Distinct effects of phyllosphere and rhizosphere microbes on invader *Ageratina adenophora* during its early life stages

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Abstract: Microbes strongly affect invasive plant growth. Interactions between soil microbes and invasive plants have received widespread attention; however, it remains to be determined how phyllosphere and rhizosphere soil microbes affect seedling mortality and the growth of invasive plants across ontogeny under varying soil nutrition levels. In this study, we used the invader *Ageratina adenophora* to evaluate these effects in plant growth chambers. We found that leaf litter harboured more potential pathogens and thus had more adverse effects on seed germination and seedling survival than soil inoculation. Microbial inoculation at different growth stages altered the microbial community and functions enriched in seedlings, and earlier inoculation had a more adverse effect on seedling survival and growth. In most cases, the soil nutrient level did not affect microbe-mediated seedling growth and the relative abundance of the microbial community and functions involved in seedling growth. Some microbial genera have distinct effects on seedling survival from those on growth. Moreover, fungal strains isolated from dead seedlings inoculated with litter exhibited significant phylogenetic signals to seedling mortality, in which strains of *Allophoma* and *Alternaria* caused high seedling mortality. Our study stresses the essential role of *A. adenophora* litter microbes in population establishment by regulating seedling density and growth.

Introduction

Plant-modified soil properties in turn affect the performance of plants, which are termed 'plant–soil feedbacks (PSFs)'. PSFs can affect species coexistence (Bever et al., 1997; van der Putten et al., 2013) and local plant community composition and dynamics (Bauer et al., 2017; Kardol et al., 2007; Teste et al., 2017). For plant invasion, PSFs have positive effects by escaping soil pathogens and recruiting some beneficial microbes (Liu et al., 2023; Mitchell & Power, 2003; Xu et al., 2012) or negative effects by accumulating local pathogens (Callaway et al., 2013; Flory & Clay, 2013; Zhang et al., 2020).

Similar to soil, leaf litter could also affect plant growth, species diversity and community structure (Liu et al., 2017; Ma et al., 2020; Olson & Wallander, 2002), thus playing important roles in population establishment and community dynamics (Jessen et al., 2023; Lamb, 2008; Xiong & Nilsson, 1999). However, related research has focused mainly on physical (e.g., maintaining soil moisture and temperature, increasing nutrition and reducing light) or chemical effects (e.g., releasing allelochemicals) (Demey et al., 2013; Jessen et al., 2023; Möhler et al., 2018; Zhang et al., 2017) but has rarely focused on leaf microbial effects. Until 2017, Whitaker et al. (2017) extended the PSF to aboveground tissues (including leaf, stem and floral tissues), termed "plant–phyllosphere feedbacks (PPFs)", and found that all four Asteraceae species experienced stronger negative PPFs than PSFs. Subsequently, this team further verified that all ten tested Asteraceae plants experienced negative PPFs (Zaret et al., 2021). The lack of strong mutualists and relatively high abundance of pathogens in the phyllosphere may account for the negative PPFs.

In addition to microbial sources (i.e., soil vs leaf litter), ontogeny (seedling growth stage) and soil nutrient levels could affect plant–microbe interactions. For example, seedlings showed distinct sensitivity over the growth stage, and younger seedlings were more susceptible to infection by a single soil microbe because of fewer defense resources (Geisen et al., 2021; Jevon et al., 2020). Interestingly, leaf litter has an adverse

effect on seedling emergence but a positive effect on later plant growth (Abbas et al., 2023; Möhler et al., 2021; Zhang et al., 2022); litter also has a stronger negative effect on earlier vegetation growth than the elder (Loydi et al., 2013; Wang et al., 2022; Xiong & Nilsson, 1999). Moreover, plants enrich distinct microbes under different nutrient conditions and affect plant performance (Dostál, 2021; Gustafson & Casper, 2004; Widdig et al., 2020). For example, the bacterial diversity in duckweed plants was reduced under nutrient–deficient conditions, but the abundance of Firmicutes increased (Bunyoo et al., 2022), and members of Firmicutes have been reported to promote plant stress tolerance (Xu et al., 2018). Ai et al. (2018) reported that nutrient additions caused crops to enrich some bacteria and fungi from soil and increase yield.

