Microbial community dynamics in the recirculating nutrient solution of tomato plug seedlings cultivated under ebb-and-flow system

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Abstract

Background: The ebb-and-flow system has ability to recirculate water and nutrients, and offers a good method to control nutrient leaching from greenhouses into the environment. However, the potential for the rapid spread of bacterial and fungal pathogens is the main hindrance for its adoption in vegetable seedlings production. Natural microflora has often shown a certain ability to suppress diseases.

Results: Here, through 16S rRNA- and ITS1-targeted Illumina sequencing, the dynamic changes in bacterial and fungal communities in the recirculating nutrient solution were characterized for tomato plug seedlings cultivated in an ebb-and-flow system in summer and winter. Both bacterial number and microbial diversity in the nutrient solution increased with recirculating irrigation, and these changes differed between summer and winter. Pseudomonas was among the most predominant bacterial genera in the nutrient solution; its relative abundance gradually increased with recycling in summer but decreased dramatically in winter. In summer, the predominant bacteria also included Comamonas and Sediminibacterium , whose relative abundances in the nutrient solution decreased and increased, respectively. In winter, the nutrient solution was also colonized by Sphingomonas , predominantly at the late stage of irrigation. Among fungi, Amanita and Trichoderma were predominant in both summer and winter. Amanita gradually increased in summer but decreased rapidly after the first irrigation cycle in winter, whereas Trichoderma accumulated rapidly at the late stage of irrigation in both summer and winter. Alternaria and Fusarium were predominant in the nutrient solution only in summer, with the former decreased and the latter increased with recirculating irrigation. Moreover, Pseudomonas mediterranea , a potentially pathogenic bacterium, and Fusarium oxysporum , a fungal pathogen were present predominantly in winter and summer, respectively. Some potentially beneficial microbes functioning in plant-growth promotion and water self-purification, such as Sphingobium xenophagum , Trichoderma harzianum , and T. virens , were identified to be increased in the recirculating nutrient solution.

Conclusions: The present data elucidate the bacterial and fungal dynamics in an ebb-and-flow system and provide useful information for pathogen control during tomato seedling production.

Background

Ebb-and-flow systems, one of the first commercial forms of hydroponic systems, are emerging as a technology to replace traditional top irrigation systems. These systems use an automatic flood and drain watering technique, in which plants are flooded temporarily and periodically [1]. The water or nutrient solution in the reservoir ascends to the growth tray via a timer-activated water pump, accumulates to a certain level, and remains in the growth tray for a set amount of time, providing water and nutrients to the plants. After a predetermined time, the solution is drained back into the reservoir through a tubing system [2,3]. The principal advantage of this ebb-and-flow system is the ability to recirculate water and nutrients [4,5], thereby improving the water and fertilizer use efficiency and reducing the environmental pollution caused by the discharge of the waste nutrient solution. The other benefits of the ebb-and-flow system include minimal costs in labor and automatic management [2]. In addition, these subirrigation systems

can overcome the “umbrella effect” (i.e., water redirection by leaves) and ensure that the plants absorb water and nutrients evenly [6]. As a result of these benefits, the supply of and the demand for ebb-and-flow systems have dramatically increased in agricultural production, especially in the intensive cultivation of plug seedlings.

In the ebb-and-flow systems, recirculating nutrient systems offer a good method to control nutrient leaching from greenhouses into the environment. However, the potential for the rapid spread of diseases is the main hindrance to adoption of recirculating nutrient systems by the greenhouse industry. Firstly, the recirculating nutrient solution are inhabited by a large number of indigenous microorganisms. Furthermore, different sources, including air, seeds, plants, substrates, and personnel, can lead to contamination by various microorganisms [7]. In addition, almost all ebb-and-flow systems are located indoors in greenhouses [3,8]. The environmental parameters are all set to optimize plant growth and minimize plant stresses. Microorganisms in the recirculating nutrient solution are also exposed to this narrowly defined environment, which supports microbial growth and production. In the ebb-and-flow system, all plant roots are in contact with the recirculating nutrient solution, giving rise to a potential problem in which the microorganisms, including plant pathogens, would be dispersed rapidly [9–11]. Common water-borne plant pathogens, such as *Xanthomonas, Fusarium, Phytophthora,* and *Pythium,* have been detected in ebb-and-flow systems [4,12–14]. Therefore, most ebb-and-flow systems also include a filtration or sterilization step for the recirculating nutrient solution [15,16].

Currently, various approaches including physical and chemical methods were used for controlling pathogens in the ebb-and-flow systems, and of them, the most commonly used disinfection methods for nutrient solution include ozonation, ultraviolet light irradiation, and heating [16–18]. However, these active methods eliminate most of the nonpathogenic microflora, most of which are beneficial to plants or, at least, do not cause significant inhibition of plant growth [2]. Additionally, in agricultural production, the nutrient solution is often disinfected after each irrigation cycle. Excessive disinfection not only increases production costs but also causes damage to the environment. Also, high cost for installation and periodic maintenance of the disinfection systems are challenging [2,19–20]. Analysis of the dynamic changes in the microbial community in the recirculating ebb-and-flow system, and especially depicting the distribution patterns of the beneficial and pathogenic microbes, will be needed to develop a more rational and economic strategy for the disinfection of recirculating nutrient solution.

Tomato (*Solanum lycopersicum)* is one of the most commonly cultivated vegetables in the world (http://faostat.fao.org), and it also serves as a model plant for studying plant-microbe interactions [21]. Ebb-and-flow systems have been used widely for the commercial cultivation of tomato plug seedlings because of its notable advantages. Unfortunately, reports on the microbial community in the recirculating ebb-and-flow system used in tomato seedling cultivation are sparse. Some reports discussing the microbial changes in the nutrient solution in hydroponic culture have mainly focused on the responses to the introduction of pathogenic or plant-growth-promoting microbes, and most of these studies only identified the microbial changes at the endpoint of cultivation without considering dynamic changes [22–25]. In the present study, Illumina MiSeq sequencing technology was used to monitor the dynamic

changes in bacterial and fungal communities in two distinct habitats within an ebb-and-flow system—the recirculating nutrient solution and substrate—in both summer and winter. In addition, the changes in the relative abundances of beneficial and pathogenic bacteria and fungi were analyzed. This work will provide a comprehensive understanding of the microbial dynamics and seasonal variations in an ebb-and-flow system and may be useful for the development of a rational disinfection strategy and the selection of suitable microflora for biological control in tomato seedling cultivation.

Results

Dynamic changes in the number of culturable bacteria

In the recirculating nutrient solution, the number of culturable bacteria showed similar changing patterns in both summer and winter (Fig. 1a). In the beginning, the numbers of culturable bacteria in the nutrient solution were much lower (less than 0.4×10^4 CFU/mL), and after the first irrigation, the numbers increased significantly to more than 1.2×10^4 CFU/mL. This increase was possibly due to the entrance of bacteria from the substrate. Subsequently, the culturable bacteria number continued to increase and then maintained a relatively stable level. At the end of cultivation, the culturable bacteria number exceeded 10^5 CFU/mL. However, in the substrate, no significant differences in the number of culturable bacteria between the original and final samples were observed, both in summer and winter (Fig. 1b).

Data analysis by Illumina sequencing

Using Illumina sequencing of *16S rRNA* amplicons, the bacterial communities in the nutrient solution and substrate samples were analyzed. In summer, from the 42 nutrient solution samples (seven input and seven output solution samples with three replicates) and six substrate samples (two samples × three replicates), 3,470,449 and 483,670 raw reads were obtained, respectively. From these raw reads, 3,252,422 and 462,306 clean reads were obtained after the low-quality sequences and errors were removed. Then, after subsampling at a minimum sampling depth of 38,848 reads per sample, 1,900 and 1,260 OTUs were obtained after BLAST and taxonomic assignment (Additional file 1: Table S1). In winter, 5,005,108 and 483,564 raw reads were obtained from 60 nutrient solution samples (10 input and 10 output samples with three replicates) and six substrate samples, respectively. Of these sequences, 4,702,819 and 459,005 clean reads and 1,752 and 1,181 OTUs were obtained, respectively (Additional file 2: Table S2).

Illumina sequencing was also performed on the fungal ITS1 genes. In summer, from the nutrient solution and substrate samples, 3,385,433 and 490,218 raw reads were obtained, and 3,365,847 and 481,295 clean sequences were yielded, respectively. After subsampling and taxonomic assignment, 1,355 and 709 OTUs were obtained (Additional file 3: Table S3). In winter, 1,624 and 470 OTUs were obtained from the nutrient solution and substrate samples, respectively (Additional file 4: Table S4). At these sequencing

depths, the rarefaction curves began to level off, suggesting that the microbial communities were reasonably well characterized with our sampling effort (Additional file 5: Fig. S1).

