recruited and enriched plant-beneficial soil bacterium *Bacillus subtilis* FB17 by secreting malic acid under infection by the pathogenic *Pseudomonas* species [41]. Similarly, our results also showed that two genera, *Enterobacter* and *Acinetobacter,* previously known to suppress peanut wilt caused by *R. solanacearum* [42, 43], significantly negatively correlated with the *Ralstonia* (Fig. 2D), thus suggesting the scenario of antagonistic microbial interactions in the presence of soil-borne pathogen.

Soil available phosphorus mediates the co-occurrence pattern among *Ralstonia* and other microbial members

How soil properties affect the severity of plant disease is an active area of research,

352 and there is some evidence regarding the role of soil AP and nutrients in disease
353 emergence and suppression [44, 45]. Nevertheless, the relationship between soil
354 nutrients and pathogen invasion remains complex due to several confounding factors
355 related to plant, soil, and microbial properties. In this study, we have also attempted to
356 discern the relative significance of various soil- and plant-related factors in the invasion
357 of rhizospheric microbial communities and networks by *Ralstonia* using ROS and SEM
358 approaches. The ROC analysis revealed that soil AP clearly predicted the incidence of
359 peanut wilt (Fig. 3). Moreover, the SEM model revealed that the most likely role of the
360 soil AP in peanut wilt is via promoting the abundance and then influencing co-
361 occurrence pattern of *Ralstonia* with other microorganisms in the rhizosphere soil. Our
362 microcosm experiment clearly confirmed our prediction that high soil AP increased the
363 enrichment of *Ralstonia* in the rhizospheric environment (Fig. 5), thus suggesting its
364 capability to competently exploit soil resources and invade rhizospheric microbial
365 communities and networks under *copiotrophic* conditions. Our results, for the first time,
366 mechanistically prove that soil phosphorus mediates the invasion of *Ralstonia* by
367 altering microbial interactions since it is one of the major limiting factors for microbial
368 growth, community composition and functioning in the soil environment [46, 47].

369 However, under low AP, we did not observe the invasion of rhizosphere
370 microbiome and microbial networks by *Ralstonia* in our microcosm experiment.
371 Moreover, *Ralstonia* and other microorganisms were highly negatively correlated in the
372 HB in which the AP was also low. Our results from field and microcosm experiments
373 clearly predicted an intense competition of *Ralstonia* with different members of soil

374 microbiome under low soil AP conditions. Nevertheless, soil microbial communities
375 and their key members appeared to be competitive than *Ralstonia* in terms of resource
376 exploitation and occupying the soil niche under resource-limited conditions. It is very
377 likely that *oligotrophic* conditions are less favorable for *Ralstonia*, and it can only
378 effectively invade microbial networks under nutrient-rich conditions due to a poor
379 connectivity and mutual interactions of *copiotrophic* taxa. Our results provide
380 interesting analogy between the *copiotrophic* metabolism of soil AP and easily
381 digestible fast food in the rhizosphere and human digestive systems, respectively.
382 Studies from both systems have suggested that *copiotrophic* conditions represent
383 species poor communities with few dominant taxa that are often weakly connected to
384 each other [48, 49]. Thus, these conditions may favor pathogen invasion while converse
385 is true for *oligotrophic* conditions.
386

387 ## Conclusions

388 Based on our results, we propose a conceptual paradigm to describe the potential
389 linkage between soil AP and the incidence of peanut wilt (Fig. 6). First, a greater
390 availability of soil phosphorus alters the co-occurrences between *Ralstonia* and other
391 members of soil microbiome, whereas its lower availability generates intense microbial
392 competition and networking that suppress *Ralstonia* in the rhizosphere system. Second,
393 the transfer, colonization and proliferation of *Ralstonia* in the rhizosphere soil were
394 suppressed if the PNCRM were highly negative, thus suggesting the significance of
395 microbial antagonistic interactions in the suppression of soil-borne pathogens or

pathobiome in the peanut rhizosphere. Third, a high-enrichment of *Ralstonia* in the rhizosphere can lead to the peanut wilt, whereas a greater enrichment of plant-beneficial bacteria may protect plants from soil-borne pathogens.

Although our findings offer novel insight into soil-disease suppression from the perspective of microbial co-occurrences, we acknowledge some limitations in our study as well. For example, the microbial interactions and their implications were derived from the network analysis. Therefore, future experiments testing microbial interactions in complex microbial communities will be needed to discern their relative importance in soil and plant health though it is not easy to assess and comprehend individual species level interactions in complex microbial communities.

Materials and Methods

Soil sampling

The soil samples were collected by the end of June 2019 from peanut fields in Yujiang (Jiangxi Province, China; 116°41' E to 117°09' E, and 28°04'N to 28°37' N). Sampling sites have subtropical monsoon climate with abundant sunshine and rainfall (mean annual sunshine hours, 1,739.4 h; mean annual temperature, 17.6°C; mean annual precipitation, 1,750 mm).

The disease suppressive soils are hard to locate, and their level of disease-suppressive in any field site is highly sensitive to the soil and crop management practices such as fertilization, cropping systems, and their duration [50]. Therefore, we were highly cautious regarding the sampling date and soil collection from the desired

418 field sites. We developed the following criteria to address the sampling related issues
419 and then strictly followed it. First, we selected the fields for soil sampling, which were
420 under the continuous cultivation of peanuts. Second, we defined the soil of a peanut
421 field as disease-conducive if those showed more than 50% of infected plants. Third, we
422 defined the soil of a peanut field as disease-suppressive if those showed no infected
423 plants at all. Forth, within each sampling site, the distance between wilt-conducive
424 fields and disease-suppressive fields should be < 50 m. Fifth, field management,
425 including cropping system and fertilizer applications, should be almost uniform. On the
426 basis of these strict criteria, we selected six sites that included three disease-conducive,
427 and three disease-suppressive fields. From each field, we harvested ten peanut plants
428 from each field using a spade, while the soil attached to the roots was collected and
429 pooled to represent a composite rhizosphere soil sample. Within the same field, ten 20-
430 cm-deep soil cores (6-cm diameter, 30-cm away from peanut roots) were collected
431 randomly and pooled to represent a composite bulk soil sample. Rhizosphere and bulk
432 soil samples from healthy and infected plants were marked as HR (rhizosphere soil
433 from disease-suppressive or healthy peanut field), IR (rhizosphere soil from disease-
434 conducive or infected peanut field), HB (bulk soil from healthy peanut field), and IB
435 (bulk soil from infected peanut field), respectively. In total, we collected 72 samples (6
436 sites × 4 sample types × 3 replicates). Samples from each field were homogenized, and
437 then subsampled for the subsequent analyses. Subsamples for the analysis of
438 physiochemical properties were air dried, ground, and sieved through 2-mm mesh. The
439 subsamples for profiling microbial communities and their properties were stored at -

40 ℃. Fresh biomass of each plant was measured immediately after sampling, and is reported (Additional file 1: Figure S3).

Characterization of soil physicochemical properties

The soil physiochemical properties were determined using the previously reported methods [51]. Soil pH was assayed using a pH meter (FE30; Mettler-Toledo) with 1:2.5 soil-water suspension. The soil organic matter (SOM) was determined using the sulfuric acid-potassium dichromate oxidation method, while the total and available nitrogen (TN and AN) contents were measured as Kjeldahl-N. The total phosphorus (TP) and available phosphorus (AP) contents were determined using the HF–$HClO_4$ digestion and sodium bicarbonate extraction with the molybdenum blue method, respectively. Lastly, total potassium (TK) and available potassium (AK) were determined using a HF-$HClO_4$ digestion and ammonium acetate extraction, respectively. The soil physicochemical properties are given (Additional file 1:Table S3).

Microcosm experiment

A microcosm experiment was conducted to test whether soil AP would affect the enrichment of potential pathogens. Thirty-six 250 ml aseptic plastic bottles were used to conduct the microcosm experiment. Each of air-dried HB soil samples (18 samples) was subsampled into two parts, and 20 g of soil was added into each bottle. We mixed 1.733 g of $Na_2HPO_4 \cdot 12H_2O$ into 1 L of pure water. Then, 4 ml of phosphorus solution was added into each of 18 bottles (30 mg/kg AP). These 18 bottles were marked as

462 'High AP' samples. While, we added 4 ml of equivalent pure water into the rest of 18
463 bottles, and mark them as 'Low AP' samples.

464 The microcosms were then amended drop-by-drop with 4 ml of peanut root
465 exudates every 5 d to simulate the rhizospheric environment. All microcosms were then
466 maintained in an incubator at 25 ℃ and 70% relative humidity. The soil samples were
467 collected after 45 d of incubation. Samples were stored at -40 ℃ after homogenization.

468

469 **Soil DNA extraction, amplification, Illumina sequencing, and sequence processing**

470 The soil DNA was extracted from 0.5 g of soil (fresh weight) using a Fast®DNA
471 SPIN Kit (MP Biomedicals, CA, USA), and then we subsequently purified DNA using
472 a PowerClean® DNA Clean-up Kit (MoBio, CA, USA) according to the manufacturer's
473 instructions. The concentrations and quality of the extracted DNA were measured using
474 a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, DE, USA).

475 Two commonly used primer sets were used to sequence and amplify bacterial 16S
476 rRNA genes [52] and fungal ITS genes [53]. Detailed information about primers and
477 PCR conditions for each primer set are given (Additional file 1: Table S4). The purified
478 amplicons were pooled in equimolar concentrations and sent for paired-end sequencing
479 using an Illumina MiSeq 2 × 300bp (Majorbio Bio-Pharm Technology Co. Ltd.,
480 Shanghai, China).

481 The raw sequence data were analyzed using the Quantitative Insights into
482 Microbial Ecology (QIIME) pipeline (v1.9.1) (http://qiime.org/) [54]. Paired-end reads
483 were merged using FLASH [55]. Reads with the length of <200 bp or with average

quality scores of <25 were removed. UPARSE was used for chimera removal, and operational taxonomic units (OTUs) were clustered at 97% sequence similarity. The representative sequence of each OTU was selected and used for taxonomic assignments [56]. The OTU data was rarefied to an even sequencing depth based on the sample with the minimum numbers of reads [57]. The taxonomic identity of the bacteria and fungi was determined based on comparisons against the RDP classifier (http://rdp.cme.msu.edu/classifier/) with the confidence threshold of 80% and UNITE database (v7) (https://unite.ut.ee/), respectively.

Statistical analysis

The alpha-diversity indices including richness and Shannon-Wiener were calculated in QIIME using the "*alpha_diversity.py*" script. While, the Bray-Curtis dissimilarity was calculated in QIIME using the "*beta_diversity.py*" script. Statistically significant differences across treatments were determined by one-way analysis of variance (ANOVA), along with the use of Duncan's test for multiple comparisons ($P < 0.05$). If the data were not normally distributed, or the variances of observations were heterogeneous, tEhe nonparametric Mann-Whitney *U* test was used to determine the statistical significance. The Principal coordinate analysis (PCoA) combined with one-way permANOVA was used to compare the structural difference of microbial communities among treatments.

A network analysis was performed to explore the microbial co-occurrence patterns using the plugin CoNet in Cytoscape 3.5.1 [58]. Robust correlations between two OTUs

were defined as those with Spearman's correlation coefficients >0.6 and with false discovery rate-corrected *P* values <0.01. With these, we formed a correlation network in which each node represented one OTU, and each edge represented a strong and significant correlation between two nodes. The networks were visualized in Gephi 0.9.2 [59], and the topology parameters of each network were determined in Cytoscape 3.5.1 using NetworkAnalyzer [58].

The receiver operating characteristic curve (ROC) analysis was conducted to investigate the potential predictors of peanut wilt. According to ROC outputs, the structural equation modelling (SEM) has been used to model complex relationships between directly and indirectly observed (latent) factors [60, 61]. In our study, the SEM analysis was used to gain a mechanistic understanding of the linkages between soil properties, microbial interactions, the relative abundance of pathogens and the incidence of peanut wilt. The model was tested by the robust maximum likelihood evaluation method using the Amos 17.0 software package (Smallwaters Corporation, Chicago, USA). The $\chi 2$ values, degree freedom, and *P*-values, were adopted to evaluate the structural equation model fitness [62]. A best fitting and most parsimonious model was obtained after excluding all non-significant parameters.

