

三、研究計畫內容（以中文或英文撰寫）：

(一) 研究計畫之背景。請詳述本研究計畫所要探討或解決的問題、重要性、預期影響性及國內外有關本計畫之研究情況、重要參考文獻之評述等。如為連續性計畫應說明上年度研究進度。

BACKGROUND

Reciprocal interactions between plants and soil microbes, known as plant–soil feedbacks (PSF), are receiving increasing attention as a process shaping the structure of plant communities (Bever *et al.*, 2010, van der Putten *et al.*, 2013). Different plants condition the soil microbial community differently, causing the prevalence of parasitic and mutualistic soil microbes to vary among host plants (Bever *et al.*, 2012, Hu *et al.*, 2018). These plant species-specific compositional shifts in the soil microbial community will feed back on the growth of nearby plant individuals and new individuals that colonize the location (Bever *et al.*, 1997, Bever, 2003). As plants also vary in their response to soil microbial communities, plant–soil microbe interactions can modify the original performance differences among plants and thereby alter the competitive dynamics (Ke & Miki, 2015, Ke & Wan, 2020), richness (Johnson *et al.*, 2012, Eppinga *et al.*, 2018), relative abundance pattern (Klironomos, 2002, Mangan *et al.*, 2010), and assembly dynamics (Fukami & Nakajima, 2013) of the plant community.

While known to be plant-specific, a common assumption is that the development and decay of PSF happens rapidly and the strength of plant–soil microbe interactions remain constant throughout the plant’s lifespan (Kardol *et al.*, 2013). Following this premise, empirical studies usually quantify feedback strengths via short-term greenhouse experiments (Kulmatiski & Kardol, 2008), and the measured feedback strengths are usually incorporated in theoretical models as time-independent parameters (e.g., Fukami & Nakajima, 2013, Bauer *et al.*, 2015, Teste *et al.*, 2017). Such an approach operates on the assumption that microbial dynamics equilibrate instantaneously compared with the dynamics of their plant counterparts (i.e., a static PSF perspective; Fig. 1A). However, studies on microbial succession dynamics (Knelman *et al.*, 2012) and dormant strategies (Shade *et al.*, 2012, Lennon *et al.*, 2021) have challenged this typical assumption. Given that plant individuals often arrive at different times and die at different ages in the field, understanding how microbial communities and plant–soil microbe interactions vary over time is critical for predicting the consequences of soil microbes for plant community assembly (i.e., a dynamic PSF perspective; Fig. 1B).

Moving forward, studies have started to investigate the temporal development of PSF. That is, how does the strength of plant–soil microbe interactions vary with the duration of soil conditioning (i.e., the development trajectory; Fig. 1B). For example, the relationship between soil conditioning length and plant–soil microbe interactions has been investigated in the context of plant invasion, showing how the benefit of enemy release attenuates with longer resident time (Diez *et al.*, 2010, Dostál *et al.*, 2013, Day *et al.*, 2015, Speek *et al.*, 2015). The importance of soil conditioning length has also been studied in the context of successive planting in agricultural systems, demonstrating intensified negative microbial effects with

increased rounds of planting (Mazzola, 1999, Packer & Clay, 2004). More recently, studies have focused on changes in plant–soil microbe interactions over the lifespan of a plant individual (Lepinay *et al.*, 2018, Ke *et al.*, 2021). For example, in previous work we used aerial photos to reconstruct a chronosequence of soil conditioning and showed that the development trajectory varies between plant functional groups (Ke *et al.*, 2021).

However, to fully understand the temporal dimension of PSF, we also need to study its decay trajectory, i.e., what successional changes do soil microbial communities undertake and how long do their effects on plant performance persist after the death of their host plant (Fig. 1B)? This question is important because uncolonized sites resulting from plant death often remain empty for an extended period before the arrival of the next individual. Our recent theoretical study highlights the importance of the decay trajectory (Ke & Levine, 2021). In particular, if soil microbial communities decay rapidly following plant death, even host-specific pathogens, microbes once thought to promote coexistence via the Janzen–Connell hypothesis (Janzen, 1970, Connell, 1971), lose their ability to maintain plant diversity. However, the decay trajectory remains empirically unstudied because current experimental designs

often immediately transplant seedlings into conditioned soils after the conditioning phase. Moreover, the characteristics of the decay trajectory may depend on the length of soil conditioning. While few studies have examined this aspect of plant–soil microbe interactions in agricultural systems (Esch & Kobe, 2021, Esch *et al.*, 2021), to the best of our knowledge no studies have characterized the decay trajectory in natural plant communities.

Here, we propose a three-year project aiming to study the decay trajectory of plant–soil microbe interactions and the interactive effects of conditioning and decay time on plant community assembly. We propose to conduct this study at Fushan Forest Dynamic Plot, a typical submontane broadleaf forest in Taiwan. As we will elaborate later, Fushan is an ideal field site for this project because previous studies have established four full forest inventories and recent years an unique fine-scale annual mortality survey has been implemented. Given that preparing soils where host plants had died for different number of years (i.e., different positions along the decay trajectory) is often infeasible in greenhouse experiments, here we will use Fushan’s survey data to reconstruct the decay trajectory (see Ke *et al.*, 2021 for an successful example of this chronosequence approach). With an unique combination of high-

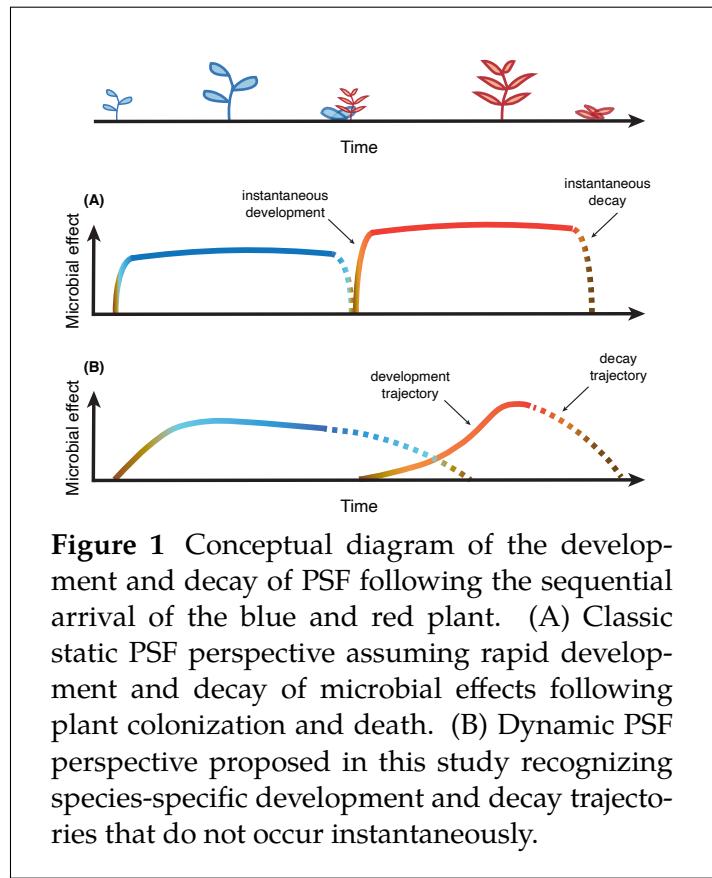


Figure 1 Conceptual diagram of the development and decay of PSF following the sequential arrival of the blue and red plant. (A) Classic static PSF perspective assuming rapid development and decay of microbial effects following plant colonization and death. (B) Dynamic PSF perspective proposed in this study recognizing species-specific development and decay trajectories that do not occur instantaneously.