Alpha- and beta-diversity analysis of the microbial communities

The Shannon index was used to assess the alpha-diversity of the microbial communities in the recirculating nutrient solution and substrate samples [26]. For the bacterial communities, in summer, the Shannon index in In1 sample was 5.57, and then increased significantly after the first irrigation cycle (Out1 sample, 6.63). Subsequently, the bacterial Shannon index in the nutrient solution fluctuated but decreased overall with the recirculating irrigation. At the end of irrigation (Out7), the bacterial Shannon index was only 4.13 (Fig. 2a). In winter, the bacterial Shannon index of sample In1 was only 3.94, much lower than that observed in the corresponding sample in summer. However, with the recirculating irrigation, the Shannon index increased. At the end of irrigation (Out10), the Shannon index reached 6.30 (Fig. 2a). For the fungal communities, in summer and winter, the Shannon index showed similar change trends. The Shannon index gradually increased during the early stage of irrigation and then decreased rapidly at the middle stage of irrigation before gradually increasing again (Fig. 2b).

In the substrate samples, the bacterial Shannon index increased significantly in summer but decreased in winter (Fig. 2c). For the fungal Shannon index, it showed no significant difference between the initial and final samples in summer, but in winter, a significant increase was observed in the final substrate sample (Fig. 2d). The other alpha-diversity indexes (Chao1, ACE, and Simposon) also showed the similar changing pattern with the Shannon index (Additional file 6: Table S5; Additional file 7: Table S6).

The overall Shannon indexes of microbial communities in the input and output nutrient solution sample groups were compared, however, no significant difference could be detected, neither for bacteria nor for fungi (Fig. 3a and 3b). However, when the summer and winter sample groups were compared, significant differences were found. The overall diversity indexes of the winter sample group were significantly higher than those of the summer sample group, for both bacterial and fungal communities (Fig. 3c and 3d). In the substrate samples, similar seasonal variations were observed for the bacterial communities (P = 0.038, Fig. 3c), but not for the fungal communities (P > 0.05, Fig. 3d).

Furthermore, the similarity of bacterial and fungal communities in the recirculating nutrient solution and substrate samples was analyzed using NMDS analysis based on the Bray-Curtis distance (Fig. 4). The NMDS plots revealed that the nutrient solution samples varied considerably, while the substrate samples clustered more closely together. In the case of nutrient solution samples, the distance between the input and output nutrient solution samples in the same irrigation cycle was close, indicating the high similarity of the microbe communities in these samples. In addition, the global NMDS plot revealed a clear distinction between the solution samples from the first irrigation (In1 and Out1) and other nutrient

solution samples, and the In1 and Out1 samples clustered more closely to the substrate group than other solution samples (Fig. 4).

Bacterial community dynamic in the recirculating nutrient solution and substrate samples

The bacterial compositions were further analyzed. In the nutrient solution samples, the predominant bacterial phyla included Proteobacteria, Bacteroidetes, and Actinobacteria (Additional file 7: Fig. S2). The relative abundance of Proteobacteria was the highest, followed by that of Bacteroidetes. The relative abundance of Bacteroidetes was low in sample In1 but increased significantly after the first irrigation, and then maintained a value of approximately 15%–20%. For the phylum Actinobacteria, its relative abundance gradually decreased in summer, but increased gradually in winter. For Cyanobacteria, this phylum was only detected in the nutrient solution in winter, with an increasing pattern. In the substrate, the bacterial communities were quite different from the nutrient solution samples, but were similar in summer and winter. The predominant phyla included Proteobacteria, Actinobacteria, and Bacteroidetes, Chloroflexi, Acidobacteria, and Verrucomicrobia. The relative abundance of Acidobacteria decreased, whereas the relative abundance of Verrucomicrobia significantly increased (Additional file 7: Fig. S2).

At the genus level, in summer, the predominant genera in the nutrient solution included *Pseudomonas, Comamonas, Sediminibacterium, Sphingobium, Massilia, Reyranella, Nevskia,*and *Acidovorax* (Fig. 5a). With the recirculating irrigation, the relative abundances of *Pseudomonas* and *Sediminibacterium* gradually increased, whereas that of *Comamonas* gradually decreased. The relative abundances of *Acinetobacter* and *Nevskia* were only higher in the In1 sample and then decreased rapidly after the first irrigation. The genus *Massilia* was only predominant in the output samples during the first three irrigation cycles (Out1-Out3). The relative abundances of the genera *Reyranella* and *Acidovorax* first increased and then decreased with nutrient solution recirculation (Fig. 5a). In winter, the bacterial communities in the nutrient solution were mainly composed of *Pseudomonas, Curvibacter, Comamonas, Zoogloea, Sphingomonas, Zymomonas, Paucimonas, Solitalea, Spirosoma,* and *Sphingobium* (Fig. 5b). The relative abundance of *Pseudomonas* decreased overall, whereas the relative abundances of the genera *Sphingomonas, Spirosoma,* and *Sphingobium* gradually increased. The genera *Curvibacter* and *Zoogloea* were predominant in both input and output solution samples only at the early stage of irrigation, after which their relative abundances rapidly decreased. However, the relative abundances of the genera *Zymomonas, Paucimonas* and *Solitalea* increased first and then decreased (Fig. 5b). In the substrate samples, the predominant bacterial genera included *Pseudonocardia* and *Streptomyces,* and the genera *Mucilaginibacter* and *Rhizomicrobium* also exhibited specific distributions in winter. The relative abundances of these predominant genera all decreased at the end of seedling periods (Fig. 5). The bacterial genera that changed in the recirculating nutrient solutions did not significantly increase or decrease in the substrate samples.

At the species level, *Comamonas testosteroni* had the highest relative abundance in the nutrient solution (Fig. 6a). In summer, the relative abundance of *C. testosteroni* gradually decreased with the recirculating irrigation (from 14.86% in In1 to 0.31% in Out7), while in winter, its relative abundance rapidly decreased after the first irrigation (from 9.52% to 2.44%). In the substrate, *C. testosteroni* was present only in winter, although its relative abundance was low, approximately 0.25% (Fig. 6a). *Sphingomonas xenophaga* was present in the nutrient solutions in both summer and winter, and its relative abundance increased (Fig. 6b). The other predominant bacterium in the nutrient solution was *Pseudomonas vancouverensis,* which was detected only in winter but not in summer (Fig. 6c). Another predominant bacterium in the recirculating nutrient solution was *Nitrosomonas oligotropha.* Its relative abundance increased significantly in both nutrient solution and substrate samples in winter, while in summer, a transient increase occurred only in samples In5 and Out5 (Fig. 6d). Different from the above bacteria, *Telluria mixta* was only detected at high levels in the output solution and the final substrate samples collected in summer (Fig. 6e). Furthermore, *Pseudomonas mediterranea,* a pathogenic bacterium causing pith necrosis in tomato, was detected predominantly only in winter, and its relative abundance was gradually reduced in the nutrient solution, while in the substrate, its abundance increased significantly in the final samples (Fig. 6f).

Fungal community dynamic in the recirculating nutrient solution and substrate

In terms of fungal communities, the predominant phyla in the recirculating nutrient solution were Basidiomycota, Ascomycota, and Chytridiomycota. However, the fungal compositions showed different change patterns in summer and winter, even at the phylum level. In summer, the relative abundance of Basidiomycota increased significantly with the recirculating irrigation, whereas the relative abundances of Ascomycota and Chytridiomycota gradually decreased. In winter, the relative abundance of the Ascomycota phylum increased significantly after the first irrigation, after which it decreased until the sixth irrigation, followed by an increase. The abundance of Chytridiomycota showed the opposite pattern, with an initial increase followed by a decrease (Additional file 8: Fig. S3).