Ageratina adenophora (Sprengel) R.M. King & H. Robinson (Asteraceae), known as Crofton weed or Mexican devil weed, has invaded more than 30 countries and areas, including South Africa, Australia, New Zealand, Hawaii, India and China (Gu et al., 2021; Julien & Griffiths, 1998). It is a perennial weed and can produce high yields of seeds with a high germination rate (Lu et al., 2006; Parsons WT, 2001). This weed commonly grows in monoculture, but a high density of seedlings is not common in the wild. Previous studies have shown that *A. adenophora* can enrich the beneficial soil microbial community to facilitate its invasion (Niu et al., 2007; Zhao et al., 2019); in contrast, *A. adenophora* leaves harbor diverse fungal pathogens that can cause adverse effects on itself seed germination and growth (Kai Fang et al., 2019; K. Fang et al., 2021). Thus, it is interesting to ask whether leaf microbes play a distinct role from soil microbes in regulating *A. adenophora* seedling density and whether these effects change with the *A. adenophora* growth stage and soil nutrition level.

In this study, we inoculated *A. adenophora* with soil or leaf litter at three stages, 0 d, 21 d, and 28 d after sowing, and transplanted seedlings to grow in soils with high or low nutrition levels. We first determined the germination, seedling survival and growth of *the A. Adenophora* plants. Then, we characterized the bacterial and fungal communities of the soils and leaf litter as inoculation sources, as well as the microbial

communities enriched in the leaves and roots of the *A. adenophora* seedlings after growing; we also isolated the fungi associated with the dead seedlings and tested their mortality to seedlings. Finally, we correlated the microbial community with *A. adenophora* seedling mortality and growth.

We hypothesized that the microbial community associated with leaf litter and rhizosphere soils can account for the differential effects on *A. adenophora* seedling mortality and growth over growth stages when growing under different nutrition levels. We expected that 1) leaf litter would have more adverse effects on seed germination, seedling survival and growth than soil, as leaf litter often harbours more plant pathogens, and 2) inoculation at different growth stages would change the microbial community enriched by seedlings and thus affect seedling growth. Earlier inoculation has a more adverse impact on seedling growth than later inoculation, as younger seedlings were more sensitive to pathogen infection than older seedlings. 3) The nutrition level influences seedlings to recruit the microbial community and thus affects seedling growth.

Results

Leaf litter and rhizosphere soils had differential effects on seedling mortality and growth of *A. adenophora* over growth stages when grown under different nutrition levels

When inoculated during the G0 period, the leaves significantly delayed germination time more than soil; relatively, the sterile leaves caused a longer germination time than non–sterile leaves (Fig. 1a, both $P < 0.05$). Leaf and soil had no distinct effects on the germination rate (Fig. 1b, $P > 0.05$). In addition, inoculation of sterile and non–sterile leaves at G0 caused a high death rate (19.7% vs 96.7%) for seedlings growing in petri dishes (Fig. 1c, Fig. S1). Only non–sterile leaves caused low seedling death (8.4%) when the seedlings were inoculated at G21 (Fig. 1c). Two weeks after transplanting these seedlings into cups, those seedlings treated by leaf inoculation,

non–sterile sample inoculation, and inoculation at an earlier stage died more than those treated by soil inoculation, sterile sample inoculation, and inoculation at a later stage. Moreover, seedlings grown in high-nutrient soil died more than those grown in low-nutrient soil. (Fig. 1d-f, Fig. S2a). Specifically, the interaction between feedback and microbe or inoculation time treatments but not nutrition level significantly affected the seedling death rate, where inoculation with only non–sterile litter at an earlier stage caused a higher death rate than inoculation at a later stage, regardless of whether the seedlings were grown at a high or low soil nutrient level (all $P < 0.05$, Fig. S2a,b).