throughput sequencing, greenhouse experiments, and theoretical modeling, we believe the proposed study has great potential to comprehensively address the temporal dimension of plant–soil microbe interactions.

RESEARCH GOAL

The goal of this proposal is to develop a thorough understanding of the dynamics of plant–soil microbe interactions during its decay process. Despite the current broad interest in how plant–soil microbe interactions influence plant communities, their temporal development and decay trajectories remain rarely studied. This knowledge gap constitutes the three research questions in this proposal:

- (1) What is the successional dynamics of microbial communities after plant death? Do microbial communities regress back to unconditioned states following the same trajectory as their development, or do they undergo different successional dynamics that depend on the length of soil conditioning?
- (2) How do microbial effects vary throughout the decay trajectory after plant death? Do microbial effects become more neutral given longer time after the death of previous host individuals, or do their directions change as microbial communities take on different successional dynamics after plant death?
- (3) How do decay rates of microbial effects influence plant community dynamics? Does accounting for multiple microbial community stages during the decay process improve our predictions on plant competitive outcome and plant community assembly processes?

SIGNIFICANCE

Plant–soil feedback is a sub-field in ecology that integrates botany, microbiology, soil science, and biogeochemistry, with great application value for agriculture, forestry, and restoration (van der Putten *et al.*, 2013). The proposed study is important and novel because of the following three reasons. First, understanding the temporal dimensions of plant–soil microbe interactions is critical for predicting how soil microbes influence plant communities in their natural context (Gundale & Kardol, 2021). Specifically, to better predict the soil environment that a new arriving seedling faces, one should study how plant–soil microbe interactions vary with the length of soil conditioning and decay. The novel experimental setup proposed here provides as the opportunity to fulfill this goal. Second, we propose to study this topic with a unique combination of methods, thereby providing a comprehensive view of the temporal dimensions of plant–soil microbe interactions. In particular, we employ high-throughput sequencing, a greenhouse experiment that preserves individual soil properties, and different ecological models to study the temporal dimension of plant–soil microbe interactions. We go even further by combining a chronosequence approach with short-time longitudinal data to validate our prediction (Walker *et al.*, 2010, Meiners *et al.*, 2015). Finally, in the literature of plant–soil feedback, most studies are from either grasslands or

temperate forests (Crawford *et al.*, 2019); few studies are conducted in subtropical forests and local research in Taiwan remain scarce. However, understanding how plant–soil microbe interactions vary through time is of great importance for Taiwan’s subtropical forest because natural disturbances (e.g., typhoons) occur frequently, providing new sites for plants to colonize and for microbial effects to operate (Zee & Fukami, 2015, Nagendra & Peterson, 2016). Under such scenario, it is even more critical to study how these microbial effects decay following natural disturbance and how their decay influence the recovery trajectory of plant communities (Ke & Levine, 2021).

(二) 研究方法、進行步驟及執行進度。請分年列述：1.本計畫採用之研究方法與原因。2.預計可能遭遇之困難及解決途徑。3.重要儀器之配合使用情形。4.如為須赴國外或大陸地區研究，請詳述其必要性以及預期效益等。