In the nutrient solution, the major genus in the Basidiomycota phylum was *Amanita.* In summer, the relative abundance of *Amanita* gradually increased with the recirculating irrigation, and it became the most abundant genus in the nutrient solution (Fig. 7a), while in winter, the relative abundance of *Amanita* was only higher in sample In1 (80.62%) and then rapidly decreased and was maintained at approximately 20% (Fig. 7b). The other predominant genera in the Basidiomycota phylum included *Rhodosporidium* and *Rhodotorula,* but these genera were predominant only during the winter seedling period. The relative abundance of *Rhodosporidium* decreased with the recirculating irrigation, while the relative abundance of *Rhodotorula* increased (Fig. 7b). For the Ascomycota phylum, the predominant genera in the nutrient solution included *Alternaria, Fusarium,* and *Trichoderma.* Of these genera, *Alternaria* and *Fusarium* werepresent predominantly in summer. With the recirculating irrigation, the relative abundance of *Alternaria* gradually decreased (from 12.69% to 1.65%), whereas that of *Fusarium* gradually increased

(from 0.22% to 3.07%; Fig. 7a). *Trichoderma* was present in the nutrient solution in both summer and winter, and its relative abundance increased rapidly during the late stage of irrigation (Fig. 7). In the substrate samples, the fungal distribution was significantly different from that observed in the recirculating nutrient solution. The predominant fungal genera included *Phaeoacremonium, Phialemonium,* and *Trichoderma.* During both summer and winter, the relative abundance of *Phaeoacremonium* decreased after seedling growth, whereas the genera *Phialemonium* and *Trichoderma* both increased (Fig. 7). Furthermore, in summer, the genera *Clitopilus* and *Alternaria* werepredominant in the substrate samples; the relative abundance of *Clitopilus* decreased, and the relative abundance of *Alternaria* increased (Fig. 7a). However, the genus *Cladosporium* was only predominant in the winter substrate samples, and its relative abundance increased significantly after seedling growth (Fig. 7b).

At the species level, *Amanita fulva* was the most abundant fungus in the nutrient solution. Especially in summer, more than 80% of fungi were *A. fulva* at the end of irrigation. However, its relative abundance was quite low in the substrate samples and showed no significant change before and after seedling growth (Fig. 8a). We also identified two *Trichoderma* species: *T. virens* and *T. harzianum.* In winter, the relative abundance of *T. virens* increased considerably both in the nutrient solution and substrate samples. In summer, although the relative abundance of *T. virens* in the nutrient solution did not significantly change, its relative abundance in the final substrate sample was 2.25 times higher than that observed in the initial sample (Fig. 8b). And for *T. harzianum,* it accumulated to a large extent in nutrient solution and substrate samples in both summer and winter (Fig. 8c). In addition, we found that *Phialemonium inflatum* was primarily present in the tomato plug seedling substrate. Its relative abundance in the initial substrate sample was approximately 2% and increased significantly after seedling growth, reaching to 5.41% and 10.33% in summer and winter, respectively (Fig. 8d). Furthermore, we also identified three potentially pathogenic fungi, including *Fusarium oxysporum, Alternaria alternate* and *Phaeoacremonium krajdenii.* In the recirculating nutrient solution, both *F. oxysporum* and *A. alternata* were predominant only in summer. The relative abundance of *F. oxysporum* gradually increased and reached 3.03% by the end of irrigation (Fig. 8e), while the relative abundance of *A. alternata* decreased gradually in the nutrient solution, but it accumulated considerably in the substrate (Fig. 8f). For *P. krajdenii,* it was only predominant in the initial substrate samples (10.98% and 6.42% in summer and winter, respectively), and decreased significantly after seedling growth (Fig. 8g). In the nutrient solution, the abundance of *P. krajdenii* was high only in the Out1 and Out2 samples, and then its abundance decreased rapidly (Fig. 8g), indicating that *P. krajdenii* could not colonize in the nutrient solution.

Discussion

In this study, through the Illumina sequencing of *16S rRNA* and *ITS1* genes, the bacterial and fungal community dynamics in the recirculating nutrient solution were monitored for the tomato plug seedlings cultivated in an ebb-and-flow system. The indigenous microbes in the irrigation water is one of the resources of the microbes in nutrient solution [7]. Moreover, in this study, both the microbial number and diversity increased immediately after the first irrigation cycle (Figs. 1 and 2), indicating that the substrate might be the primarily source of microbial transfer to the nutrient solution. This finding was consistent

with the previous reports that the microbe propagules can be leached from the substrate in pots and transported by the recirculated irrigation water [27]. This conclusion was also confirmed by the NMDS analysis, which revealed that Out1 sample was clustered more closely to the substrate group than the In1 sample (Fig. 4). For the nutrient solution, the input and output samples seemed to become more similar with recirculating, and no significant difference could be detected between the bacterial and fungal communities in the input and output sample groups (Fig. 3).

The bacterial and fungal communities in the nutrient solution were further analyzed (Figs 5–8). *Pseudomonas* was the most predominant bacterial genera in the nutrient solution; its relative abundance gradually increased in summer but decreased in winter with the recirculating irrigation. In summer, the predominant genera also included *Sediminibacterium, Comamonas, Acinetobacter* and *Nevskia.* And of them, *Sediminibacterium* gradually increased, and the others decreased. While in winter, the bacterial genera *Sphingomonas, Spirosoma,* and *Sphingobium* gradually increased, and the genera *Curvibacter* and *Zoogloea* decreased (Fig. 5). The fungal genera in the nutrient solution included *Amanita, Rhodosporidium, Rhodotorula, Alternaria, Fusarium,* and *Trichoderma.* Among these genera, *Amanita* gradually increased in summer but decreased rapidly in winter, whereas *Trichoderma* accumulated rapidly in both summer and winter, and *Alternaria* and *Fusarium* were onlypresent predominantly in summer (Fig. 7). These genera compositions were only partially consistent with previous findings [28–30]. which was explained by the reason of the unique growth substrate and irrigation water used in this study. Also, the compounds in nutrient solution, plant root exudates and dead plant materials may also affect microflora in ebb-and-flow systems. Additionally, the microbial communities in the nutrient solution showed different compositions and change patterns in summer and winter. Specifically, the environmental parameters in the greenhouse and nutrient solution temperature might be the reasonable explanation for the seasonal variations.

One of the concerns of closed ebb-and-flow system is the risk of pathogen attacks. Phytopathogens can easily grow due to high nutrient concentrations and then they can ruin the entire crop through rapid spreading in solution circulation system [2,28]. In this study, although the tomato plug seedlings did not show any disease symptoms, some potentially pathogenic microbes were indeed detected in the nutrient solution and substrate samples. *P. mediterranea,* a pathogen responsible for pith necrosis, was detected predominantly in winter, with decreasing in the nutrient solution but increasing in the substrate. Pith necrosis is a disease of tomato that causes severe crop losses [31,32]. Additionally, some potentially pathogenic fungi including *F. oxysporum* and *A. alternate,* were increased considerably in the nutrient solution or substrate samples. Of them, *F. oxysporum* can cause *Fusarium* wilt in tomato, and *A. alternate* is responsible for many tomato diseases, e.g., stem canker, black mold rot, leaf spot, early blight, and black shoulder disease [33–35]. We also identified *P. krajdenii,* a pathogenic fungus causing petri disease in grapevine [36]. However, *P. krajdenii* was only predominant in the initial substrate and output solution samples, indicating that *P. krajdenii* might be unable to colonize in an ebb-and-flow system of tomato plug seedlings cultivation. The presence of these potentially pathogenic microbes in the ebb-and-flow system indicated the necessary for the disinfection of nutrient solutions.

Several effective methods, such as heat treatment, ozonization, and ultraviolet radiation, have been proposed for the disinfection of nutrient solutions, and have been proven to disinfect nutrient solution effectively in tomato and cucumber plants [28,37–39]. Unfortunately, these commonly used disinfection methods affect the total microflora by destroying not only the target pathogens, but also nontarget microorganisms [20,28]. Finding methods that eliminate pathogens without destroying the natural microflora has become a major challenge. In fact, natural microorganisms have often shown a certain ability to suppress diseases, and microbial inoculation methods are less disruptive of the microflora [37]. In this study, during tomato plug seedlings cultivation, some potentially beneficial bacteria and fungi candidates were also detected (Figs. 6, 8). For example, *P. vancouverensis* was identified predominantly in this system, despite only in winter. It has been reported that inoculation of *P. vancouverensis* in tomato and red pepper plants could improve plant growth and induce resistance to chilling and salinity stresses [40,41]. Another bacterium candidate was *T. mixta,* which can function as an active polysaccharide degrader [42]. Here, *T. mixta* in the nutrient solution and substrate could facilitate the carbon utilization of plants and other microorganisms. However, *T. mixta* is strictly aerobic [42], which could partially explain why this bacterium was present only in the substrate and output solution samples. Furthermore, many plant growth-promoting fungi were also detected. For example, two *Trichoderma* species, *T. virens* and *T. harzianum* were identified to be accumulated. *T. virens* is an avirulent symbiont with the ability to control plant disease [43,44], and *T. harzianum* can improve the biomass and nutrient uptake, and confer resistance to the soil-borne pathogen *Rhizoctonia solani* in tomato plants by producing the secondary metabolite harzianic acid [45,46]. The increasing abundance of *T. virens* and *T. harzianum* would contribute to the health and rapid growth of the tomato plug seedlings. In addition, we found that *P. inflatum* could accumulate in the tomato plug seedling substrate. *P. inflatum* is a foliar-isolated endophytic fungus and functions as a defensive symbiosis against the root-knot nematode and hemipteran sucking bugs in cotton [47,48]. In addition to these plant-growth promoting microbes, some bacteria used in sewage treatment, including *C. testosteroni* [49–51], *S. xenophaga* [52,53] and *N. oligotropha* [54,55] were identified. These bacteria would be the indigenous bacteria in irrigation water and then colonized in the nutrient solution, and would aid in the self-purification of the recirculating nutrient solution. Especially for *N. oligotropha,* which showed the ammonia-oxidizing activity even when the temperature was below 7°C [55], it was found to be increased significantly only in winter, and might provide a good candidate during winter growth season.