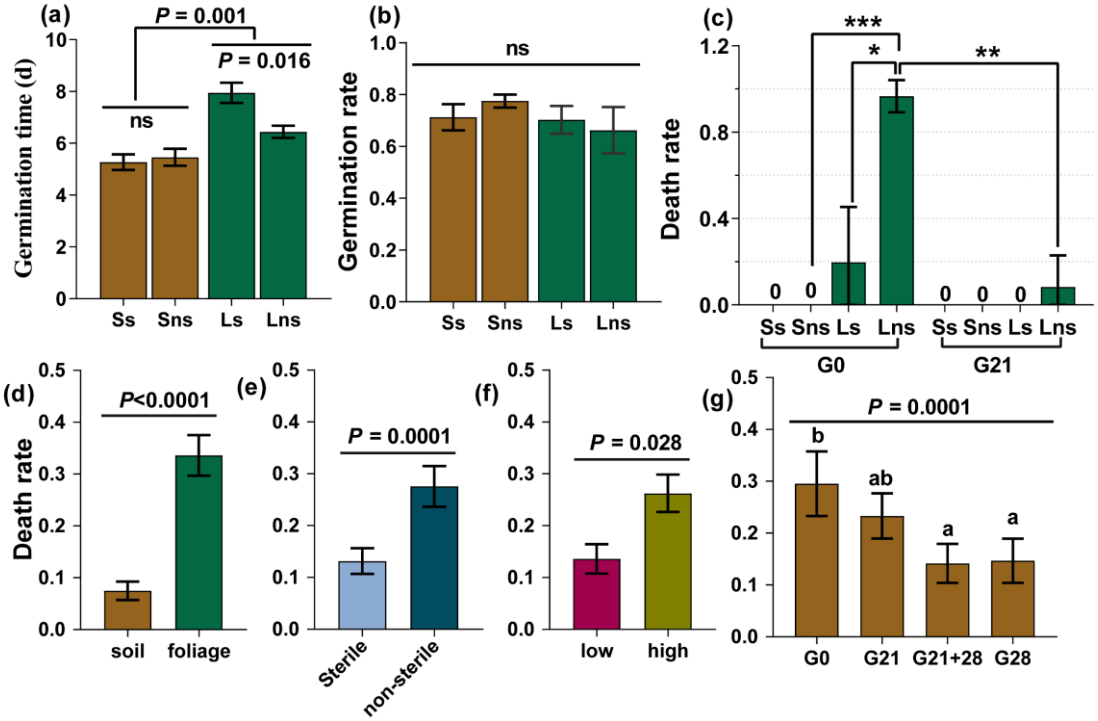

Figure 1. Seed germination and seedling survival of *A. adenophora*. Seed germination time and rate after inoculation of soil or leaves in the G0 period (a-b) and seedling death rate after inoculation of soil or leaves in the G0 and G21 periods and subsequent growth in Petri dishes (c). Seedling death rate after two weeks when seedlings were transplanted into soil under different feedback conditions (d), under sterile or non–sterile inoculation (e), at different nutrient levels (f) and at different inoculation time treatments(g) based on all the treatments. Ss: sterile soil; Sns: non–sterile soil; Ls: sterile leaf; Lns: non–sterile leaf; G0, inoculated on the day of germination; G21, inoculated on the 21st day after germination; G28, inoculated on the 28th day after germination; G21+28, inoculated on both the 21st and 28th days after germination. * $P < 0.05$, *** $P < 0.001$. Error bars depict the standard error.