RESEARCH PLAN

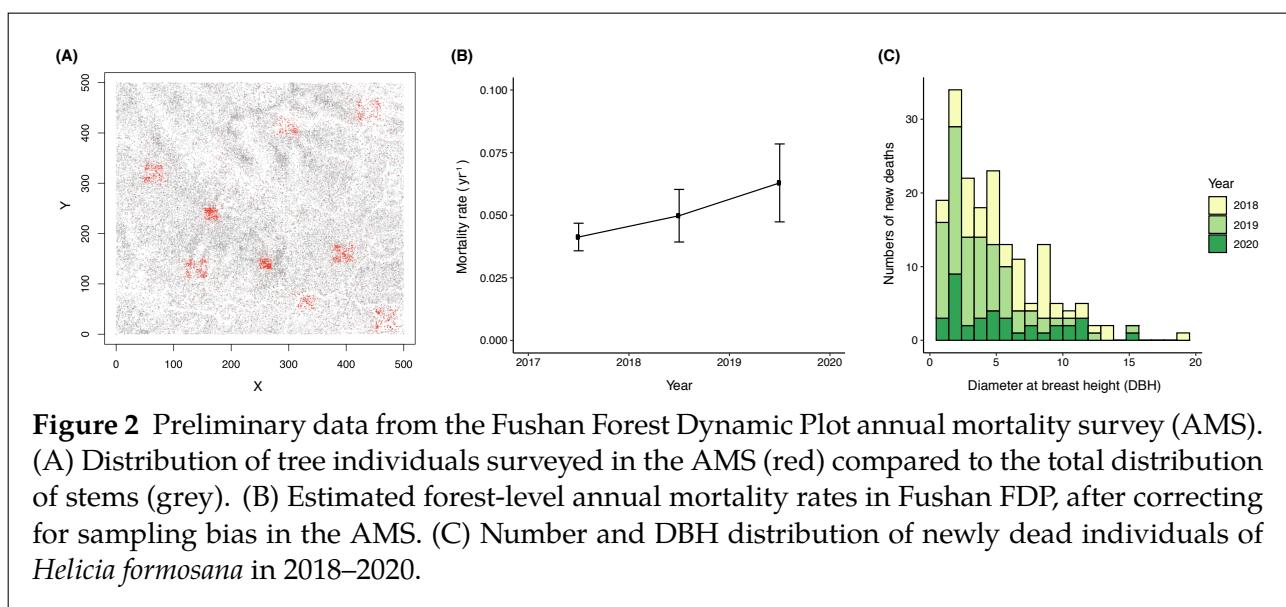
Field site and target species

We will conduct this study in the 25-ha Fushan Forest Dynamics Plot (FDP; 24°45'N, 121°35'E) in northeastern Taiwan. Fushan FDP was established in 2004, following the Smithsonian’s Forest Global Earth Observatory (ForestGEO) standardized protocol. Fushan FDP ranges in elevation from 600 to 750 m, with a climate strongly influenced by typhoons in summer and the northeastern monsoon in winter. The forest has an annual mean temperature of 18.2°C, mean annual precipitation of 4270 mm, and mean relative humidity of 95.1% (Peereman *et al.*, 2020). The vegetation type of Fushan FDP is a submontane evergreen broadleaf forest, with a total of 114,508 individuals (110 woody species from 39 families) being recorded in the first census in 2004 (Su *et al.*, 2007, Chang-Yang *et al.*, 2013). The most abundant species, in terms of the total number of individuals, are: *Blastus cochinchinensis* (Melastomataceae), *Helicia formosana* (Proteaceae), *Engelhardtia roxburghiana* (Juglandaceae), *Pyrenaria shinkoensis* (Theaceae), and *Castanopsis cuspidata* (Fagaceae), with the first two species accounting for 34.3% of the total abundance. Since its first survey in 2004, to date Fushan FDP has completed three additional full surveys (i.e., in 2009, 2014, and 2019). A more detailed description of Fushan FDP can be found in Su *et al.* (2007).

In addition to being a representative subtropical forest in Taiwan, we plan to conduct this research at Fushan FDP because it is one of the eight ForestGEO plots around the world where a series of annual mortality and damage survey (AMS) were conducted. Since 2017, the AMS assessed and followed the fate of a subset of 5,492 stems with DBH (i.e., diameter at breast height) ≥ 1 cm (Fig. 2A); the mode and cause of individual death were also recorded and this monitoring program is expected to continue for five years until 2022. Preliminary analysis suggest that the annual mortality rate at Fushan FDP was $5.15\% \text{ yr}^{-1}$ across 2017–2020, a value higher than other tropical forests in ForestGEO’s network due to natural disturbances such as typhoons (Chang Yang C.-H., *personal communication*; see

also Fig. 2B). This extremely valuable dataset, along with the four full surveys, provide the unique opportunity to study the decay rates of plant–soil microbe interactions.

In this study, we will focus on three species at Fushan FDP: *B. cochinchinensis* (柏拉木), *H. formosana* (山龍眼), and *E. roxburghiana* (黃杞). We selected these three species as they are the most abundant species at Fushan FDP and thrive in the subcanopy. Moreover, based on the AMS, the three species have high numbers of newly dead individuals each year, a necessary criterion for studying the effects of decay time. For example, across 2017–2020, on average 59 individuals of *H. formosana* died each year; the wide DBH range of these dead individuals allowed us to study the interactive effects of conditioning time and decay time (Fig. 2C; see also later section). Finally, the three species are chosen as they belong to different mycorrhizal groups. In particular, *B. cochinchinensis* forms association with arbuscular mycorrhizal fungi (Gurmessa *et al.*, 2019), *E. roxburghiana* is ectomycorrhizal (Haug *et al.*, 1994), whereas *H. formosana* is non-mycorrhizal (Owen, 2013).



Individual selection and soil sampling

As we are interested in the interactive effects of conditioning and decay time on plant–soil microbe interactions, we will select dead individuals as soil source based on: (1) how long did the individual conditioned nearby soil before it died and (2) how long has the individual died. With a close inspection of the survey data, for all three species we will select multiple plant individuals that fall into each combination of conditioning time \times decay time.

For the duration of soil conditioning by plant individuals, we will use tree size (i.e., DBH) as a proxy for individual age. To clearly demonstrate the potential effects of conditioning time, within each species we will select individuals with the smallest and largest DBH to represent individuals that had imposed short and long conditioning effects, respectively. Qualitatively separating individuals into two extreme size/age classes, while seemingly arbitrary, increases our confidence in using DBH to indicate the duration of soil conditioning. We caution that given the innate differences in species' size, the DBH cutoff

for small and large individuals would differ between the three species. Based on available individuals within the AMS dataset, we consider *H. formosana* individuals with DBH < 3.5 cm as young individuals (with short conditioning time) and those with DBH > 7.5 cm as old individuals (with long conditioning time). For *B. cochinchinensis*, individuals with DBH < 1.1 cm are considered as young individuals and those with DBH > 1.8 cm are considered as old individuals. For *E. roxburghiana*, the DBH threshold for young and old individuals is DBH < 10 cm and DBH > 20 cm, respectively.

To study the decay of plant–soil microbe interactions, we will calculate the number of years since the plant individual died. Based on available data from the full survey and AMS, we will classify individuals into five groups based on their year of death: (1) 2009–2014 (i.e., died 7 to 12 years ago), (2) 2015–2017 (i.e., died 4 to 6 years ago), (3) 2018–2019 (i.e., died 2 to 3 years ago), (4) 2020–2021 (i.e., died last year or less than a year), and (5) plant individuals that are still alive during the start of this project (i.e., still alive in late 2021).

For *H. formosana* and *B. cochinchinensis*, we will select 10 individuals as replicates within each combination of conditioning × decay time; however, only 5 individuals per combination will be selected for *E. roxburghiana* due to limited number of individuals in the AMS dataset. We will prioritize selecting dead individuals whose nearby soil are not densely recolonized by other understory species. In total, we will select 250 individuals (Table 1). For each selected plant individual, we will use a sterilized soil core sampler to collect soil from three different locations beneath the individual. The three samples will then be homogenized in a sterile plastic bag to create a representative soil sample for that specific individual (in total about 300 mL). Finally, we also plan to collect soil samples from 20 locations that are near a random subset of selected individual but not colonized by any of the three species; these soils represent “naive” soils without a conditioning history by the three species and will serve as controls. All soil samples will be stored at 4°C before being processed back in the lab. Each soil sample will be passed through a sterilized disposable sieve and further homogenized thoroughly in a sterile plastic bag. We will subsample from the collected bulk soil to characterize microbial communities and soil abiotic properties (Question 1), and the rest of the collected soil will be used in a greenhouse experiment (Question 2); information gained from these two research questions will be used for model development (Question 3).

Table 1 Number of sampled individuals (in total 250 individuals) belonging to each conditioning time (i.e., plant size as a proxy of age) × decay time (i.e., year of death) combination.

	Alive	Year of individual death			
		2021-2020	2019-2018	2017-2015	2014-2009
<i>Helicia formosana</i>					
short conditioning (DBH <3.5)	10	10	10	10	10
long conditioning (DBH >7.5)	10	10	10	10	10
<i>Blastus cochinchinensis</i>					
short conditioning (DBH <1.1)	10	10	10	10	10
long conditioning (DBH >1.8)	10	10	10	10	10
<i>Engelhardtia roxburghiana</i>					
short conditioning (DBH <10)	5	5	5	5	5
long conditioning (DBH >20)	5	5	5	5	5

Q1: What is the successional trajectory of microbial communities after plant death?