Conclusion

In conclusion, the distribution of bacteria and fungi showed obvious dynamic changes in the recirculating nutrient solution and substrate in an ebb-and-flow system for tomato plug seedlings growth. The results indicated that the irrigation water and substrate might be the primary sources of bacteria and fungi transfer to the nutrient solution. The environmental conditions could have influenced the bacterial diversity in different seasons. Furthermore, we particularly focused on the changes of some potentially pathogenic and beneficial microorganisms. The relative abundances of pathogenic fungi such as *F. oxysporum* significantly increased in both recirculating nutrient solution and substrates, and the

pathogenic bacterium *P. mediterranea,* was also detected predominantly in winter, indicating that disinfection of the nutrient solution was necessary for recycling. Additionally, some beneficial bacteria and fungi, such as *S. xenophagum, T. harzianum,* and *T. virens,* could accumulate gradually in the recirculating nutrie1nt solution and substrate. These microbes could function in plant-growth promotion and water self-purification. The comprehensive characterization of the bacterial and fungal dynamics in a recirculating ebb-and-flow system will lay a foundation for further research and applications in tomato plug seedlings cultivation.

Methods

Cultivation of the tomato plug seedlings

Tomato (*S. lycopersicum* cv. “Zhongza 105”) seeds were purchased from China Vegetable Seed Technology Co. Ltd. (Beijing). The seeds were immersed in deionized water for 6 h at room temperature, disinfected in a 5% NaClO solution for 10 min, washed with sterile water five times, and then germinated at a constant temperature of 28°C. Seeds with uniform sprouting were selected and sown in the 105-well plug trays containing a commercial substrate mixture (peat: vermiculite: perlite = 3:1:1, v/v) at a depth of 1.0 cm. Each plug hole contained one seed and was covered with vermiculite after sowing. The plug trays with the sown seeds were placed in a simplified ebb-and-flow seedling plate (1,515 mm × 560 mm × 40 mm, L×W×H). The experiments were performed in a greenhouse at the Institute of Vegetables and Flowers from June 18 to July 16, 2017 (summer) and from November 5 to December 17, 2017 (winter). In the greenhouse, the temperature was reduced in summer using a sunshade net with a fan and wet curtain system, and in winter, the temperature was increased using a heater. The environmental parameters during the seedling period were monitored in real time with a “Wenshiwawa” system (GIS–4-CE–3V, NERCITA, Beijing). Before use, the seedling plates, plug trays, and nutrient solution reservoirs were disinfected (2% NaClO for 30 min), after which they were flushed with sterile water and then allowed to air dry.

During seedlings cultivation, the water-soluble “20–10–20” fertilizer (Wintong, Shanghai) containing 20% N, 10% P_2O_5, and 20% K_2O was used as the nutrient solution. In each irrigation, the nutrient solution (input) was pumped from a reservoir into the seedling plates to a height of 2 cm and then remained for 10 min. Subsequently, the output nutrient solution was collected back into the nutrient solution reservoir. After adding additional water and fertilizer to the defined volume and electrical conductivity (EC) value, the output nutrient solution was reused as the input solution for the next irrigation cycle. In summer and winter, seven and 11 irrigation cycles were performed, respectively (Additional file 10). Three biological replicates performed in three independent ebb-and-flow systems were included in the experiment.

Counting of the culturable bacteria

The input and output solutions from each irrigation cycle were collected and filtered through three layers of sterile gauze. Culturable bacteria in the solutions were counted as described in a previous report [56]. Colony-forming units (CFUs) of culturable bacteria were estimated by the dilution plate method using nutrient agar medium (NA; 1% peptone, 0.3% beef extract, 0.5% NaCl, 1.5% agar, pH 7.2) and are expressed as averages of five replicate plates for each duplicate sample with 95% confidence intervals.

For the substrate, the initial samples were collected before sowing, and the final samples were collected at 27 days post sowing (dps) and 46 dps in summer and winter, respectively. At these timepoints, 20 tomato plug seedlings were randomly selected and the root substrates were collected. After being evenly mixed, 5 g of the substrates was collected and placed in a flask containing 50 mL of sterile water. After incubating at 28°C with shaking at 160 rpm for 2 h, the extraction solution was collected and filtered through three layers of gauze. The number of culturable bacteria in the extraction solutions was estimated by the dilution plate method using the NA medium. In addition, 5 g of each substrate sample was dried in the oven at 80°C until reaching a constant weight. The dry weight (DW) of each substrate was recorded, and the number of culturable bacterial CFUs in each gram of substrate was calculated (CFU/g DW).

PCR amplification of 16S rRNA and ITS1 for Illumina sequencing

During each irrigation cycle, 1 L of each input and output nutrient solution was collected and filtered through a 0.22-μm filter (Millipore Corp., Bedford, MA). The filter membrane was then minced using a pair of sterile scissors, and the genomic DNA was extracted using an E. Z. N. A.® Water DNA kit (OMEGA, Georgia, USA) according to the manufacturer's instructions. Genomic DNA from the substrate samples was extracted using a Fast DNA™ Spin kit for soil (MPbio, CA, USA). The extracted genomic DNA was purified using a TIANquick Midi Purification kit (TIANGEN, Beijing), and the DNA concentration was measured using a NanoDrop™ One instrument (Thermo Scientific, MA, USA). DNA integrity was examined via 1% agarose gel electrophoresis.

From the extracted genomic DNA samples, the V3-V4 region of the 16S rRNA gene and fungal ITS1 gene was amplified with primer pair 341F (5'-CCTAYGGGRBGCASCAG–3')/806R (5'-GGACTACHVGGGTWTCTAAT–3') and ITS5–1737F (5'-GGAAGTAAAAGTCGTAACAAGG–3')/ ITS2–2043R (5'-GCTGCGTTCTTCATCGATGC–3'), respectively, including the barcode sequences for a Multiplex Identifier tag. PCR amplification was performed with an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s, with a final incubation at 72°C for 5 min. The amplifications were confirmed by electrophoresis on a 2% agarose gel. PCR amplicons were then purified individually with a GeneJET Gel Extraction Kit (QIAGEN, Hilden, Germany) and then pooled together in equal amounts. The sequencing library was generated using an Ion Plus Fragment Library Kit (Thermo Fisher Scientific, USA) following the manufacturer's instructions and quantified with an Agilent Bioanalyzer 2100 system. Illumina sequencing was performed

by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China), using the Ion S5^{TM} XL system (Thermo Fisher Scientific) with the generation of 2 × 250 base pairs (PE250). The sequencing data have been deposited in the NCBI Sequence Read Archive (PRJNA563805).

Sequencing data analysis

The reads were assigned to each sample according to the unique barcodes. The raw sequences were trimmed off the adapter at the ends of the sequences and primer sequences and quality filtered using Cutadapt (v1.9.1) and UCHIME software packages [57,58]. Subsampling was performed using the "single_rarefaction.py" module of QIIME (v1.7.0), to account for variation in read depth across samples [59], at a minimum sampling depth. The subsampled reads were clustered to the OTUs at 97% identity using the UPARSE pipeline [60]. Taxonomies were assigned to each OTU using the RDP Naïve Bayesian Classifier [61] with custom reference databases. OTUs whose RDP classifications did not match their expected taxonomic kingdoms (bacteria and fungi) were removed.

Also, the subsampled OTU table was used for downstream alpha- and beta-diversity analyses. The diversity index (Shannon) was calculated using the Mothur program [26]. The intrasample rarefaction curves were generated using a resampling without replacement approach [62]. Distances were calculated using the "vegdist" function of the package "vegan" for Bray-Curtis. Nonmetric multidimensional scaling (NMDS) was performed using "vegan" packages [63]. The major microbial player analyses and graphics were performed using the average relative abundance and relative frequency of each OTU in three replicates.