With the exception of nutrient level, feedback and inoculation time, as well as their interaction with nutrient level, significantly affected the microbial role in the total dry biomass of *A. adenophora* ($P < 0.05$, Fig. 2a-c, S3a). Leaf microbes were significantly more positive for *A. adenophora* growth than soil microbes ($P < 0.0001$, Fig. 2a). The nutrient level did not change the intensity of microbe-mediated seedling growth ($P = 0.068$, Fig. 2b). Moreover, microbes had a significantly negative effect on *A. adenophora* growth at G0 inoculation but had a positive effect at the other three inoculation time ($P < 0.0001$, Fig. 2c). Microbial effects on seedling biomass interact with soil nutrient level and inoculation period: when inoculated at G21 period, both soil and leaf inoculation showed a more positive effect under high nutrition than under low nutrition; when inoculated at G21+28 period, leaf inoculation produced a more positive effect under low nutrition than under high nutrition but soil inoculation was a contrast effect (Fig. S3a,b).

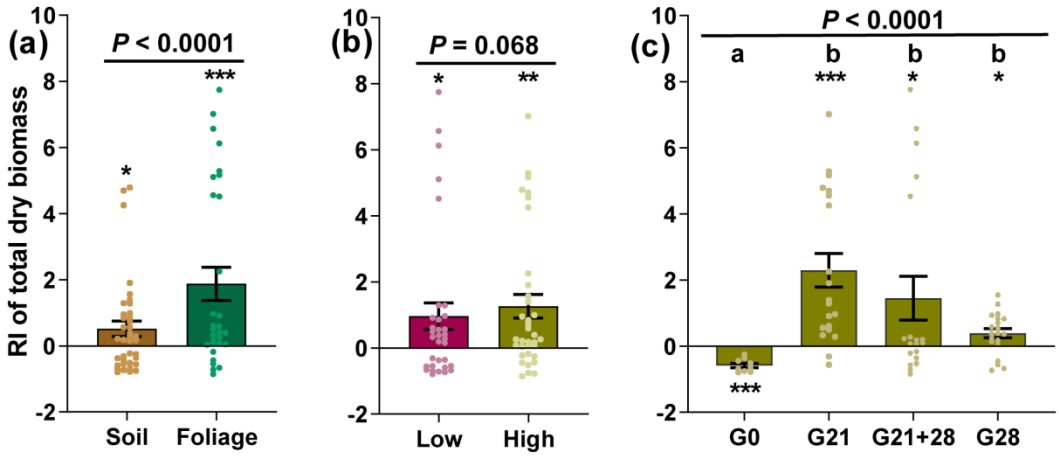

Figure 2. Effects of feedback (a), nutrient level (b), and inoculation time (c) on the microbial role in *A. adenophora* growth based on total dry weight. For the abbreviations G0, G21, G28, and G21+28, see Fig. 1. Error bars depict the standard error. The asterisks indicate that the RI is significantly different from zero. Different lowercases represent significant differences among the different inoculation time treatments.

The microbial community composition and potential functions associated with leaf and soil inocula explained distinct seedling mortality patterns at the early stage

The soil and leaf inocula had distinct microbial diversity, community compositions and potential functions (Figs. 3,4). The microbial diversity and richness were greater in the soils than in the leaf litter (Fig. 3a-c). The bacteria Actinobacteriota and Acidobacteriota and the fungi Zygomycota had higher relative abundances in the soil than in the litter, while the abundance of Proteobacteria was greater in the litter than in the soil (Fig. 3d,e). The relative abundance of the top 30 genera also differed between the soil and litter (Fig. 4a,f).