(1-1) Hypothesis

We hypothesize that the microbial community would progressively change over time, becoming more and more different from unconditioned state with longer duration of soil conditioning (i.e., development trajectory, Fig. 1; see also Fig. 3). When the host plant individual dies, the microbial community will go through a decay trajectory, for which we hypothesize the following three different patterns.

- Q1. H_0 : Microbial communities regress back to unconditioned states following the same pathway as their development trajectory, independent to how long the previous host plant conditioned the soil (Fig. 3A). This hypothesis represents the case where plant death results in the lost of host-specific microbes, therefore the microbial community composition regresses back to the unconditioned state.
- Q1. H_1 : Microbial communities undergo successional trajectories that are different from their development trajectory, independent to how long the previous host individual conditioned the soil (Fig. 3B). This hypothesis represents the case where the death of plant individuals triggers alternative microbial succession trajectories (e.g., litter input causes the increase of saprotrophs and the decrease of symbiotrophs).
- Q1. H_2 : There is interaction between conditioning time and the decaying trajectory, such that the decaying trajectory depends on how long the previous plant conditioned the soils (Fig. 3C). This hypothesis represents the case where only the death of old individuals (with long conditioning time) can trigger alternative decay trajectories, whereas microbial communities associated young individual regress to unconditioned state following the same development trajectory.

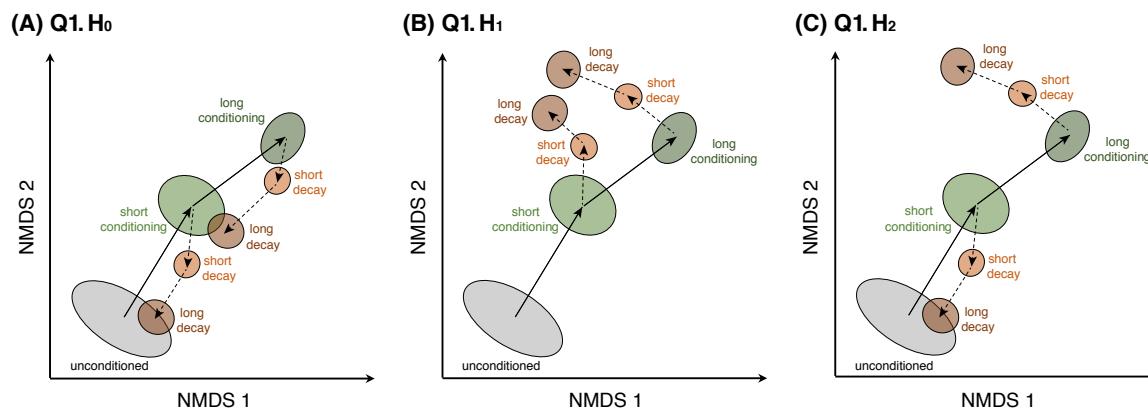


Figure 3 Expected results based on different hypothesis for Q1, visualized with NMDS plots of microbial community composition (for simplicity, only the response of one plant species is shown). Ellipses represent SE among samples that belong to the same conditioning × decay time combination. Solid arrows linking green ellipses depict the community development trajectory, whereas dashed lines linking brown ellipses depict the community decaying trajectory after the death of host plant individuals. Darker green and brown colored ellipses represent longer duration of conditioning and decay, respectively.

(1-2) Approach: Next generation sequencing

We will characterize fungal and bacterial communities from the 0.25 g soil subsampled from the 270 field-collected soil samples. We will extract microbial DNA with PowerSoil DNA Isolation Kit (Qiagen) following the manufacturer's protocol. We will amplify the bacterial 16S ribosomal DNA region, with primer pair 515f (5'- GTG YCA GCM GCC GCG GTA A -3') – 806r (5'- GGA CTA CNV GGG TWT CTA AT -3') (Caporaso *et al.*, 2012), and the fungal internal transcribed spacer 1 region (ITS1), with primer pair ITS1-F_KYO1 (5'- CTH GGT CAT TTA GAG GAA STA A -3') – ITS2_KYO2 (5'- TTY RCT RCG TTC TTC ATC -3') (Toju *et al.*, 2012). Here, each primer will be concatenated with 3–6-mer Ns (Lundberg *et al.*, 2013) and an Illumina sequencing primer region, resulting in a fusion primer for our PCR reactions (forward: 5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG – [3–6-mer Ns] – [515f or ITS1-F_KYO1] -3'; reverse: 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G – [3–6-mer Ns] – [806r or ITS2_KYO2] -3').

We will conduct PCR reactions in two steps. Following previous experience, although absolutely open to necessary adjustments, the first PCR process will contain 3.2 μ L of MQ water, 5 μ L of DNA polymerase mastermix, 0.4 μ L of each primer (10 μ M for both forward and reverse primer), and 1 μ L of extracted DNA (i.e., in total 10 μ L). We plan to run the reactions at 95°C for 2 min, followed by 36 cycles of 95°C for 20 sec, 52.5°C for 16S (or 50°C for ITS1) for 20 sec, 72°C for 50 sec, and a final extension at 72°C for 10 min. After this first PCR process, we will run a subsequent second PCR for sample identification. The fusion primers used in this second PCR concatenates P5/P7 Illumina adaptors, 8-mer index sequences, and the sequencing adaptor (Hamady *et al.*, 2008) (forward: 5'- AAT GAT ACG GCG ACC ACC GAG ATC TAC AC – [8-mer tag] – TCG TCG GCA GCG TC -3'; reverse: 5'- CAA GCA GAA GAC GGC ATA CGA GAT – [8-mer tag] – GTC TCG TGG GCT CGG -3'). We will run this second PCR for 8 cycles with the same protocol as our first PCR but with an annealing temperature of 50°C for both bacterial and fungal amplicons. After a purification/equalization process, 5 μ L of PCR product will be taken from each sample to create a pooled library, which will be sequenced using the Illumina MiSeq sequencer (2 \times 300 cycle sequencing kit).