Additional file

Additional file 1: Figure S1. Species composition (**A**) at class level, and (**B**) alpha-diversity and (**C**) beta-diversity of fungal communities. **Figure S2.** Co-occurrences among microorganisms in (**A**) HB, (**B**) HR, (**C**) IB, and (**D**) IR treatments. **Figure S3.**

Plant biomass of healthy and infected peanuts. **Table S1.** One-way permANOVA investigating the structural difference of bacterial community. **Table S2.** One-way permANOVA investigating the structural difference of fungal community. **Table S3.** Soil physicochemical properties of each treatment. **Table S4.** Detailed information about primers and PCR conditions.

Acknowledgements

Not applicable

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Availability of data and materials

The raw 16S rRNA and ITS sequences reported in this study were submitted to the NCBI Sequence Read Archive (SRA) under the accession numbers SRP287597 and SRP288029, respectively.

Authors' contributions

ZL conceived and designed the experiments. PL, ML, GL, KL, TL, and MW performed the experiments. PL and MS analyzed the data and wrote the paper.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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748 **Figure captions**

749

750 **Fig. 1** Species composition and diversities of bacterial communities. Species
751 composition at (a) phylum and (b) genus level. Alpha-diversity (c) and beta-diversity
752 (d) of bacterial communities. Different lowercases in (b) and (c) represent significant
753 difference at P <0.05. Best-diversity was calculated based on the Bray-Curtis
754 dissimilarity, and was plotted based on the first two axes of the principle coordinate
755 analysis.

756

757 **Fig. 2** Microbial ecological network showing the co-occurrences among *Ralstonia* and
758 other microorganisms in each treatment. Co-occurrence patterns among *Ralstonia* and
759 other microorganisms in HB (a), HR (b), IB (c), and IR (d); and network parameters
760 including degree (e), betweenness centrality (f), and closeness centrality (g) of each
761 network. The red lines represent negative correlations, and the blue lines represent
762 positive correlations. Each network was constructed with 18 samples under the
763 parameters r >0.6 and P <0.01. Lowercase letters above the boxes indicate a significant
764 difference at P <0.05 according to a nonparametric Mann-Whitney U test.

765

766 **Fig. 3** Predictors of the incidence of peanut wilt determined by receiver operating
767 characteristic curve analysis. A higher AUC indicates better prediction. PNCRM:
768 proportion of negative co-occurrences among *Ralstonia* and other microorganisms.

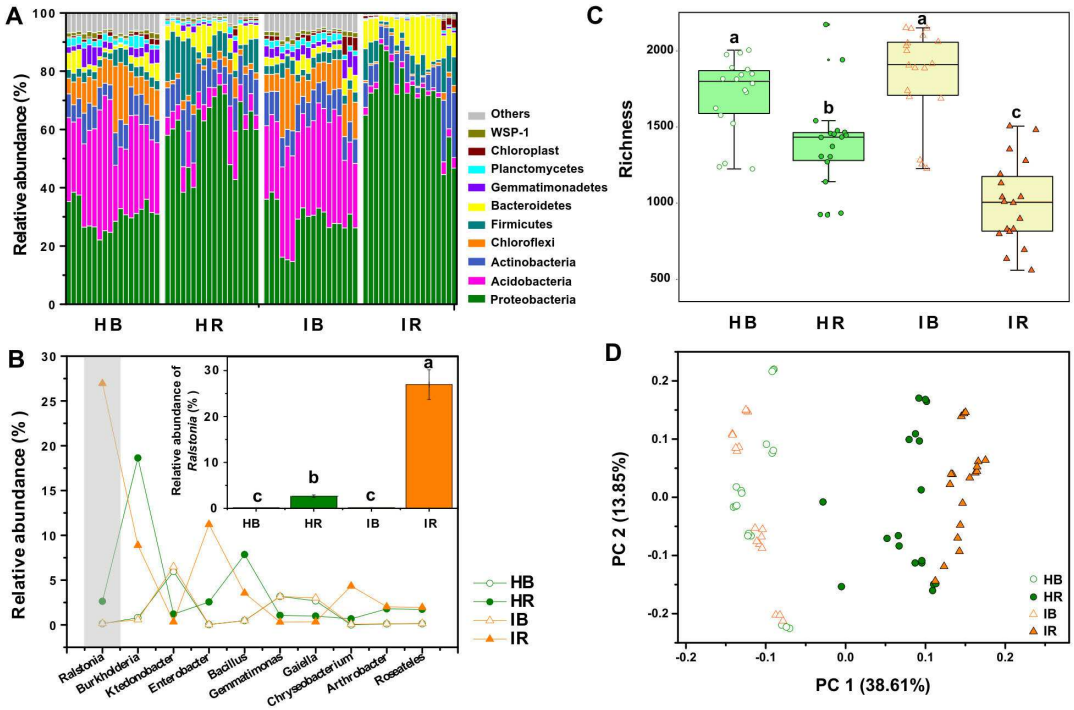
769

Fig. 4 The linkages between soil properties, biological parameters, and the incidence of peanut wilt. The linkages were determined by structural equation modeling (SEM). The red and blue lines indicate negative and positive linkages, respectively. The width of arrows indicates the strength of significant standardized path coefficients ($P < 0.05$).

Fig. 5 The ln-transformed relative abundance of *Ralstonia* in low AP and high AP soils. ** indicates a significant difference at $P < 0.01$.

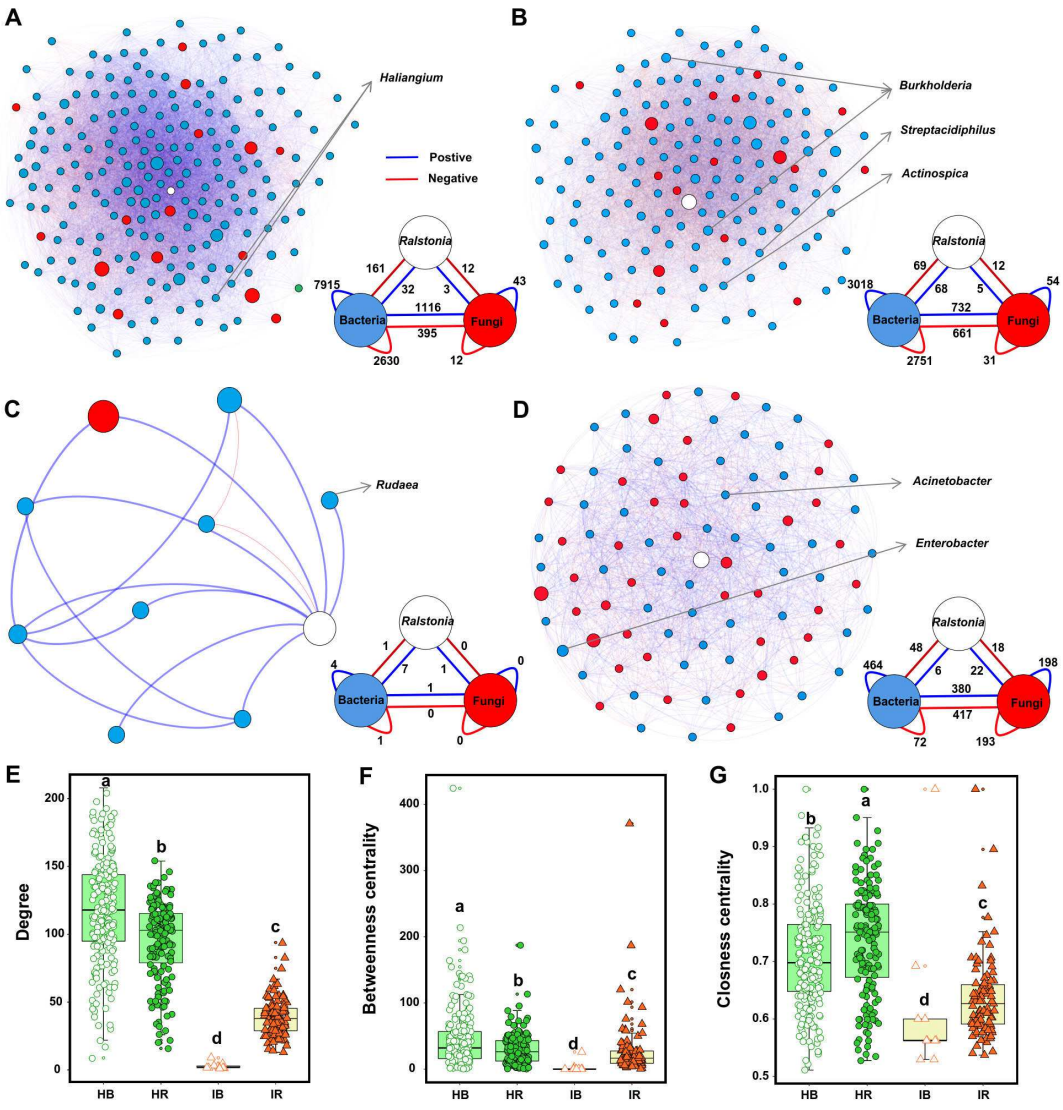
Fig. 6 A conceptual paradigm showing the potential mechanism explaining the incidence of peanut wilt.

792 **Fig. 1**


A
Relative abundance (%)
HB
HR
IB
IR
Others
WSP-1
Chloroplast
Planctomycetes
Gemmatimonadetes
Bacteroidetes
Firmicutes
Chloroflexi
Actinobacteria
Acidobacteria
Proteobacteria
B
Relative abundance (%)
Relative abundance of Ralstonia (%)
Ralstonia
Burkholderia
Ktedonobacter
Enterobacter
Bacillus
Gemmatimonas
Gaiella
Chryseobacterium
Arthrobacter
Roseateles
C
Richness
D
PC 2 (13.85%)
PC 1 (38.61%)