Statistical analysis

All of the data are expressed as the mean ± SD. Statistical analysis was carried out using the software package SPSS (version 21.0, Chicago, USA). Paired student's *t*-tests were calculated for all pairwise comparisons, and *P* values were adjusted using the FDR correction for multiple testing.

Abbreviations

CFU: colony forming unit; dps: days post sowing; DO: dissolved oxygen content; DW: dry weight; EC: electrical conductivity; ITS: Internal Transcribed Spacer; NA: nutrient agar medium; NCBI: National Center for Biotechnology Information; NMDS: nonmetric multidimensional scaling; NS: nutrient solution; OTU: operational taxonomic unit; PCR: polymerase chain reaction; rRNA: ribosomal RNA; SRA: Sequence Read Archive.

Additional Files

Additional file 1: Table S1. Statistical analysis of the 16S rRNA sequencing data in the samples collected in summer.

Additional file 2: Table S2. Statistical analysis of the 16S rRNA sequencing data in the samples collected in winter.

Additional file 3: Table S3. Statistical analysis of the ITS1 sequencing data in the samples collected in summer.

Additional file 4: Table S4. Statistical analysis of the ITS1 sequencing data in the samples collected in winter.

Additional file 5: Figure S1. Rarefaction curves of the microbial communities in the nutrient solution and substrate samples. (a, b) The curves based on bacterial OTUs in summer (a) and winter (b); (c, d) curves based on fungal OTUs in summer (c) and winter (d).

Additional file 6: Table S5. Estimation of α-diversity indexes in the microbial communities in the samples collected in summer.

Additional file 7: Table S6. Estimation of α-diversity indexes in the microbial communities in the samples collected in winter.

Additional file 8: Fig. S2. Distribution of bacterial communities at the phylum level. The recirculating nutrient solution and substrate samples were collected in summer (a) and winter (b).

Additional file 9: Fig. S3. Distribution of fungal communities at the phylum level. The recirculating nutrient solution and substrate samples were collected in summer (a) and winter (b).

Additional file 10: Table S7. The irrigation frequency and fertilizer concentrations for tomato plug seedlings cultivation.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data and materials
The datasets generated and analysed during the current study are available in NCBI Sequence Read Archive (PRJNA563805), or included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions
CJD carried out the experimental design, data analysis, and manuscript writing. QL carried out all experiments and data analysis. LLW collaborated in bioinformatics analysis. QMS participated in the design of the study and in the manuscript editing. All authors have read and approved the final manuscript.

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Figures

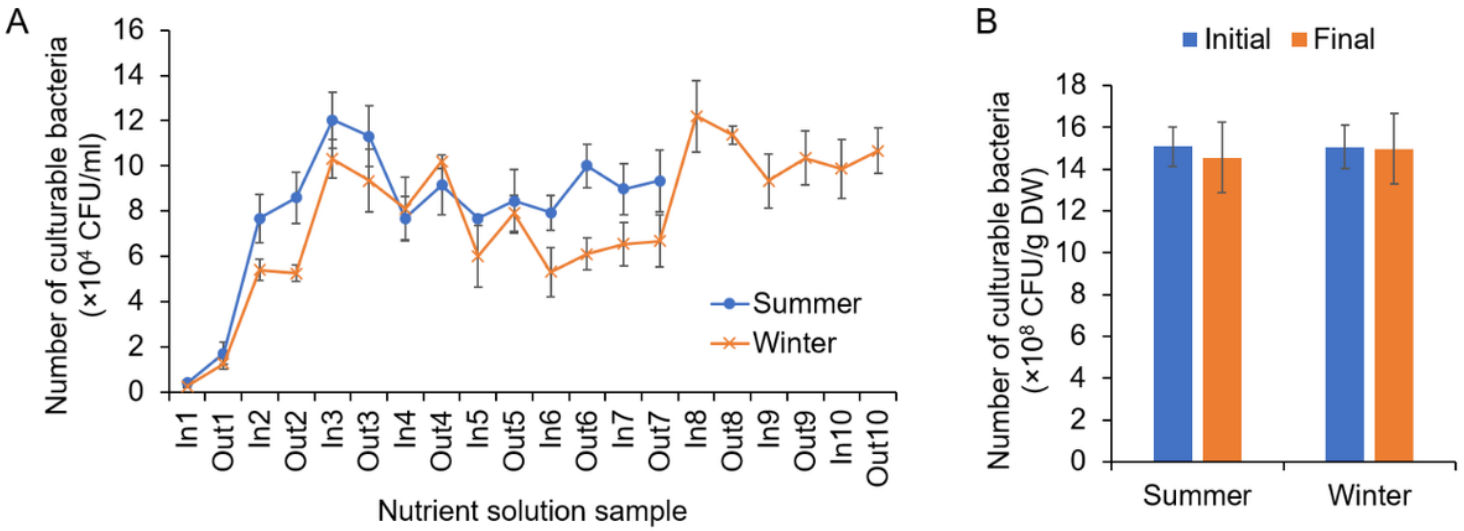

Figure 1

Changes in the number of culturable bacteria in the ebb-and-flow system for tomato plug seedlings. The recirculating nutrient solution (a) and substrate (b) samples were collected in summer and winter.

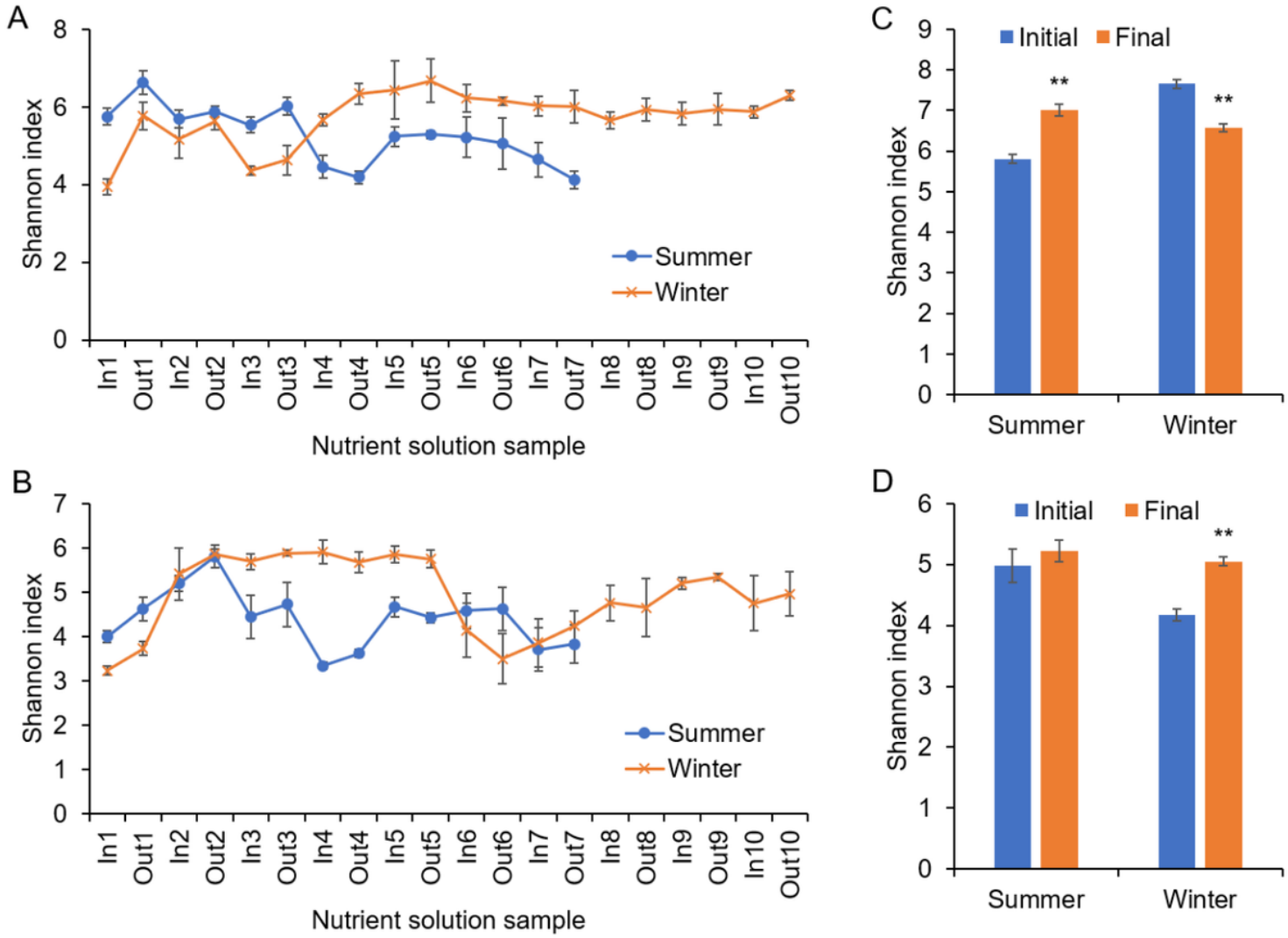

Figure 2

Dynamic changes in the Shannon index of microbial communities in the ebb-and-flow system. (a, b) Shannon indexes of the bacterial (a) and fungal (b) communities in the nutrient solutions. (c, d) Shannon indexes of the bacterial (c) and fungal (d) communities in the substrate samples. **, $P < 0.01$ compared to the corresponding initial values.