The raw Illumina MiSeq sequencing reads will be processed with the Claident pipeline (Tanabe & Toju, 2013). In short, we will convert raw Miseq BCL data into FASTQ data, demultiplex sequences, remove low quality reads, and then fuse forward and reverse reads using PEAR (Zhang *et al.*, 2014). Low quality reads, potentially chimeric, and noisy reads will be eliminated with UCHIME (Edgar *et al.*, 2011). Sequencing reads that passed through all filtering processes will then be clustered into Operational Taxonomic Units (OTU) using VSEARCH Rognes *et al.*, 2016, with a cutoff sequence similarity of 97%. After clustering, we will assign taxonomy to the OTUs using the RDP Naive Bayesian rRNA Classifier (Wang *et al.*, 2007) trained on either the 16S rRNA training set 16 for bacteria or the Warcup Fungal ITS training set 2 (Deshpande *et al.*, 2016) for fungi. Based on the taxonomic assignment results, incorrect Kingdom classifications as well as statistically-identified potential contaminant OTUs will be removed (Davis *et al.*, 2018). If necessary, we will also perform bioinformatics using the DADA2 pipeline (Callahan *et al.*, 2016) and use Amplicon Sequence Variants (ASVs) instead of OTUs. However, we expect minor differences as studies have shown that general ecological patterns remain robust to this decision (Glassman & Martiny, 2018).

(1-3) Analysis and expected results

To compare compositional differences among microbial communities belonging to different conditioning time \times decay time combinations, we will use non-metric multidimensional scaling (NMDS) to ordinate microbial communities based on Bray-Curtis dissimilarities. Effects of different conditioning time \times decay time combinations will be tested with permutational multivariate analysis of variance (PERMANOVA). Based on this analysis, Q1. H_0 would be supported if the microbial composition becomes more similar to that of the unconditioned soils with longer time since the death of the host plant individual (Fig. 3A); Q1. H_2 would be supported if the microbial composition does not converge to that of the unconditioned soil but instead becomes progressively different with longer time since the death of the host plant individual (Fig. 3B); Q1. H_3 would be supported if the decaying trajectory depends on how long the previous plant conditioned the soils (Fig. 3C).

Question 2: How do microbial effects vary across the the decay trajectory?

(2-1) Hypothesis

We hypothesize that soil microbes have a negative effect on plant performance during the development trajectory of microbial communities, a common pattern observed in natural systems (Kulmatiski *et al.*, 2008, Crawford *et al.*, 2019). Moreover, we expect such negative effects to aggravate with longer plant conditioning length (see also Ke *et al.*, 2021). After the death of host plant individuals, we hypothesize the following three patterns for microbial effects during the decay trajectory.

- Q2. H_0 : Microbial effects regress from having a negative impact on plant performance to having a weak neutral impact. For soils with longer conditioning time, their microbial effect follow the same pattern but attenuate at a slower rate (Fig. 4A). This hypothesis corresponds to Q1. H_0 (Fig. 3A), where microbial communities regress back to unconditioned states following their development trajectory.
- Q2. H_1 : Microbial effects change from having a negative effect to having a positive effect (Fig. 4B). This hypothesis corresponds to Q1. H_1 (Fig. 3B), where litter input from dead plants triggers alternative microbial succession trajectories dominated by saprotrophs that facilitate nutrient release from litter, thereby creating a positive microbial effect. We also hypothesize soils with longer conditioning time will reach stronger positive effects due to greater litter input.
- Q2. H_2 : There is interaction between soil conditioning time and the changes in microbial effects after plant death (Fig. 4C). This hypothesis corresponds to Q1. H_2 (Fig. 3C), where the microbial community associated with old individuals go through alternative decay trajectories but that associated with young individuals regress back to unconditioned states.

(2-2a) Approach: Greenhouse experiments

To examine how changes in the soil microbial community may affect plant performance, in the second year of this proposal we will use field-collected soil to conduct a greenhouse experiment. Specifically, we will assess plant performance in soils that differed in their host plant species (i.e., *H. formosana*, *B. cochinchinensis*, *E. roxburghiana*, or unconditioned control),

conditioning time (i.e., two size/age classes), and decay time (i.e., five levels of individual's year of death). Our greenhouse experiment aims at transplanting seedlings of *H. formosana*, *B. cochinchinensis*, *E. roxburghiana* in 170 field-collected soils (i.e., from 20 unconditioned locations and a subset of 150 previously-sampled individuals; 5 individuals from each of the 10 conditioning time \times decay time categories for each of the 3 species). Soils collected from different individuals will be kept separated throughout the experiment, thereby resulting in soils with different combinations of conditioning time \times decay time (Rinella & Reinhart, 2018, Peacher & Meiners, 2020, see also Ke *et al.*, 2021). Half of the soil volume (150 mL) collected from each individual or unconditioned locations will be autoclaved to create a sterilized treatment (120°C for 60 min, sit overnight for 24 h, and another 120°C for 60 min). This soil preparation step will result in 340 unique soil inocula.

Seeds of the three species will be collected from the field prior to the start of this project (winter 2021; Chang Yang C.-H., *personal communication*). Seeds will be surface sterilized by soaking seeds in 5% bleach for 30 sec, 95% ethanol for 30 sec, and rinsing them with DI water for 1 min. We will let the sterilized seeds germinate by spreading them evenly onto germination trays and place them in a growth chamber. After two weeks since germination, we will transplant seedlings individually into 4-inch pots (i.e., one seedling per pot) filled with 700 mL of sterilized potting material and 75 mL of either live or sterilized soil inoculum to the top. This will result in a total of 1020 pots (i.e., 170 field-collected soil \times 2 sterilization treatments \times 3 species). Transplanted pots will be placed randomly in the greenhouse and grow for 6 months, after which we will harvest and oven-dry (70°C for 72 hrs) both aboveground and belowground tissue from each pot. The resulting total dry biomass will be weighted to quantify the effects of soil microbes on plant performance.