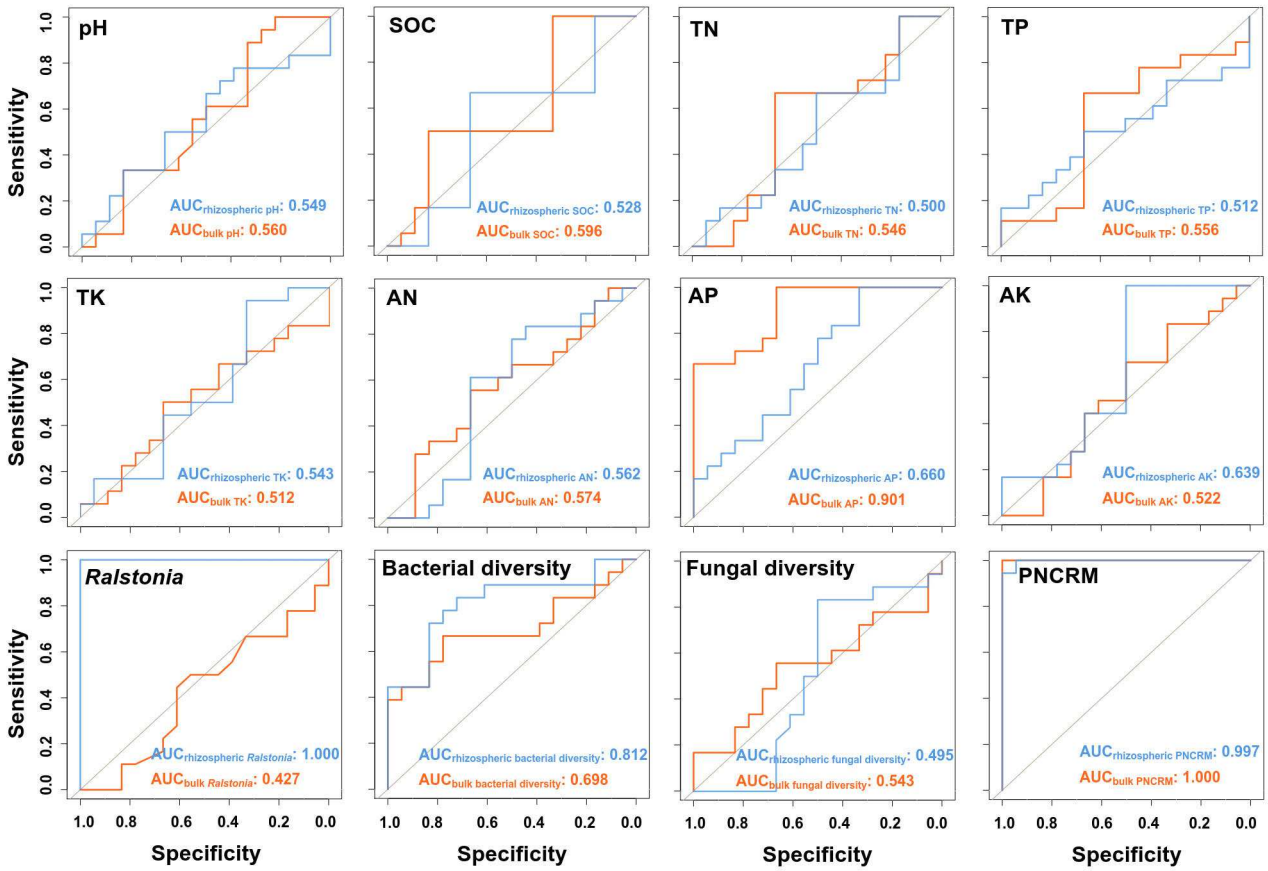
793

794 **Fig. 2**


A
Haliangium
Postive
Negative
Ralstonia
Bacteria
Fungi
161
12
7915
32
3
43
1116
395
2630
12
B
Burkholderia
Streptacidiphilus
Actinospica
Ralstonia
Bacteria
Fungi
3018
69
68
5
12
54
732
661
2751
31
C
Rudaea
Ralstonia
Bacteria
Fungi
1
0
4
7
1
0
1
0
1
0
D
Acinetobacter
Enterobacter
Ralstonia
Bacteria
Fungi
48
18
464
6
22
198
380
417
72
193
E
Degree
a
b
c
d
HB
HR
IB
IR
F
Betweenness centrality
a
b
c
d
HB
HR
IB
IR
G
Closness centrality
b
a
c
d
HB
HR
IB
IR

795

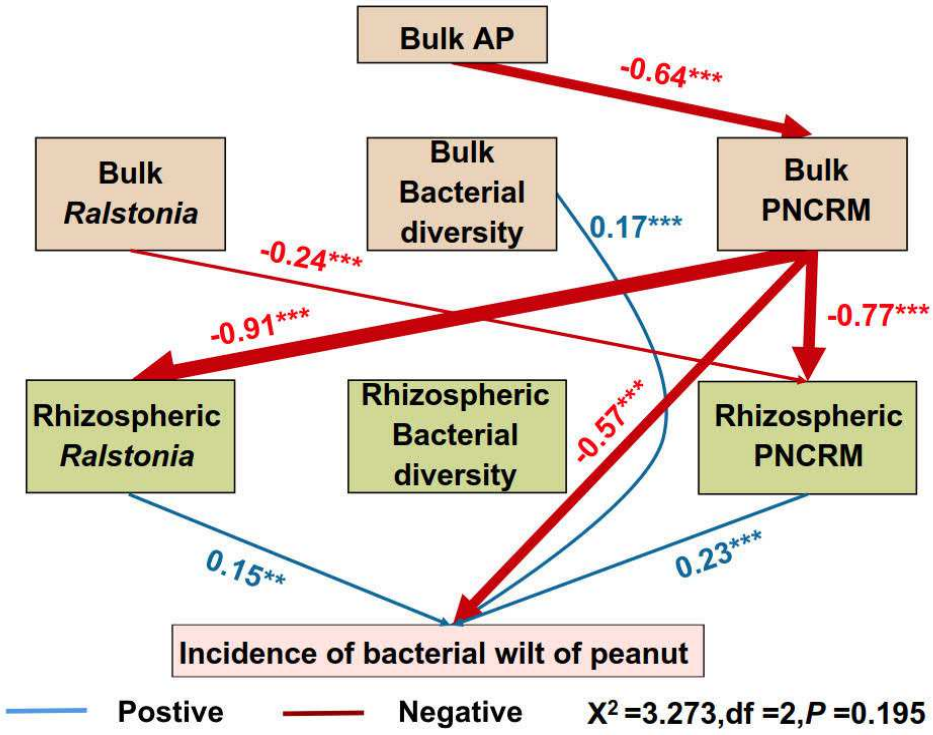
796 **Fig. 3**



797

798

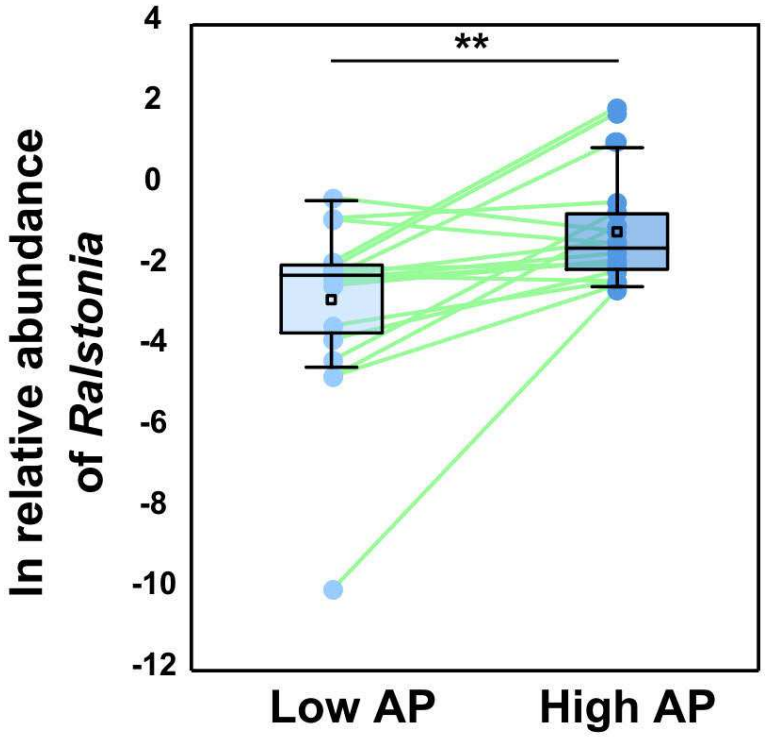
799 **Fig. 4**



800

801

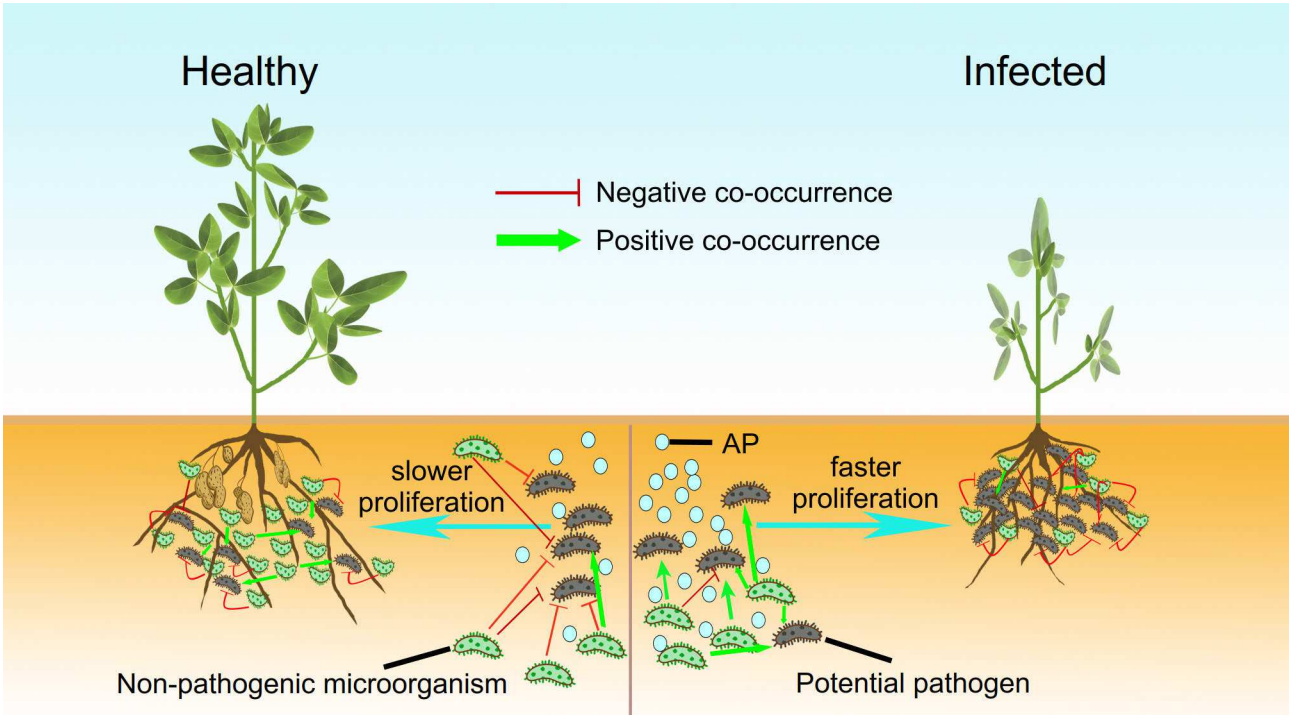
802 **Fig. 5**



803

804

805 **Fig. 6**


Healthy
Infected
Negative co-occurrence
Positive co-occurrence
slower
proliferation
AP
faster
proliferation
Non-pathogenic microorganism
Potential pathogen

806

Supplementary materials for

Soil phosphorus availability determines the invasion of rhizospheric microbial networks and peanut infection by *Ralstonia*

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Figure S1 Species composition (A) at class level, and (B) alpha-diversity and (C) beta-diversity of fungal communities.