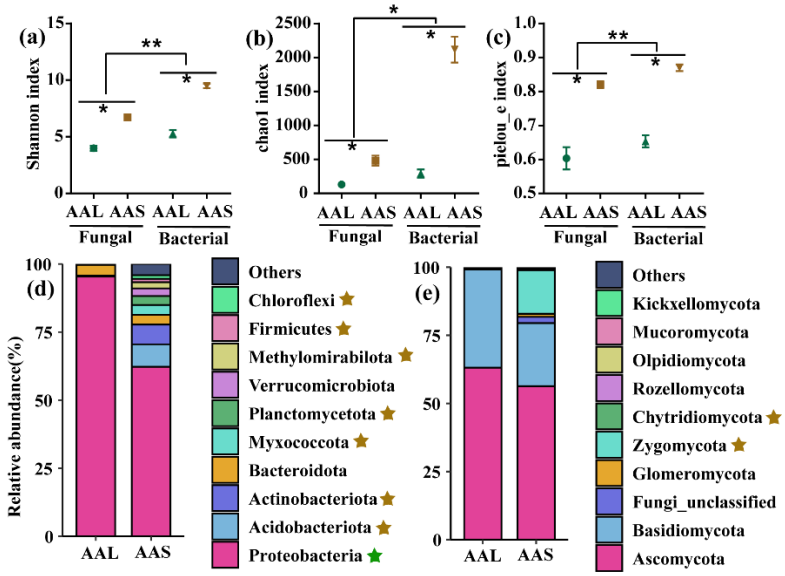

Figure 3 Microbial diversity and community composition at the phylum level in the inocula AAS and AAL. Fungal and bacterial Shannon, Chao 1 and Pielou_e indices of AAS and AAL (a-c). Relative abundances of bacterial (d) and fungal phyla (e) in the AAS and AAL. AAS: *A. adenophora* rhizosphere soil inoculum, AAL: *A. adenophora* litter inoculum. Brown stars represent significantly greater relative abundances in the AAS than in the AAL, green stars represent significantly greater relative abundances in the AAL than in the AAS, $P < 0.05$ at least.

We further correlated the microbial genera and potential functions of leaf and soil inocula with seedling mortality in response to inoculation with non–sterile inocula at G0. The abundances of both the soil and leaf microbial genera were related to the

seedling death rate (DR) but not to the germination time (GT) or germination rate (GR) (Fig. 4a,f). Specifically, the leaf core bacterial genera *Pseudomonas*, *Sphingomonas*, *Massilia*, *Variovorax*, *Aureimonas*, and *Agrobacterium* were positively correlated with DR, but the soil core bacteria, including Alphaproteobacteria_unclassified, *Rhodoplanes*, Vicinamibacteraceae_unclassified, and *Pedomicrobium*, were negatively correlated with DR (Fig. 4a-c). Plant bacterial pathogens (2.29%) were the potential function of the core bacterial taxa in leaves but not in soil; soil had a greater abundance of nitrogen circle–related function (50.98%) than leaves (17.55%) (Fig. 4d,e, Table S1-2). The leaf core fungal genera *Didymella*, Pleosporales_unclassified, *Subplenodomus*, and *Bulleromyces* were positively correlated with DR, but the soil core fungal genera *Mortierella*, *Hypocrea*, *Pochonia* and *Volutella* were negatively correlated with DR (Fig. 4f-h). The abundance of plant fungal pathogens was greater in leaves (68.20%) than in soil (33.93%), while Ecotomycorrhizal was found only in soil (16.33%) (Fig. 4i,j, Table S3-4).