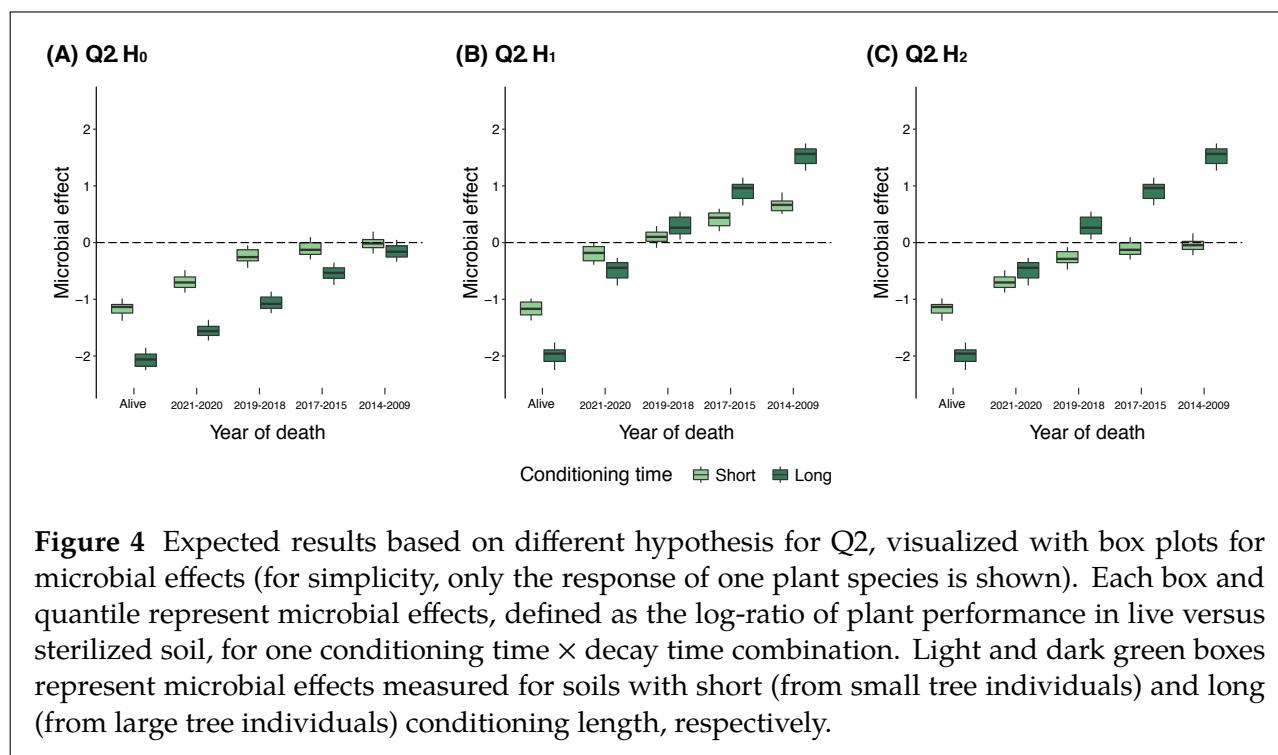


Figure 4 Expected results based on different hypothesis for Q2, visualized with box plots for microbial effects (for simplicity, only the response of one plant species is shown). Each box and quantile represent microbial effects, defined as the log-ratio of plant performance in live versus sterilized soil, for one conditioning time \times decay time combination. Light and dark green boxes represent microbial effects measured for soils with short (from small tree individuals) and long (from large tree individuals) conditioning length, respectively.

(2-2b) Approach: Extending mortality survey

This experiment was made possible because of the mortality data produced by the annual

mortality and damage survey (AMS), as well as the four full surveys conducted at Fushan FDP. To continue this valuable effort, with the support from this grant we will extend the AMS (i.e., another three years beyond its original 2022 deadline) so that future research relying on detailed forest mortality information can be conducted.

(2-2c) Approach: Verifying chronosequence results with short-term time series

The above characterization of microbial communities and corresponding greenhouse experiment rely on a chronosequence approach, i.e., sampling soils from different individuals that died at different years. To strengthen our inference based on the chronosequence approach, we will supplement it with short-term longitudinal data by repeatedly sampling the same chronosequence (Damgaard, 2019). To this end, we will repeat the aforementioned soil sampling and next generation sequencing each year throughout the funding period of this proposal (i.e., 2022–2024); we will also repeat the greenhouse experiment twice (i.e., 2023 and 2024). By doing so, not only will we further extend the chronosequence to quantify ongoing changes e.g., individuals that died in 2009 will be dead for 15 years by 2024), we will also be able to verify our chronosequence-based predictions. For instance, we can compare the microbial community and microbial effects associated with the following two individuals: (1) an individual that died three years ago during our first sampling in 2022 (i.e., died in 2018), and (2) an individual that died three years ago during the our last sampling in 2024 (i.e., died in 2021). If the microbial community and corresponding microbial effects of these two individuals are similar, this will indicate that our chronosequence approach produces informative predictions.

(2-3) Analysis and expected results

We will quantify microbial effects by comparing plant growth response in live soil to that in sterilized soil. By taking the log-ratio, a positive value would represent beneficial microbial effects (i.e., better growth performance in live soil) whereas a negative value would represent a detrimental effect (i.e., worse growth performance in live soil). When plotting the measured microbial effect for each combination of conditioning time \times decay time, Q2. H_0 will be supported if microbial effects become more neutral through time despite different conditioning time (Fig. 4A). On the other hand, Q2. H_1 will be supported if the measured microbial effects change in sign (e.g., from negative to positive; Fig. 4B) after plant death. Finally, Q2. H_2 will be supported if the soil conditioning time affects the pattern that we observed; in particular, we predict that microbial effects will become neutral for soils with short conditioning time but change signs for soils with long conditioning time (Fig. 4C).

Q3: How do the decay rates of microbial effect influence plant community dynamics?

(3-1) Hypothesis

The above two research questions focus on investigating the interactive effects of conditioning time and decay time on microbial community structure (Question 1) and plant performance (Question 2). For the final component of this proposal, we will build ecological models to study the potential long term consequences of such dynamic plant–soil microbe interactions. In particular, we will study how the gradual decay of microbial communities after plant

death, manifested in the models as multiple microbial states during the decay process, affect plant competitive outcome, community convergence pattern, and recovery from disturbance. Guiding this theoretical work are two hypothesis.

Q3. H_0 : Considering multiple microbial community states during the decay process has little impact on plant community structure. This hypothesis represents the common implicit assumption that species' interactions have fixed strength that do not vary through time.

Q3. H_1 : Accounting for multiple microbial community states during the decay process critically affects model predictions, with models incorporating such soil dynamics predicting higher likelihood of plant coexistence, slower community convergence, and more alternative trajectories during community recovery.

(3-2a) Approach: Dynamic modeling

We will build two types of models for this specific research goal. First, using ordinary differential equations (ODEs), we will build novel patch occupancy models to study how species coexistence is affected by the temporal dimensions of plant–soil microbe interactions. The models will be built upon the foundation laid by our previous work (Ke & Levine, 2021). In short, our previous model considered the sequential transition between sites with different plant–soil states and demonstrated how soil conditioning and decay rates can affect plant competitive outcome. It is the first theoretical study that explicitly considers the demographic and temporal context of plant–soil microbe interactions. Although our previous model challenged the typical assumption that soil conditioning and decay happen instantaneously following plant colonization and death, however, it still used over-simplified dynamics to represent the decay process (i.e., a single decay rate parameter). Therefore, we propose to extend our model by considering multiple states of the microbial community during the decay process (informed by our microbial community survey; Question 1), with each state having different impacts on plant performance (informed by our greenhouse experiment; Question 2). We will also extend our model from two species to multi-species, which remains a research frontier in theoretical ecology (Levine *et al.*, 2017), and further incorporate recent developments from the microbial seed bank theory (Lennon *et al.*, 2021) to model the resilience of microbial communities (Shade *et al.*, 2012).