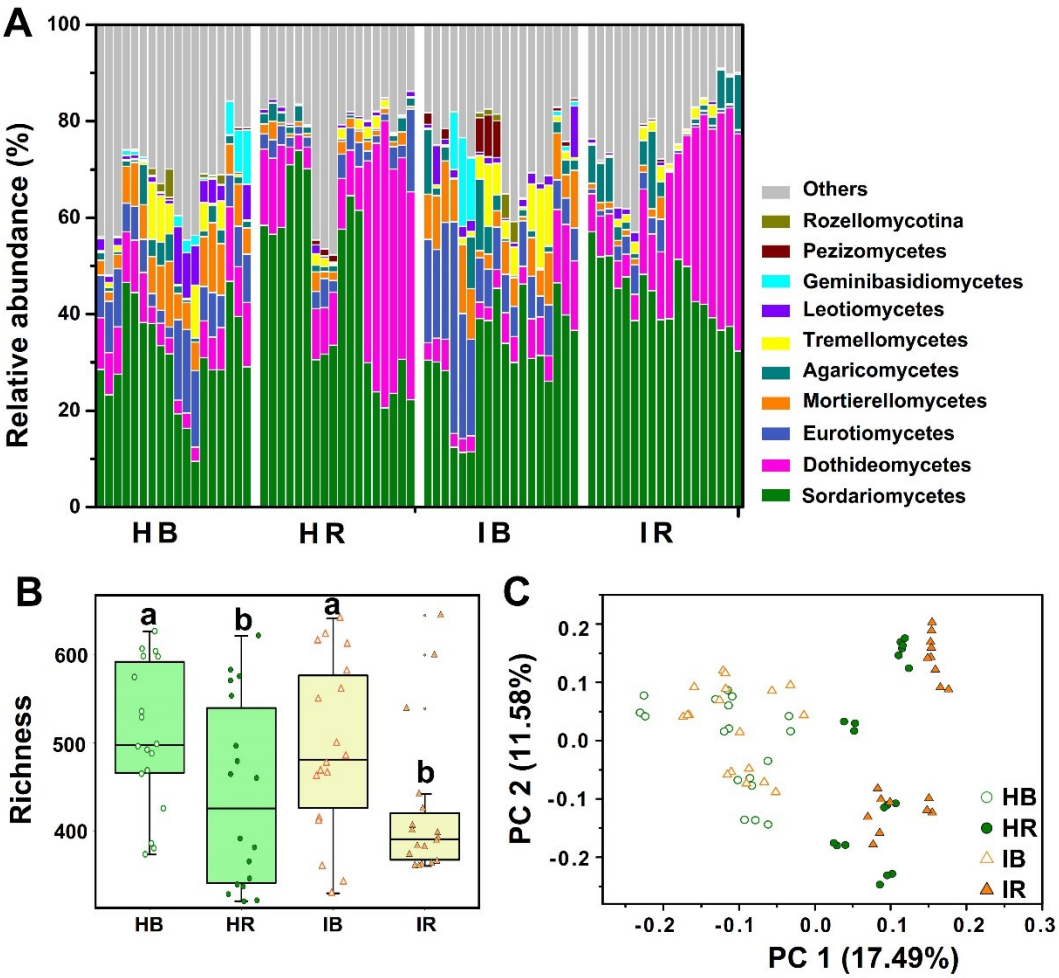


Figure S2 Co-occurrences among microorganisms in (A) HB, (B) HR, (C) IB, and (D) IR treatments. The red lines represent negative correlations, and the blue lines represent positive correlations. Each network was constructed with 18 samples under the parameters r >0.6 and P <0.01.

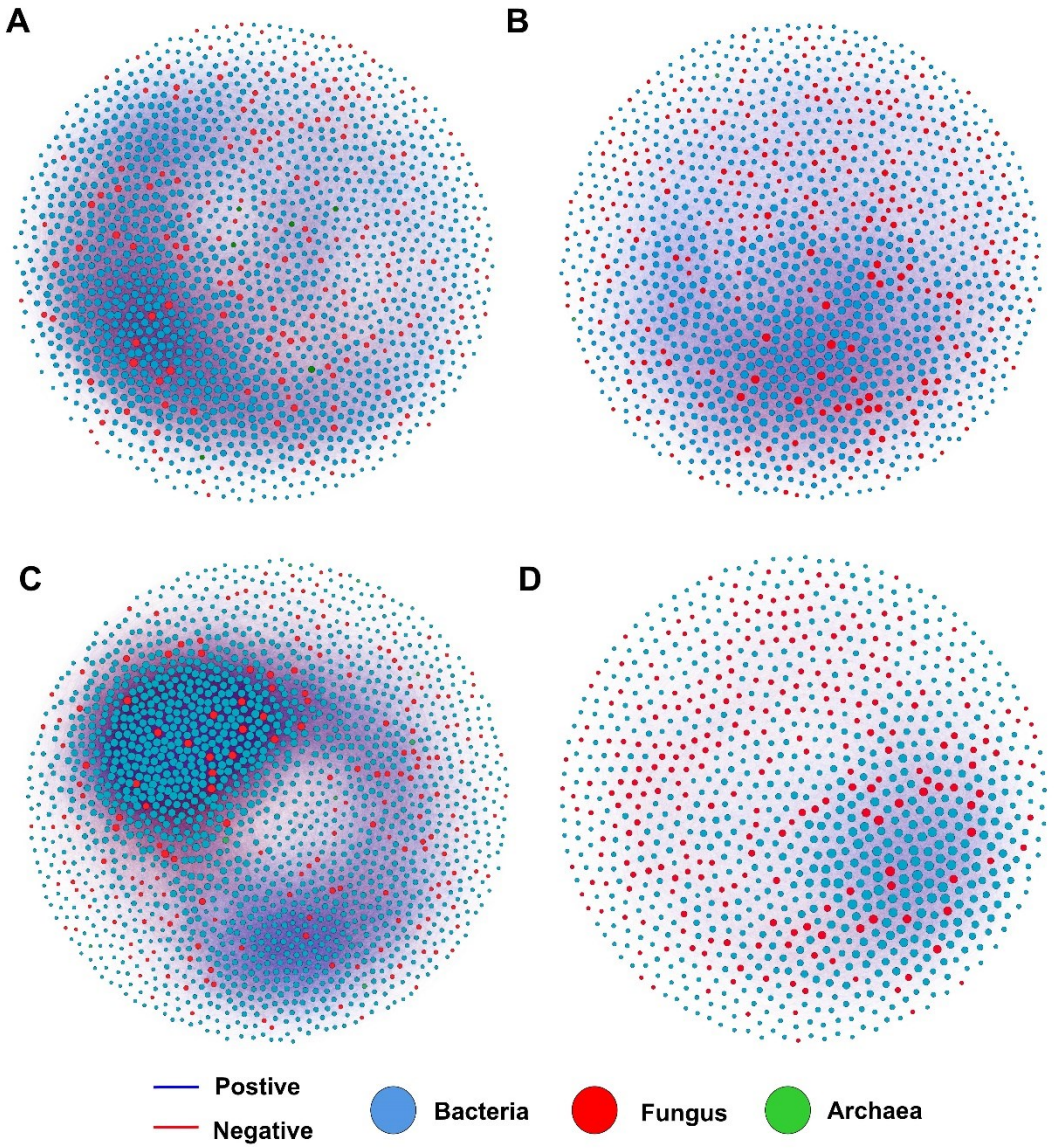


Figure S3 Plant biomass of healthy and infected peanuts. *** indicates significant difference at P <0.001.

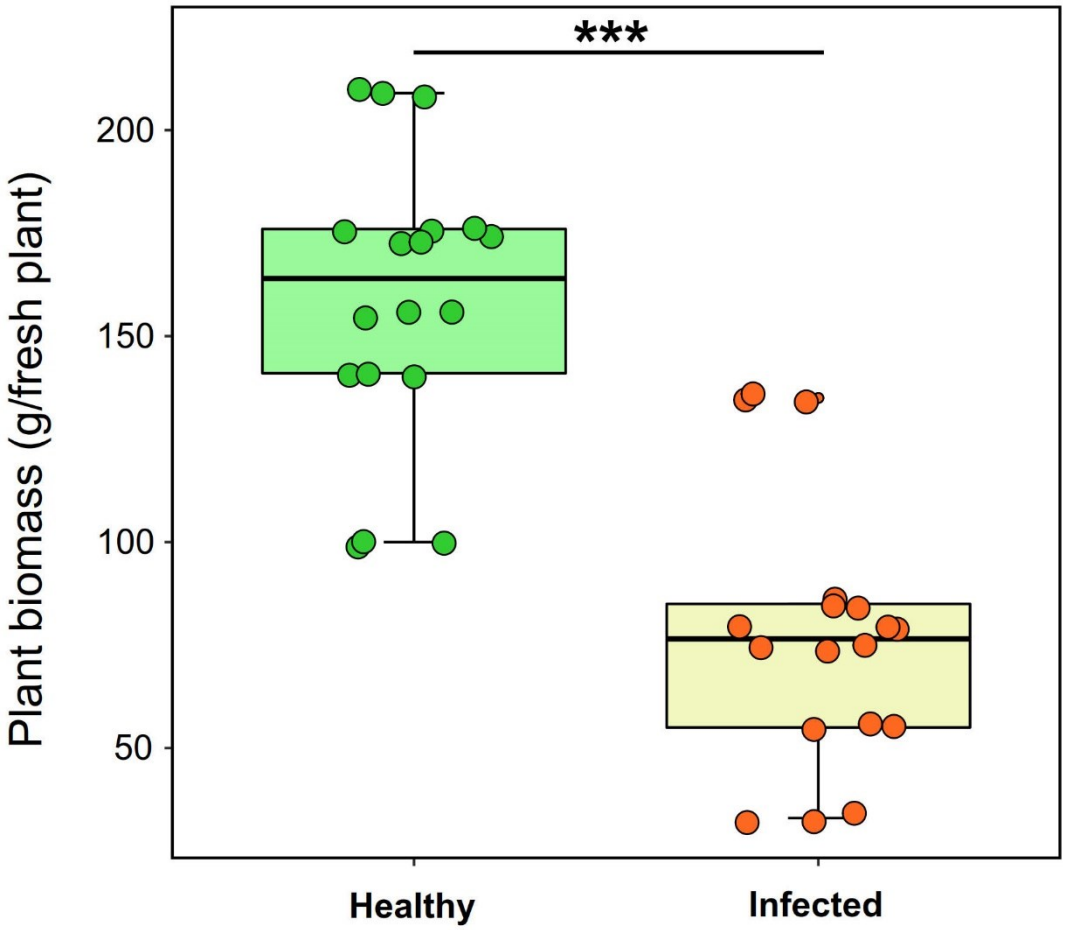


Table S1 One-way permANOVA investigating the structural difference of bacterial community. The lower triangular matrix in the table shows the *F* values of permANOVA, and the upper triangular matrix shows the corresponding *P* values.

	HR	HB	SR	SB
HR		< 0.001	< 0.001	< 0.001
HB	16.990		< 0.001	0.620
SR	10.600	28.360		< 0.001
SB	20.960	0.750	34.190	

Table S2 One-way permANOVA investigating the structural difference of fungal community. The lower triangular matrix in the table shows the *F* values of permANOVA, and the upper triangular matrix shows the corresponding *P* values.

	HR	HB	SR	SB
HR		< 0.001	0.018	< 0.001
HB	6.445		< 0.001	0.039
SR	2.493	10.020		< 0.001
SB	6.501	1.773	9.039	

Table S3 Soil physicochemical properties of each treatment. Data are the means ± standard deviation (n=18). Different letters in each row indicate significant differences among treatments at $P < 0.05$. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; TK: total potassium; AN: available nitrogen; AP: available phosphorus; AK: available potassium.

	HB	HR	IB	IR
pH	(5.52±0.17) B	(6.26±0.15) A	(5.75±0.14) B	(6.19±0.17) A
SOC(g/kg)	(12.41±1.23) A	(14.77±1.24) A	(14.34±1.08) A	(14.45±1.11) A
TN(g/kg)	(0.80±0.07) A	(0.82±0.08) A	(0.76±0.06) A	(0.83±0.08) A
TP(g/kg)	(0.80±0.08) A	(0.81±0.07) A	(0.71±0.08) A	(0.95±0.12) A
TK(g/kg)	(12.97±1.52) A	(12.74±1.55) A	(12.03±1.78) A	(12.11±1.36) A
AN(mg/kg)	(89.1±6.34) A	(80.51±6.89) A	(82.88±5.94) A	(83.89±4.90) A
AP(mg/kg)	(52.44±4.55) B	(94.15±7.1) A	(110.23±11.39) A	(117.55±9.33) A
AK(mg/kg)	(155.28±30.6) B	(200.68±25.73) A	(154.88±25.49) B	(237.35±21.05) A

Table S4 Detailed information about primers and PCR conditions.