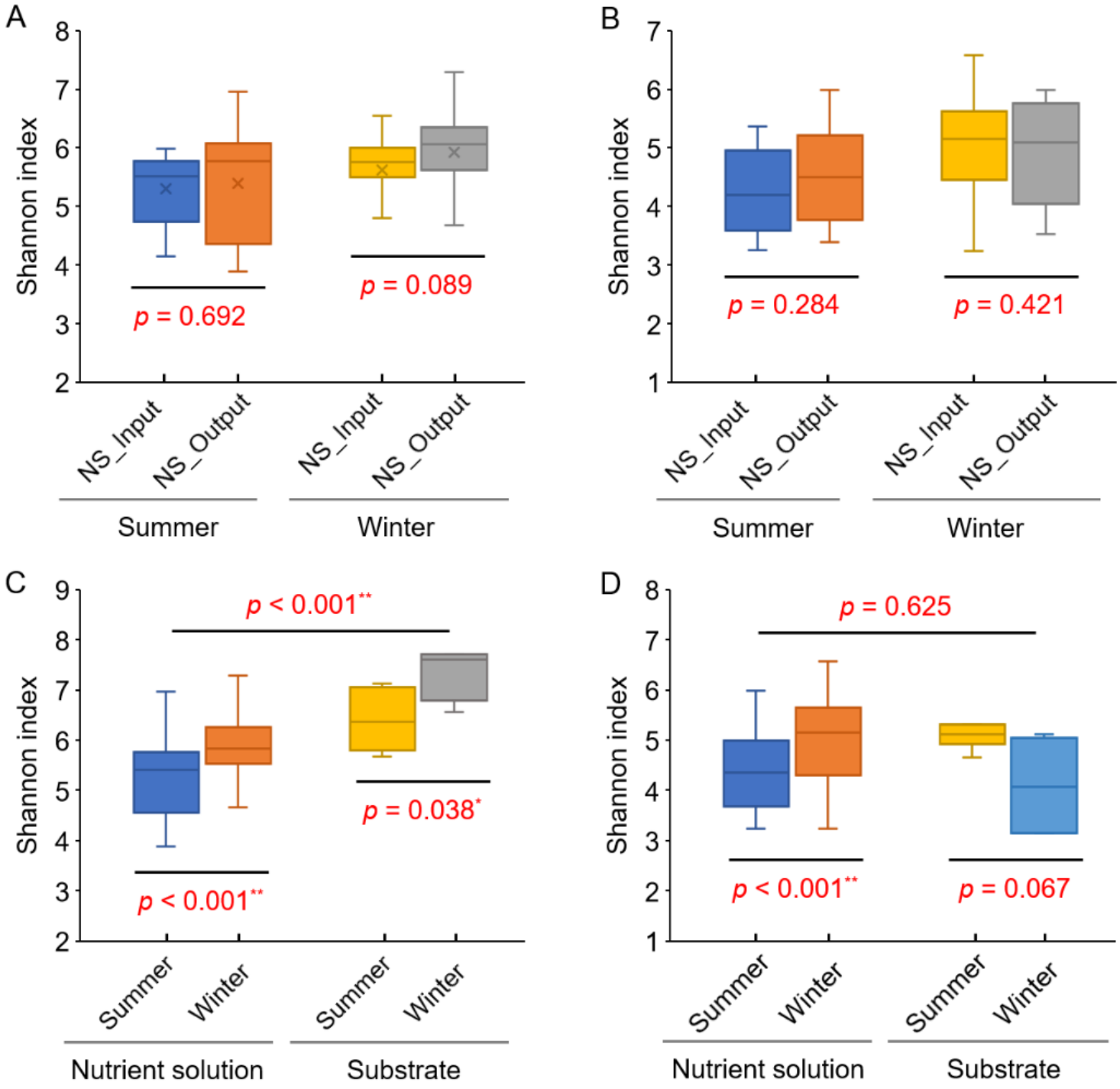

Figure 3

Comparation of Shannon indexes of microbial communities in the nutrient solution and substrate sample groups. (a, b) Comparation of Shannon indexes of bacterial (a) and fungal (b) communities between the input and output nutrient solution sample groups. (c, d) Seasonal variations in the Shannon indexes of bacterial (c) and fungal (d) communities in the recirculating nutrient solution and substrate sample groups.

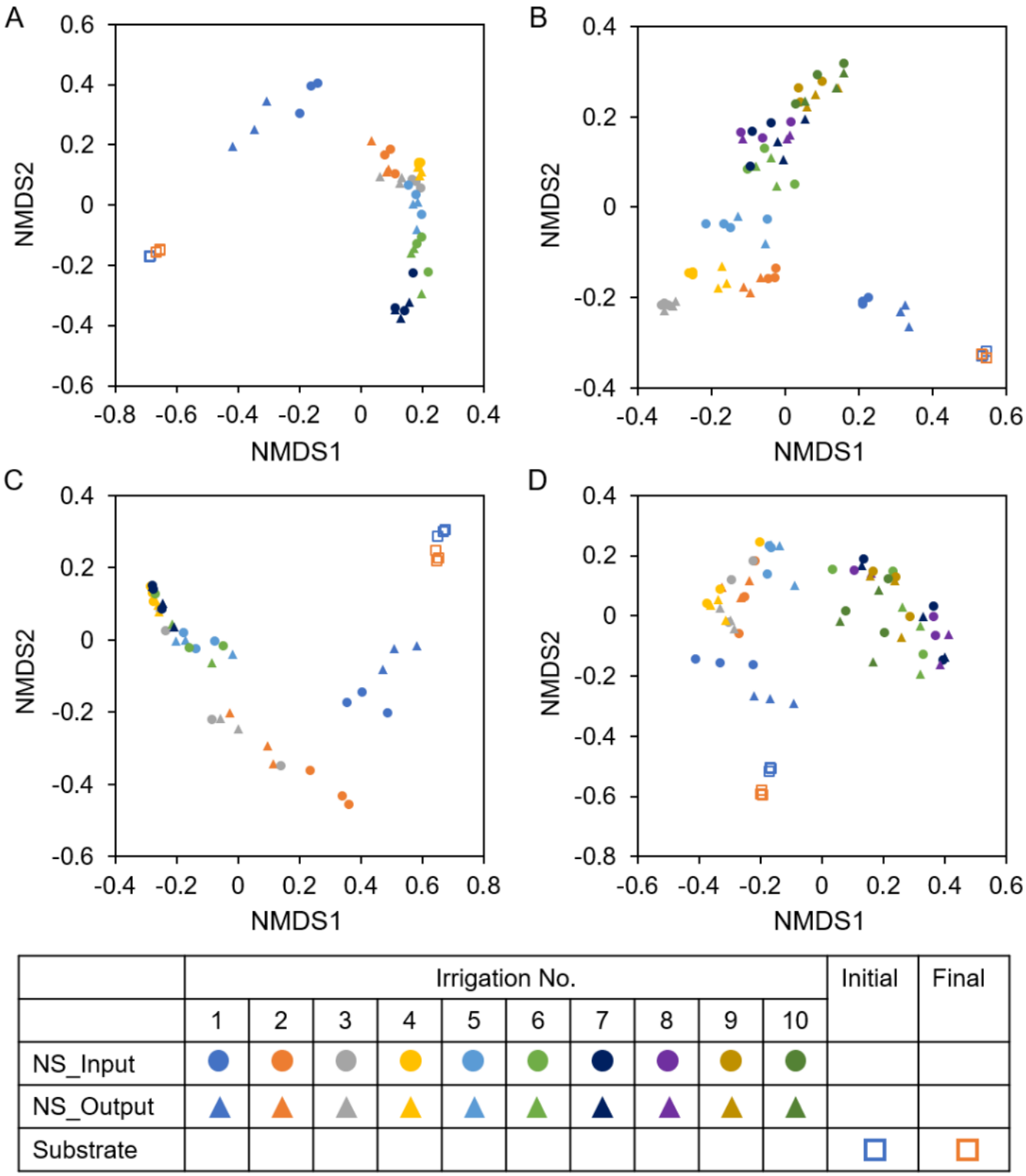

Figure 4

NMDS plots for Bray-Curtis distances of microbial communities in an ebb-and-flow system. bacterial (a, b) and fungal (c, d) communities in the recirculating nutrient solution and substrate samples were compared. Samples from a and c were collected in summer cultivation season, and samples from b and d were collected in winter.