(3-2b) Approach: Individual-based modeling

Second, using individual-based models (IBMs), we will simulate how plant community assembly processes are affected by the temporal dimensions of plant–soil microbe interactions. With collaborators in the USA, we have developed an IBM that can be further expanded for the purpose of this proposal (see also Fukami & Nakajima, 2013, Zee & Fukami, 2015, Ke *et al.*, 2021). In short, the IBM consists of a regional species pool containing multiple plant species (each with a different trait value) and patches consisting of multiple sites (each with a different habitat condition). The IBM simulates ecological processes such as immigration, reproduction, arrival, competition for establishment, and death of plant individuals (Fig. 5A). Competition for establishment at empty sites is determined not only by the match between species' trait values and local site conditions, but also by the soil microbial legacy effects created by the previously established plant. In one previous publication (Ke *et al.*, 2021), we studied how different development trajectories of plant–soil microbe interactions affect

plant community assembly; however, this IBM framework has never been used to study the decay trajectory. Here, we will extensively expand the IBM by setting soil microbial legacy effects to depend on both (1) the previous established individual's age of death and (2) the number of years the site remained empty before the arrival of a new colonizer (Fig. 5B). This will allow us to study how plant community assembly is influenced by different temporal development and decay scenarios of plant–soil microbe interactions.

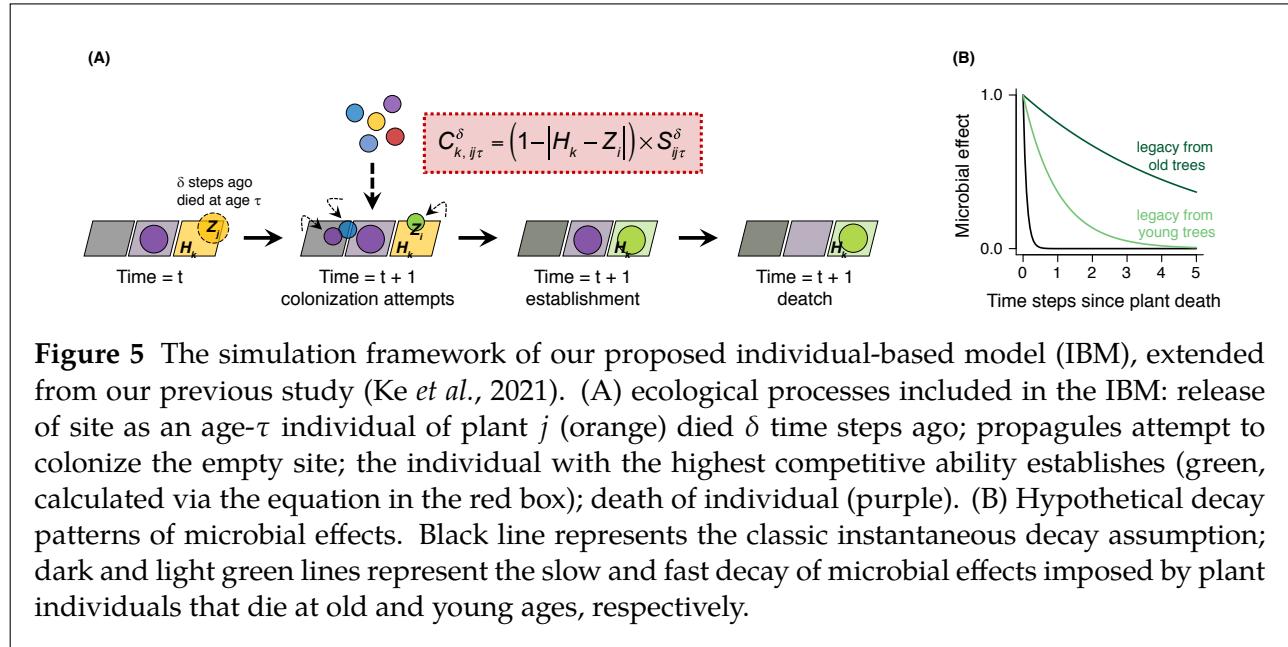


Figure 5 The simulation framework of our proposed individual-based model (IBM), extended from our previous study (Ke *et al.*, 2021). (A) ecological processes included in the IBM: release of site as an age- τ individual of plant j (orange) died δ time steps ago; propagules attempt to colonize the empty site; the individual with the highest competitive ability establishes (green, calculated via the equation in the red box); death of individual (purple). (B) Hypothetical decay patterns of microbial effects. Black line represents the classic instantaneous decay assumption; dark and light green lines represent the slow and fast decay of microbial effects imposed by plant individuals that die at old and young ages, respectively.

(3-3) Analysis and expected results

The goal of our first ODE model is to study how plant competitive outcome and regional-scale relative abundance are influenced by the gradual decay of microbial communities. To this end, we will study how the likelihood of plant coexistence varies with underlying parameter values and model structure. We will first vary the parameters that represent the transition rates among multiple microbial states, and compare corresponding competitive outcome to that obtained from the simplified model with a single decay rate parameter (i.e., Ke & Levine, 2021). We will also study how competitive outcome is impacted when the effects of microbes on plant performance varies across states (e.g., weaker detrimental effects as decay progresses, or even a reverse from detrimental to beneficial effects). Strong support for Q3. H_1 would come from a larger parameter space allowing plant coexistence when the model includes multiple microbial community states during the decay process.

For our second model, the focus would be how community transient dynamics and recovery from disturbance are influenced by the gradual decay of microbial communities. To study community assembly, we will generate 10 patches for the regional species pool to colonize independently and quantify the beta diversity among the 10 patches and its temporal pattern. We will repeat this simulation for 20 replications, where 20 independently created sets of species pools are allowed to colonize the same set of 10 patches (see also Fukami & Nakajima, 2013, Ke *et al.*, 2021). To study the recovery dynamics after disturbance, we will perturb species' abundance after an equilibrium is reached and observe the temporal trajectory of alpha and beta diversity among the 10 patches.

Importantly, we will compare the above patterns under different temporal development and decay scenarios of microbial legacy effects. For the temporal development pattern, where the microbial effects depend on the previous individual's age of death (i.e., τ in Fig. 5A), we plan to consider three scenarios: (1) instantaneous, where microbial effects immediately build up and remain unchanged despite individuals becoming older; (2) magnifying, such that the longer the previous individual lived before it died, the stronger its impact on the new individual; (3) attenuating, where the microbial effect from the previous individual weakens in strength as it dies at older ages. For the temporal decay pattern, where the microbial effects depend on how long the site remained empty after an individual's death (i.e., δ in Fig. 5A), we also plan to consider three scenarios: (1) instantaneous, where microbial effects immediately disappear after one time step (i.e., black line in Fig. 5B); (2) constant decay, such that the longer the site remains empty, the weaker the microbial effect on the new individual; (3) age-dependent decay, where the decay rate of microbial effect depends on the age of death of the previous individual (i.e., an interactive effect of the development and decay trajectories; green lines in Fig. 5B).