	Primers	Primer sequence (5'-3')	Target gene	Target subfragment	PCR reaction condition
Bacteria	515F	GTGCCAGCMGCCGCGGTAA	16S	V4-V5	An initial denaturation at 95 °C for 3 minutes, followed by 27 cycles of 30 s at 95 °C, annealing for 30 s at 55 °C and elongation for 45 s at 72 °C, the last step being extension at 72 °C for 10 minutes.
	907R	CCGTCAATTCCTTTGAGTTT			
Fungi	ITS1F	CTTGGTCATTTAGAGGAAGTAA	ITS	ITS1	An initial denaturation at 95 °C for 3 minutes, followed by 35 cycles of 30 s at 95 °C, annealing for 30 s at 55 °C and elongation for 45 s at 72 °C, the last step being extension at 72 °C for 10 minutes
	ITS2	GCTGCGTTCTTCATCGATGC			

Figures

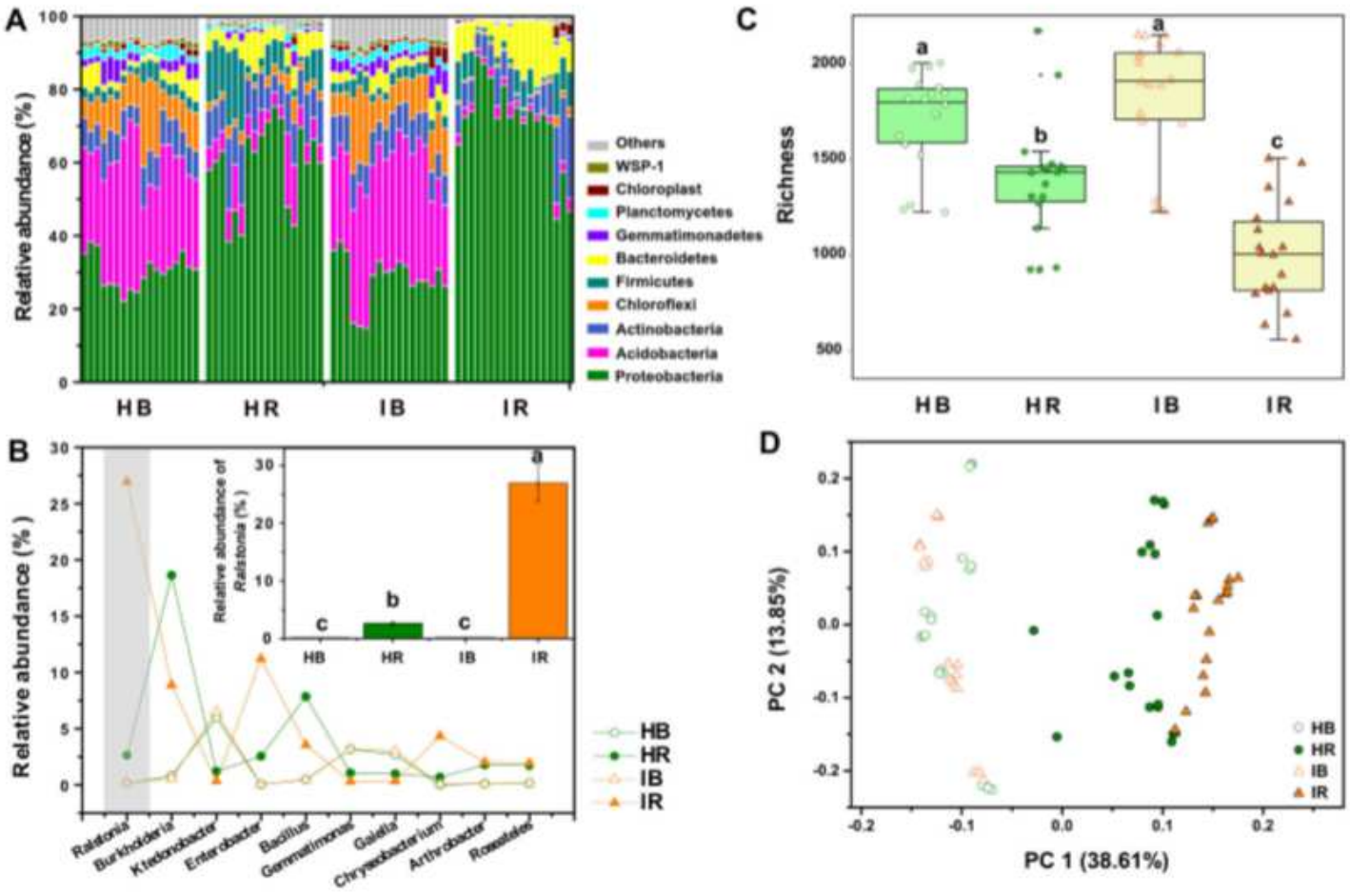


Figure 1

Species composition and diversities of bacterial communities. Species composition at (a) phylum and (b) genus level. Alpha-diversity (c) and beta-diversity (d) of bacterial communities. Different lowercases in (b) and (c) represent significant difference at P <0.05. Best-diversity was calculated based on the Bray-Curtis dissimilarity, and was plotted based on the first two axes of the principle coordinate analysis.

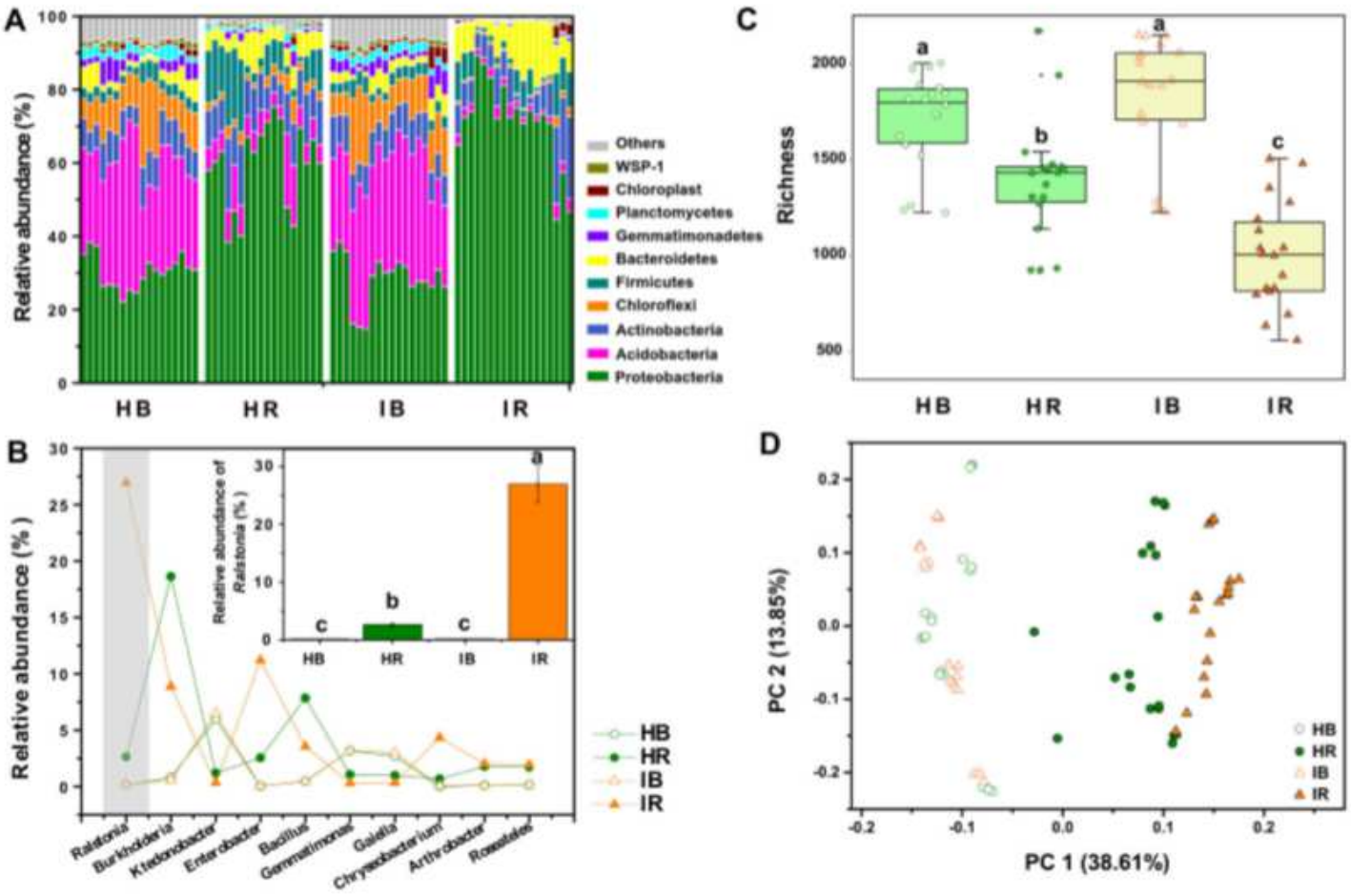


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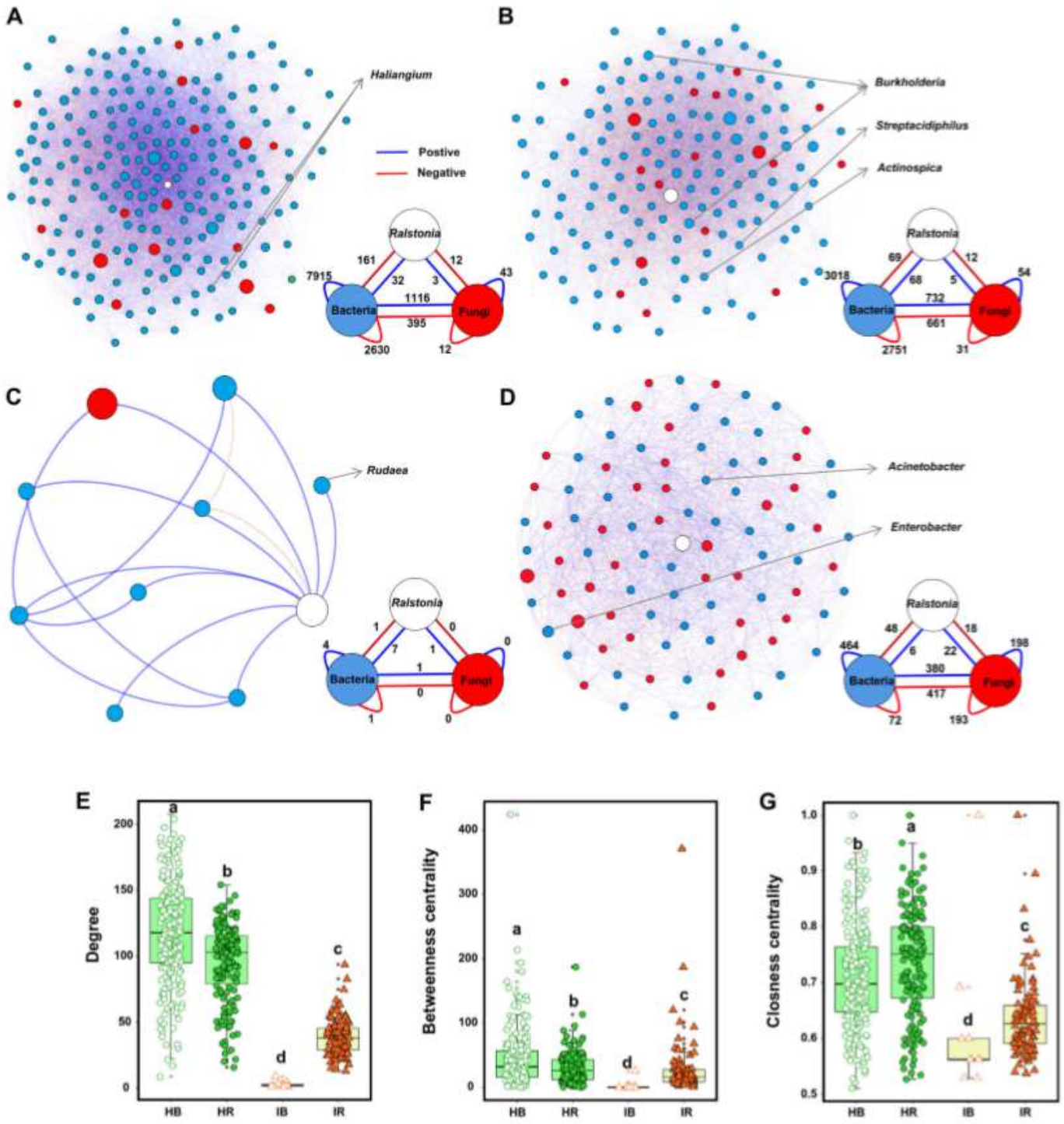


Figure 2

Microbial ecological network showing the co-occurrences among Ralstonia and other microorganisms in each treatment. Co-occurrence patterns among Ralstonia and other microorganisms in HB (a), HR (b), IB (c), and IR (d); and network parameters including degree (e), betweenness centrality (f), and closeness centrality (g) of each network. The red lines represent negative correlations, and the blue lines represent positive correlations. Each network was constructed with 18 samples under the parameters r >0.6 and P

<0.01. Lowercase letters above the boxes indicate a significant difference at P <0.05 according to a nonparametric Mann-Whitney U test.