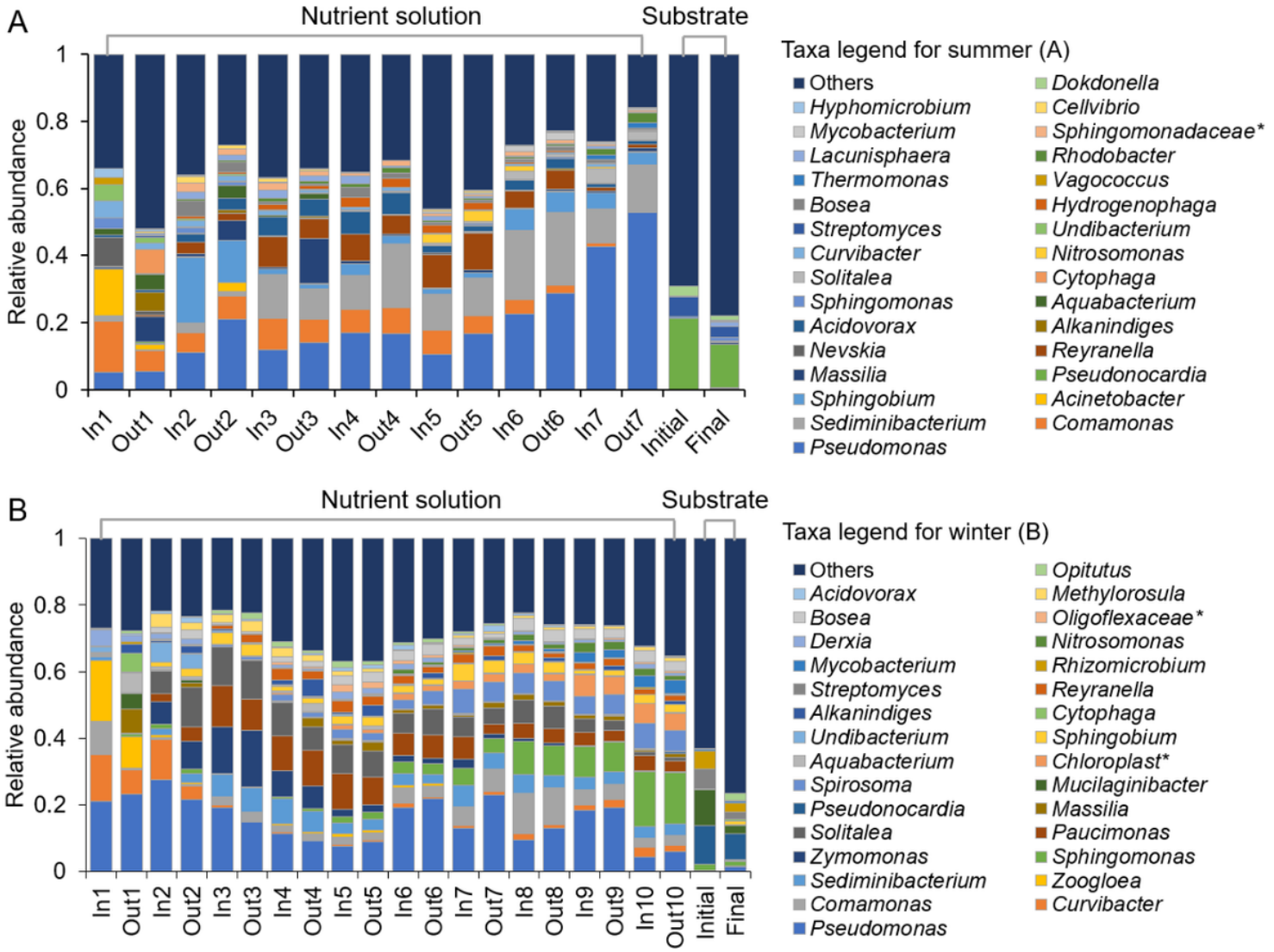

Figure 5

Distribution of bacterial communities at genus level in an ebb-and-flow system for tomato plug seedlings. The nutrient solution and substrate samples were collected in summer (a) and winter (b), respectively. The y-axis shows the relative abundances of the top 30 genera. Asterisks in the legend indicate taxonomic bins containing OTUs that could not be resolved to the genus level.

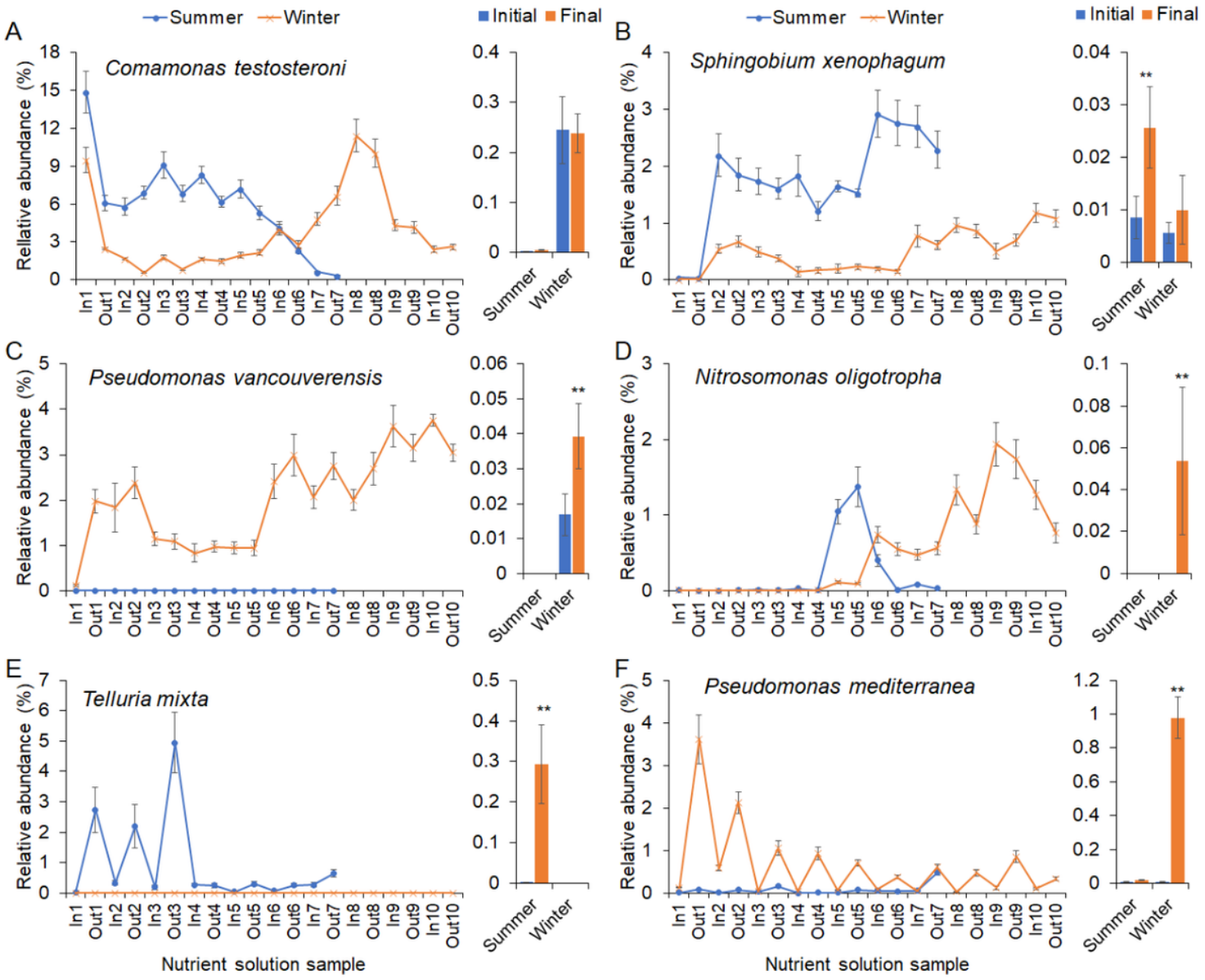

Figure 6

Dynamic changes in six predominant bacteria species in an ebb-and-flow system for tomato plug seedlings. The changing patterns in the nutrient solution and substrate samples were shown with lines and bars, respectively. **, $P < 0.01$ compared to the corresponding initial values.