POTENTIAL PROBLEMS AND SOLUTIONS

Fushan FDP is a complex system compared to other systems where plant-soil feedback experiments have been performed (e.g., grasslands and temperate forests). Therefore, while we have experience in conducting such an experiment, we anticipate necessary challenges that accompany the implementation of the research program in a new system. One challenge is that plant roots can be intermingled and therefore the soil is actually "co-conditioned" by multiple plants (e.g., understory ferns). The common assumption in the literature is that the tree species would have stronger conditioning effect than other understory plants due to their larger biomass (Grime, 1998, Peltzer *et al.*, 2009). Although this challenge makes it difficult to establish a "one-to-one" pairwise relationship between the plant species conditioning the soil and the plant species receiving the microbial effects, the novelty and rigor of our research program still holds. In particular, the collected soils and experimental design would still faithfully capture the decay process and represent the soil environment experienced by new seedlings, which is the main goal of this proposal. The other challenge is that the variability in soil microbial communities among individuals may be large. We will conduct a small-scale pilot experiment focusing on the decay trajectory of *H. formosana* to assess the underlying variability. If the variability proves to be too large to be characterized with our current design, we will adjust our sample size accordingly (with the potential to drop one species to remain feasible). We have limited ourselves to tree species that are abundant at Fushan FDP so further increasing the sample size should be doable.

SUPPORT FOR MAJOR INSTRUMENTATION

All experiments will be realized at PI Po-Ju Ke's lab at National Taiwan University. With the start up fund provided by the university, the lab has access to greenhouses and computer clusters necessary to conduct the greenhouse experiment and modeling. While the lab has

basic equipment for microbial DNA extraction, we currently borrow equipment for DNA amplification (i.e., polymerase chain reaction, PCR) and amplicon library preparation from the lab of Prof. Chih-Han Chang (located on a different floor). Therefore, in this proposal, we ask for the support to establish our own web lab. In particular, we would like to purchase our own thermal cycler and gel imaging system so that we can conduct PCR in our own lab space throughout the three years supported by this project (see table CM10).

(三) 預期完成之工作項目及成果。請分年列述：1.預期完成之工作項目。2.對於參與之工作人員，預期可獲之訓練。3.預期完成之研究成果（如期刊論文、研討會論文、專書、技術報告、專利或技術移轉等質與量之預期成果）。4.學術研究、國家發展及其他應用方面預期之貢獻。

RESEARCH TASKS TO BE CARRIED OUT EACH YEAR

Table 2 summarizes the research activities that we plan to carry out each year. Briefly, in the first year (2022), we will select tree individuals based on Fushan FPD's survey data (see Table 1). We will collect field soil, characterize the microbial community, perform a small-scale pilot experiment focusing on *H. formosana*, and study the dynamic system model. In year two (2023), we will continue soil sampling and microbial community characterization, perform the full greenhouse experiment, and develop the individual-based model. In year three (2024), we will repeat the soil sampling and the greenhouse experiment to verify the chronosequence approach. Data analysis and manuscript preparation would also be conducted throughout the whole three-year funding period.

Table 2 Shadings represent the expected time when research activities are planned to take place. Note that we will repeat the soil sampling, next generation sequencing of microbial community, and greenhouse experiment for three consecutive years. Light grey shading represents the pilot experiment.

	Year 1 (2022)				Year 2 (2023)				Year 3 (2024)			
	1-3	4-6	7-9	10-12	1-3	4-6	7-9	10-12	1-3	4-6	7-9	10-12
Select individuals	■											
Field soil sampling	■				■				■			■
Mortality survey			■				■					■
Question 1												
Microbial sequencing	■	■			■	■			■	■		
Analysis/writing		■	■			■	■			■	■	
Question 2												
Greenhouse experiment		■	■		■	■			■	■		
Analysis/writing			■			■	■			■	■	
Question 3												
Dynamic system model	■	■										
Individual-based model			■		■	■	■					
Analysis/writing									■	■	■	

TRAINING TO BE GAINED BY PARTICIPATING PERSONNEL

With this funding, each year we will train one research assistant, two Masters students, one Ph.D. student, and several undergraduate students to study plant–soil microbe interactions in forest ecosystems, with a particular emphasis on Fushan FDP. The trainees will learn a broad array of skills, including: field survey for forest dynamics, soil collection, high-throughput sequencing for characterizing microbial communities, preparing and setting up greenhouse transplant experiments, computer programming for theoretical research, and manuscript writing.

ANTICIPATED RESEARCH OUTCOMES

We anticipate the following research outputs over the three years supported by this grant. With high-throughput sequencing, we will construct a valuable time series revealing the successional dynamics of microbial communities following the death of their host plant individual. With the greenhouse experiment, we will better understand how soil microbes mediate the performance of three abundant tree species in Taiwan’s low elevation subtropical forests. The theoretical research will produce a general modeling framework to study the temporal dimensions of plant–soil microbe interactions, which will serve as a starting point for developing similar research programs in other ecosystems. Finally, we will extend Fushan’s annual mortality survey beyond its original deadline to lay the path for future studies that would rely on detailed forest mortality information.

We anticipate to publish at least four publications in top-tier SCI journals: one opinion piece on the temporal dimensions of plant–soil microbe interactions, one for microbial successional dynamics, one for plant growth response from the greenhouse experiment, and one for the theoretical models. We will present our funding in national (e.g., Taiwan’s Congress of Animal Behavior and Ecology) and international (e.g., Annual meeting of the Ecological Society of America) meetings and conferences.

CONTRIBUTION TO ACADEMICS AND NATIONAL DEVELOPMENT

As highlighted at the beginning, plant–soil microbe interaction is a sub-field in ecology that integrates botany, microbiology, soil science, and biogeochemistry, with great application value for agriculture, forestry, and restoration. Testing how soil microbes affect plant communities contributes to our understanding of forest structure and ecosystem functioning (e.g., soil carbon cycling), and can guide effective strategies for restoration and invasion control. Extending the annual mortality survey, as proposed in this proposal, will greatly improve our understanding of mortality agents and the forest’s response to natural disturbances (e.g., typhoon).

Theoretical modeling is a powerful research approach that can benefit many sub-fields of life science; however, very few principle investigators in Taiwan can provide such expertise. With the support from this grant, we can train new scientists with relevant skills

and add a new dimension to existing research projects, all of which can greatly enhance the scientific development in Taiwan. Moreover, we will also attend international conferences to present the results and invite collaborators to visit Taiwan. All of the above activities will increase Taiwan's visibility in the global scientific community and facilitate future international research collaborations.

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