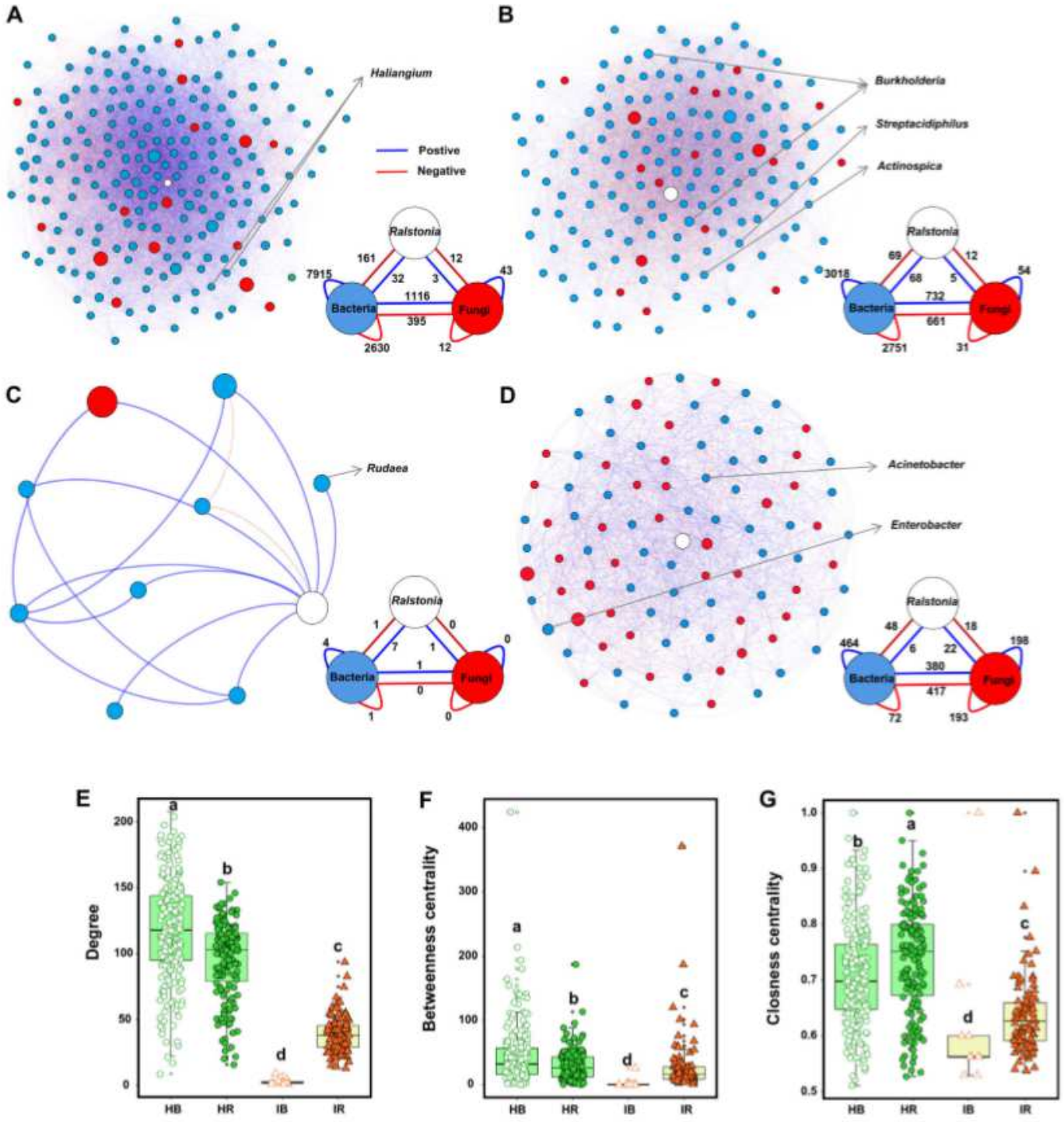


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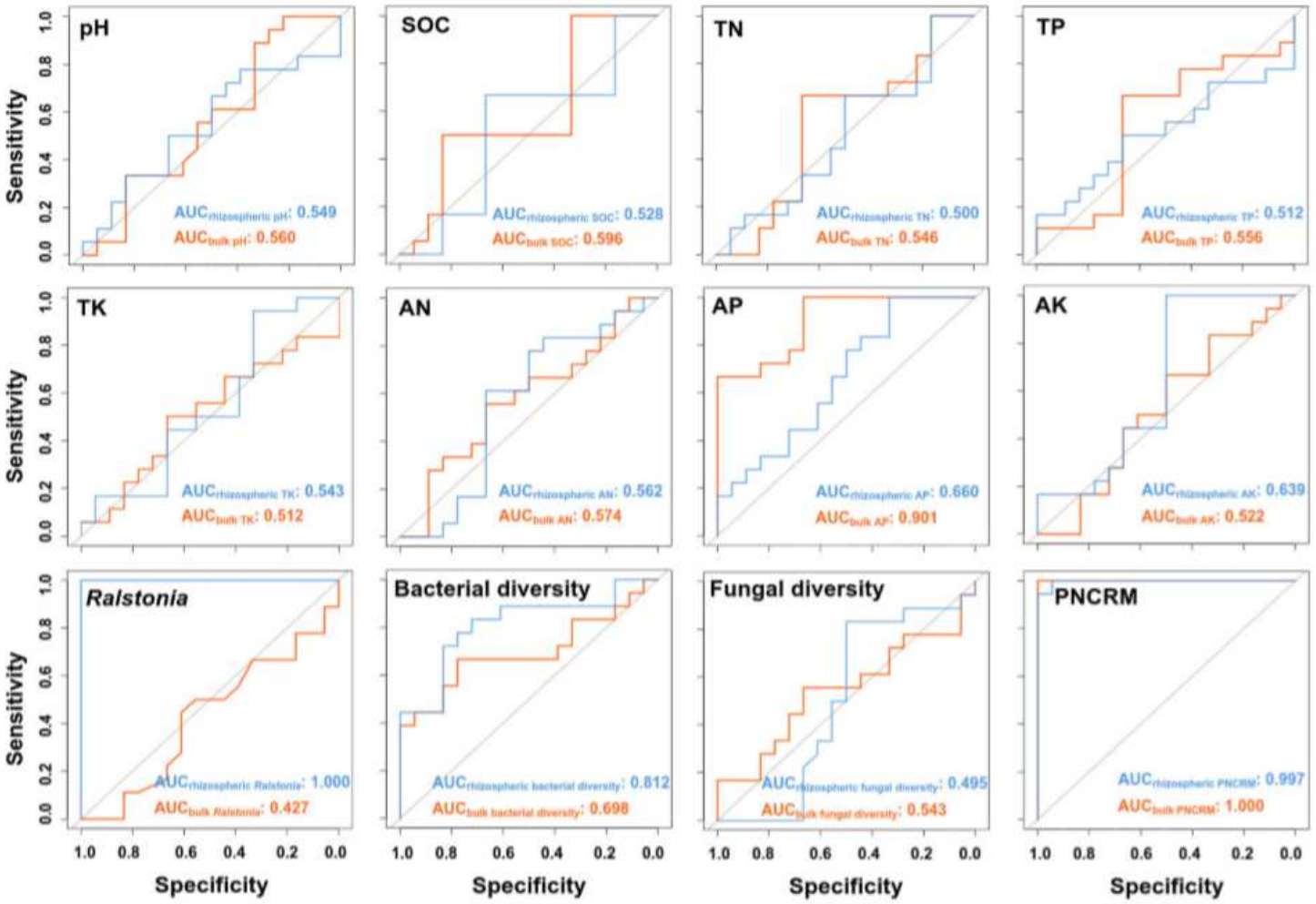


Figure 3

Predictors of the incidence of peanut wilt determined by receiver operating characteristic curve analysis. A higher AUC indicates better prediction. PNCRM: proportion of negative co-occurrences among Ralstonia and other microorganisms.