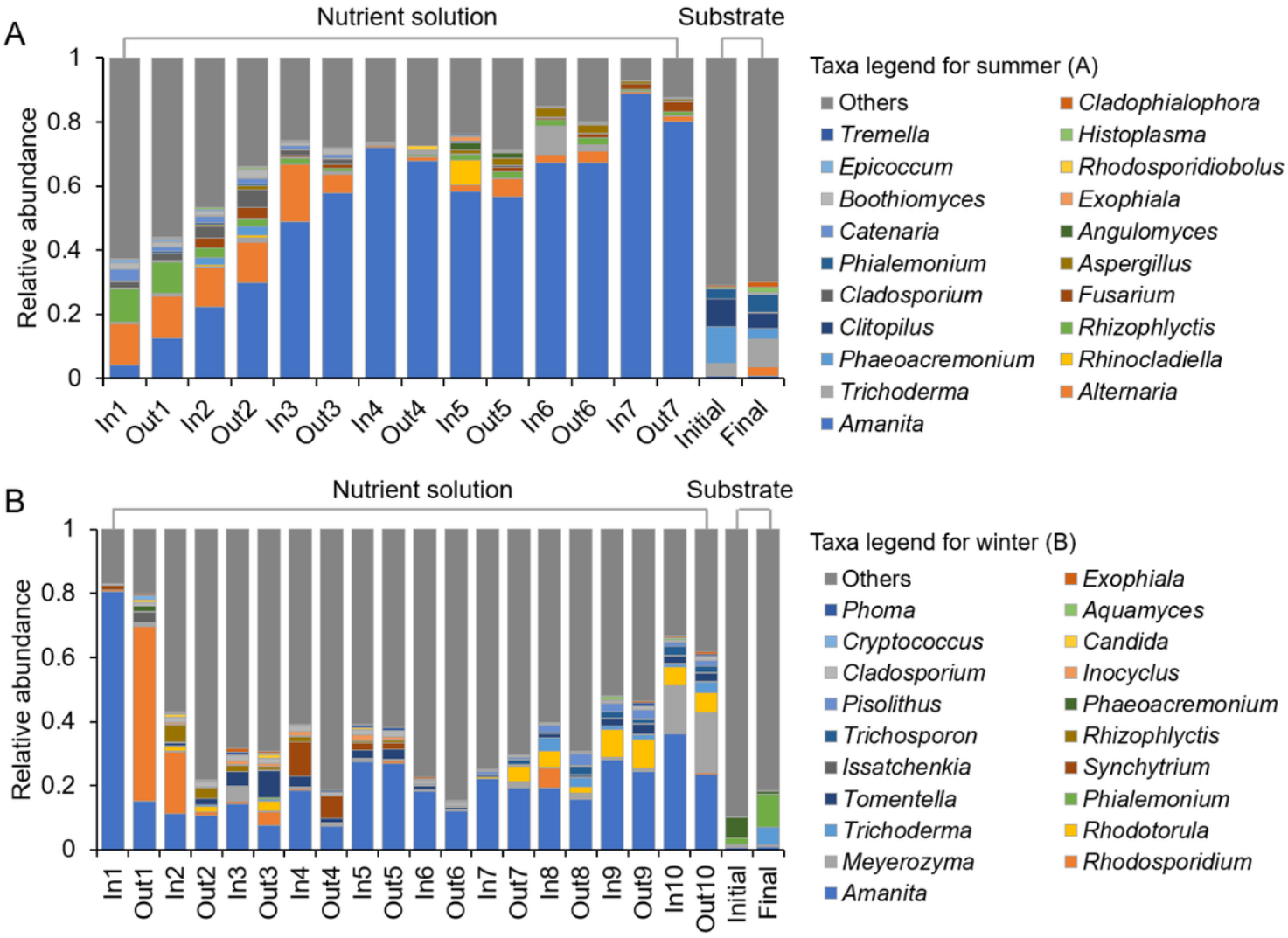

Figure 7

Distribution of fungal communities at genus level in an ebb-and-flow system for tomato plug seedlings. The nutrient solution and substrate samples were collected in summer (a) and winter (b), respectively. The y-axis shows the relative abundances of the top 20 genera.

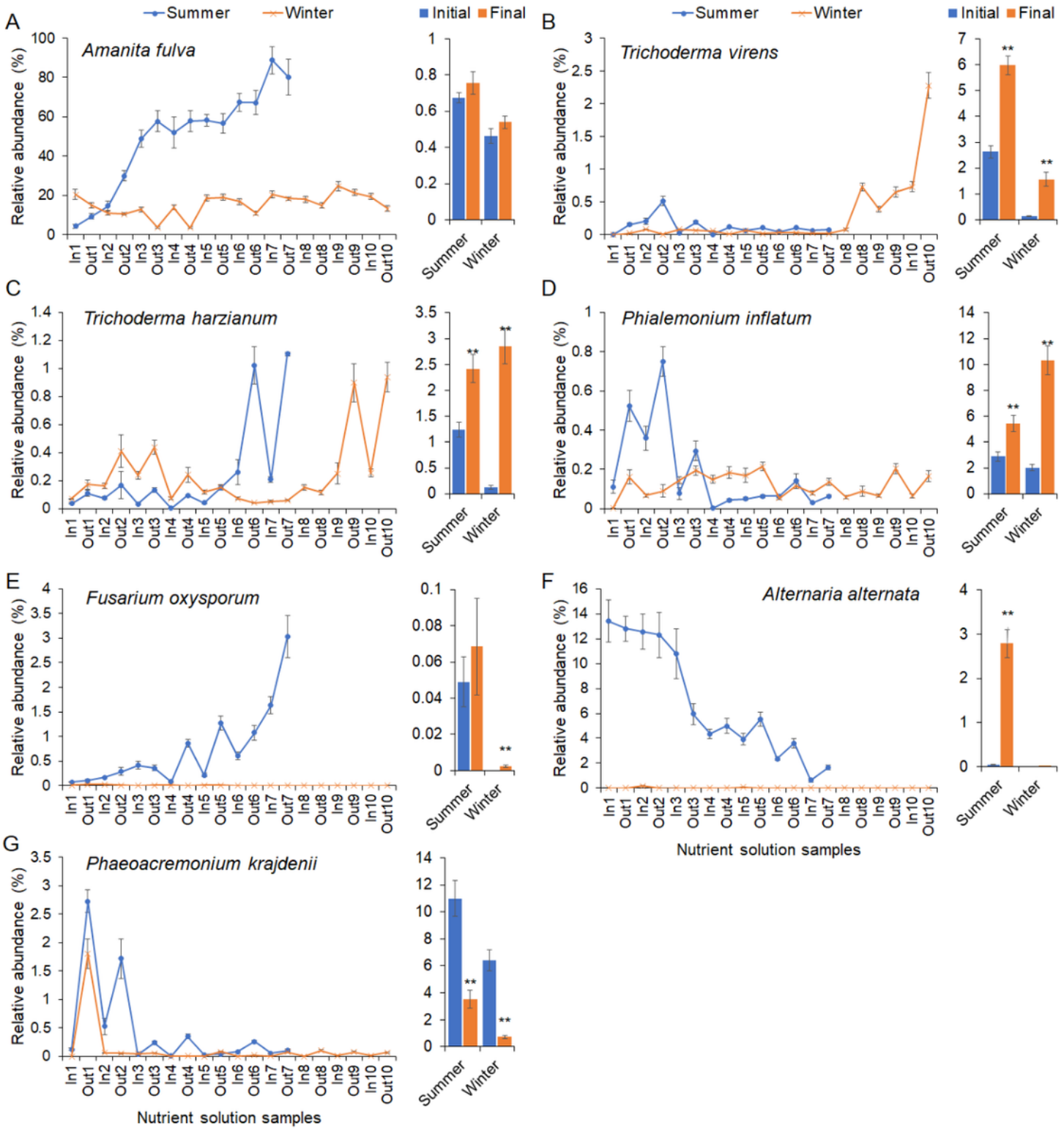

Figure 8

Dynamic changes of seven predominant fungi species in an ebb-and-flow system for tomato plug seedlings. The changing patterns in the nutrient solution and substrate samples were shown with lines and bars, respectively. **, P < 0.01 compared to the corresponding initial values.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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