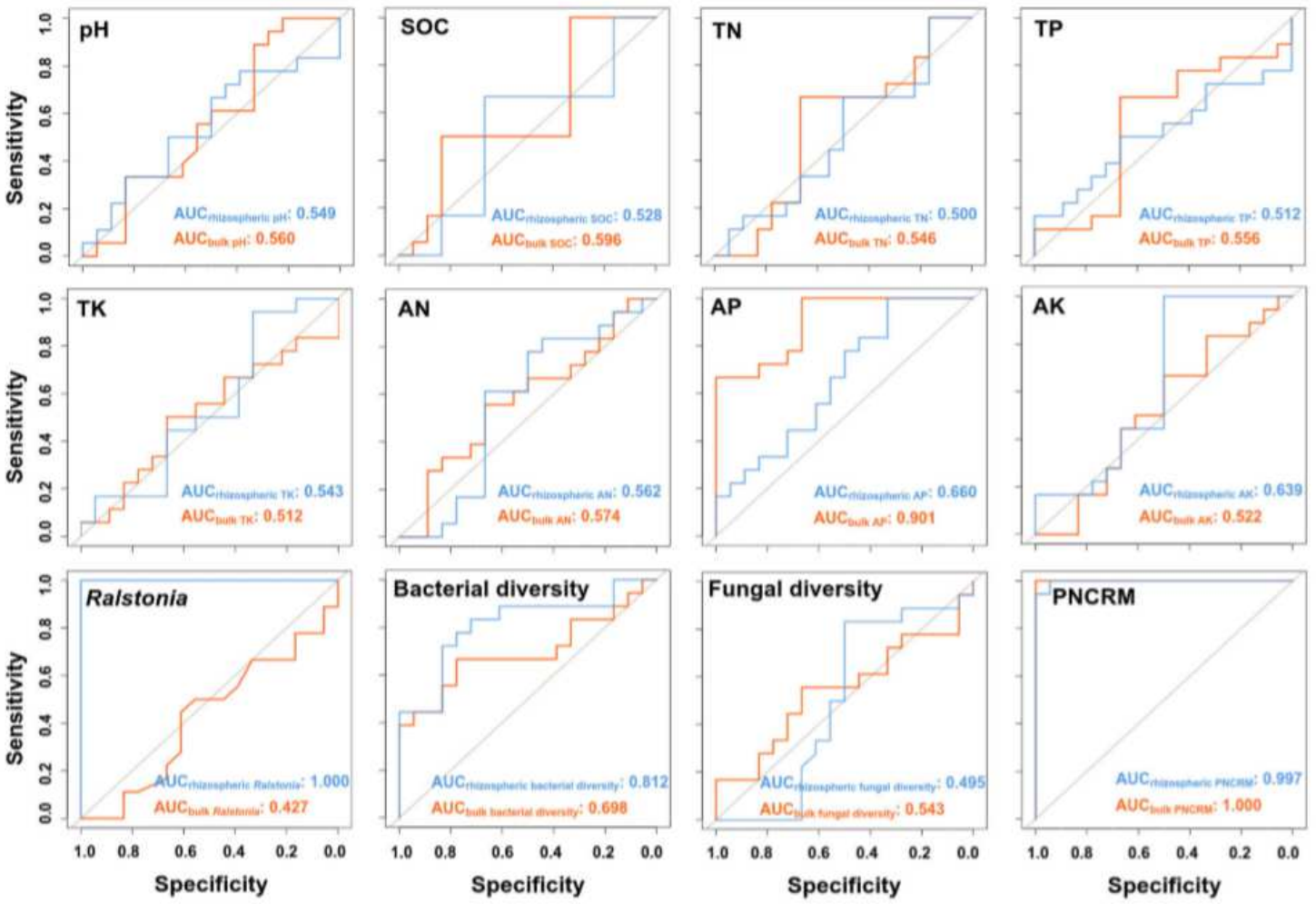


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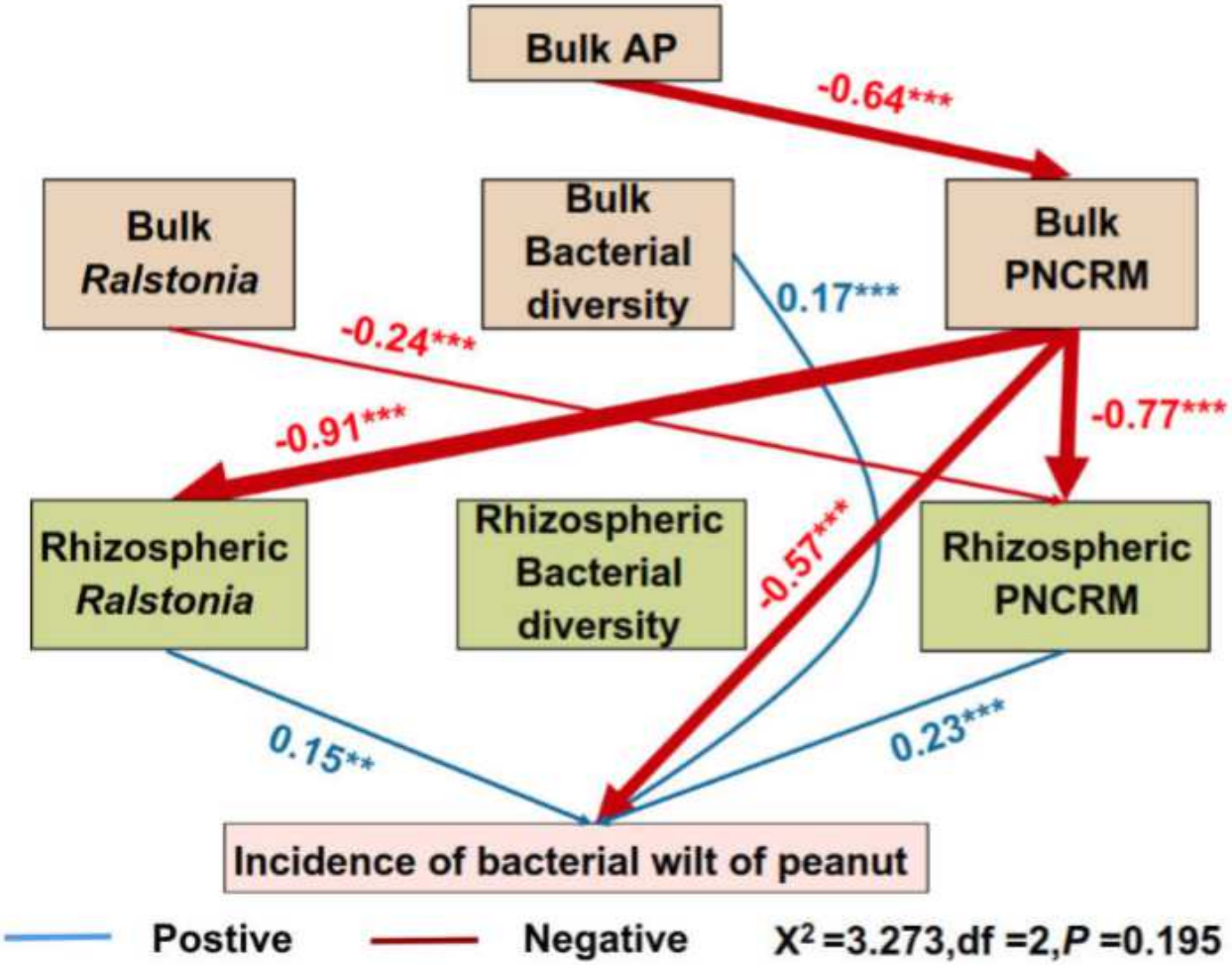


Figure 4

The linkages between soil properties, biological parameters, and the incidence of peanut wilt. The linkages were determined by structural equation modeling (SEM). The red and blue lines indicate negative and positive linkages, respectively. The width of arrows indicates the strength of significant standardized path coefficients ($P < 0.05$).

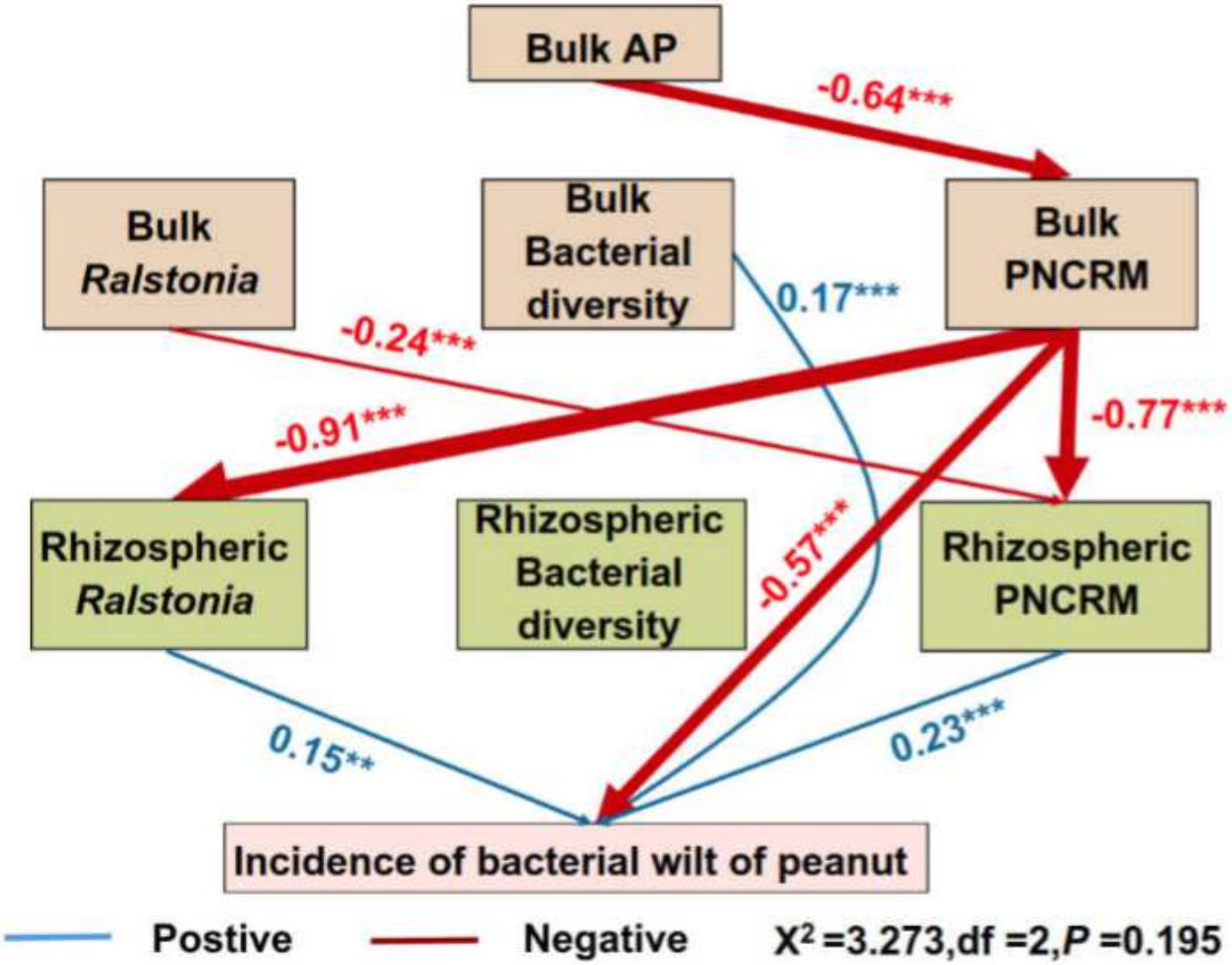


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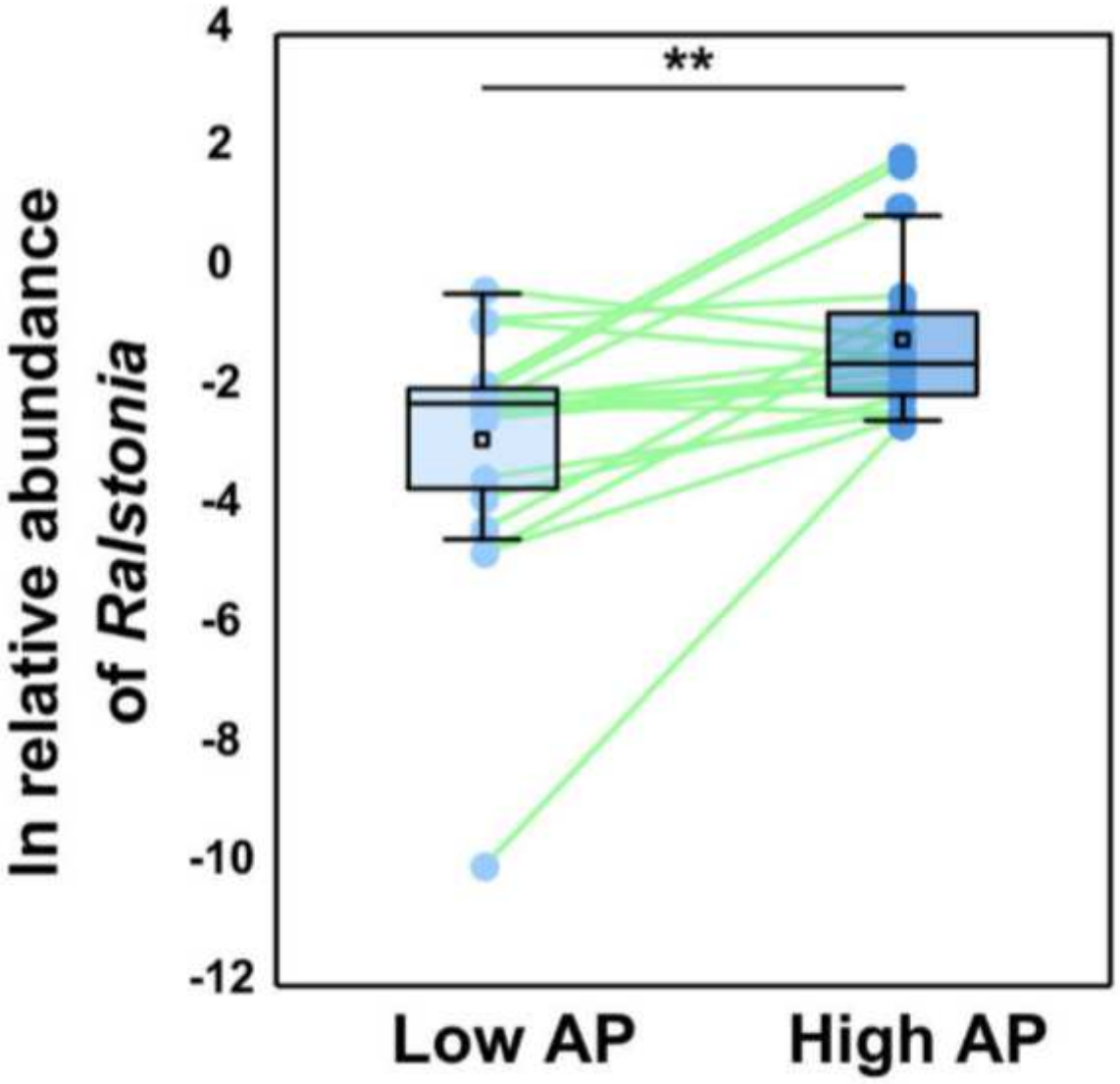


Figure 5

The ln-transformed relative abundance of Ralstonia in low AP and high AP soils. ** indicates a significant difference at P <0.01.

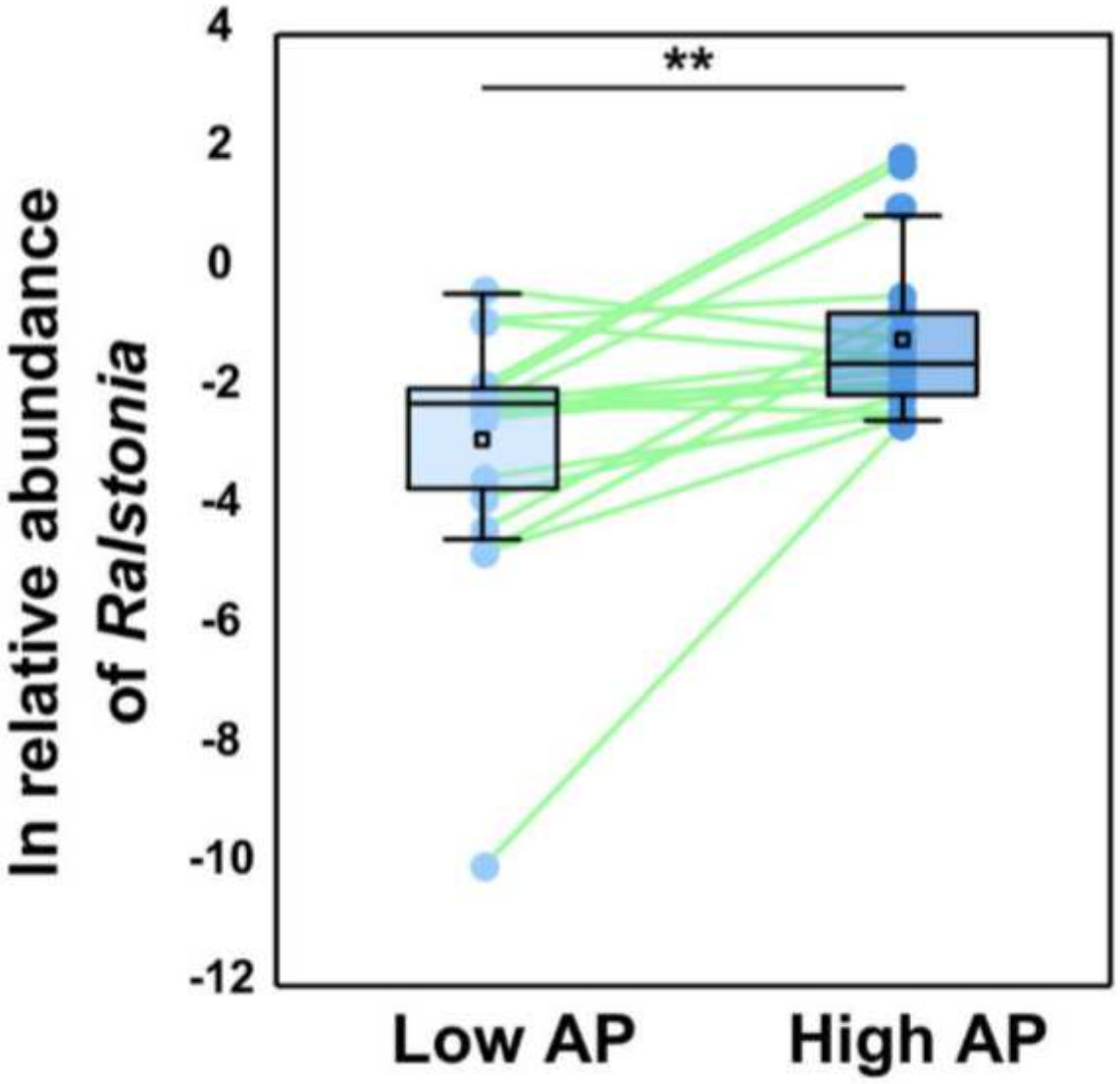


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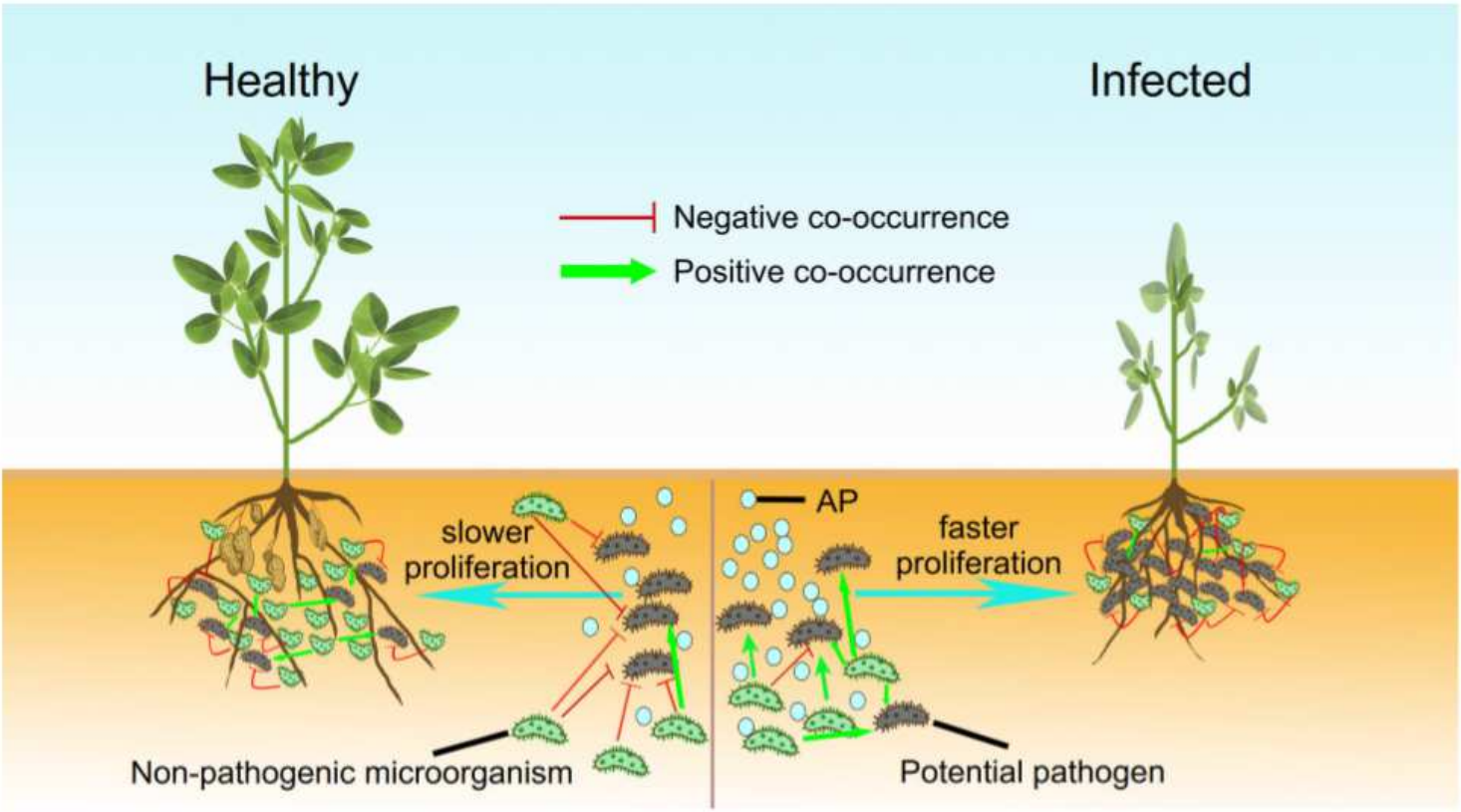


Figure 6

A conceptual paradigm showing the potential mechanism explaining the incidence of peanut wilt.

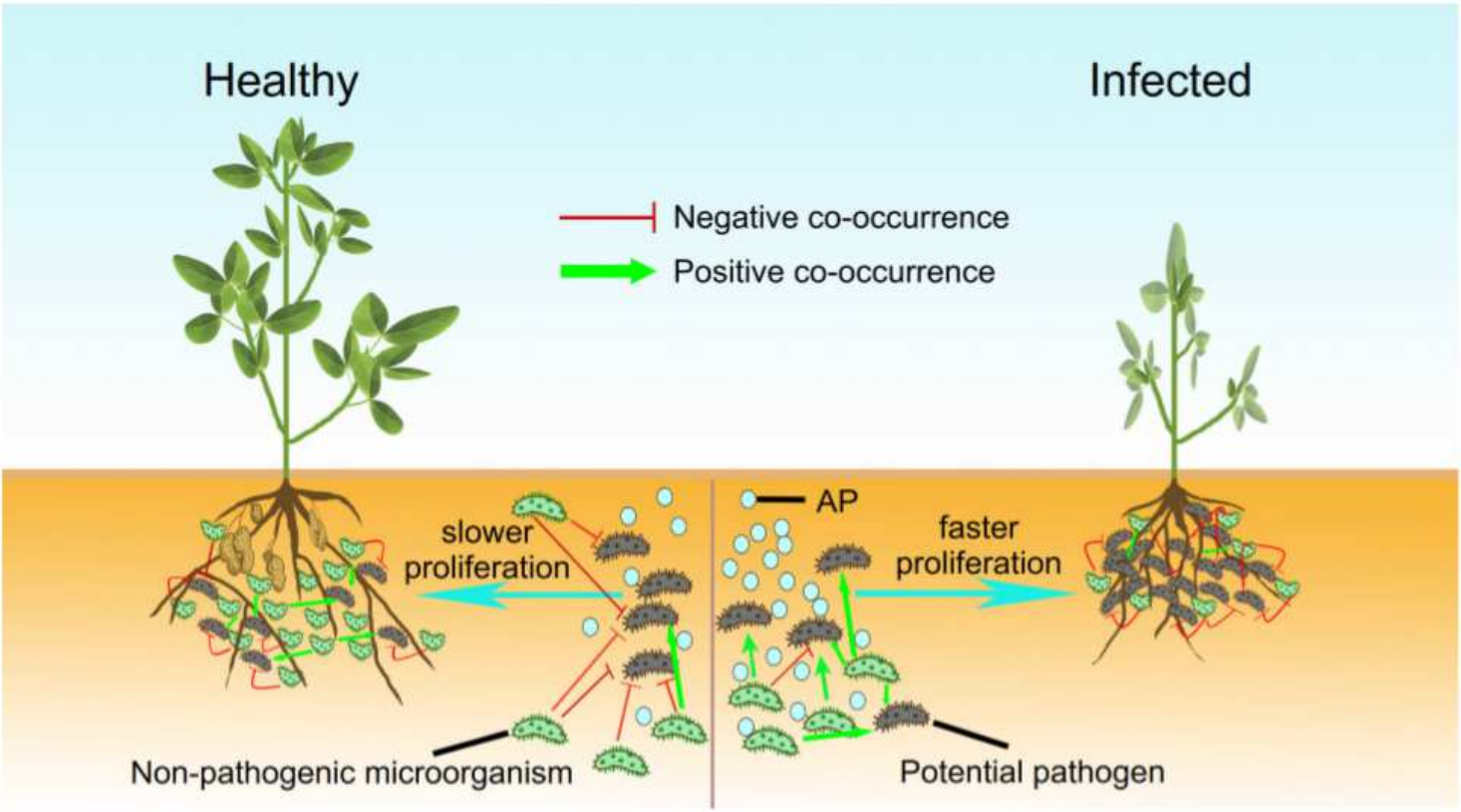


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