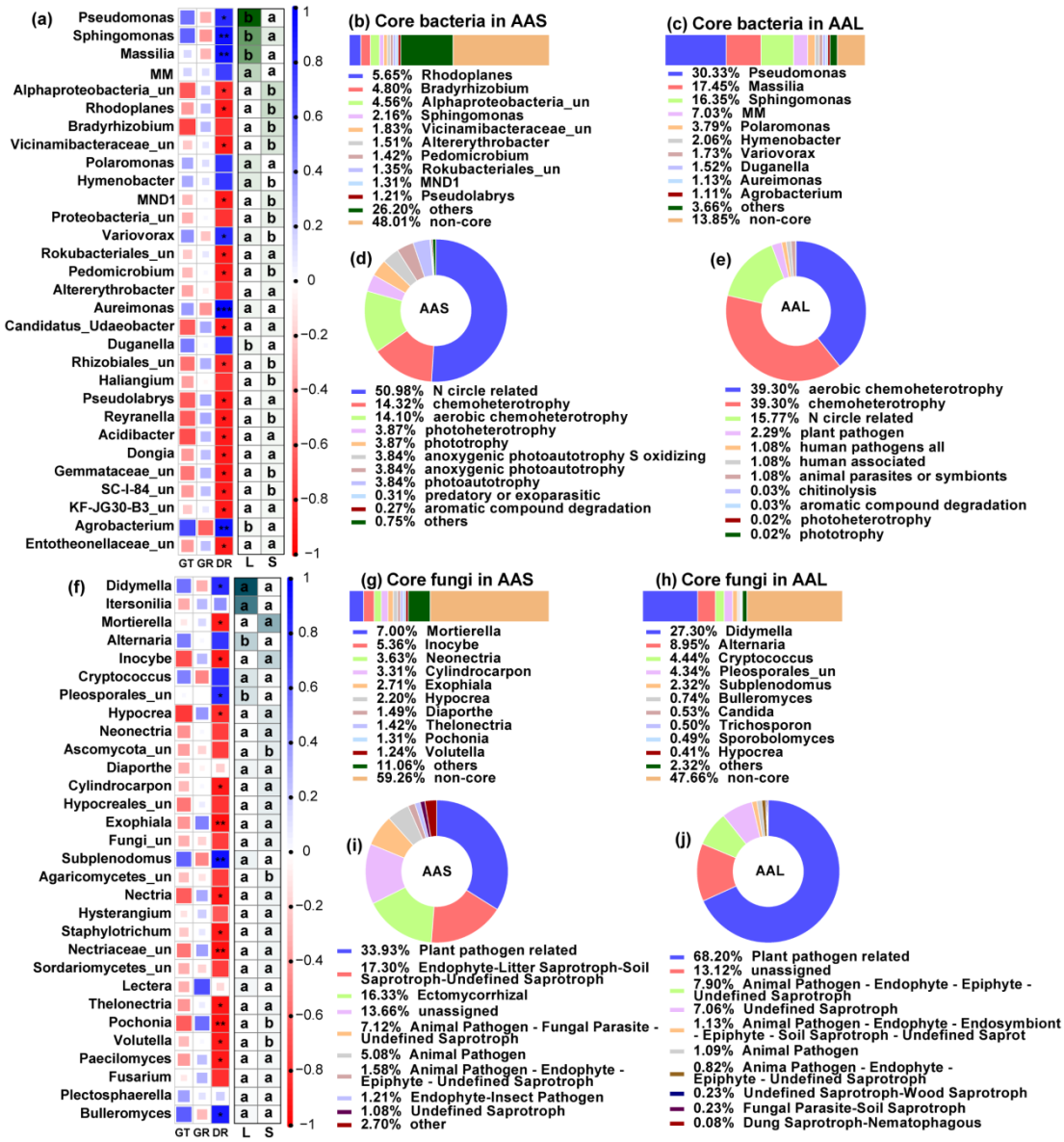

Figure 4. Microbial community composition and potential functional differences between leaf litter (AAL) and rhizosphere soil (AAS). Correlations between the top 30 bacterial (a) and fungal (f) genera and germination time (GT), germination rate (GR) and death rate (DR). Core bacteria (b-c) and fungi (g-h) in AAS and AAL. The potential functions of core bacteria (d-e) and fungi (i-j) in AAS and AAL. Only the top 10 core taxa and potential functional groups are shown in the figures. The "un" in the figures is the abbreviation for "unclassified", and the MM is *Methylobacterium-Methylorubrum*. Red and blue represent negative and positive Spearman's coefficients, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Different lowercase letters in the heatmap represent significant differences in relative abundance between AAS and AAL ($P < 0.05$). Several bacterial functions classified as N circles related to AAL and AAS are shown in Table S1-2. Several fungal guilds were classified as plant pathogen-related guilds in

the AAL and AAS (see Tables S3-4).

We obtained 192 cultivable fungal isolates from 40 dead seedlings, with an average of 4.825 isolates per dead seedling (Fig. 5b). Based on the ITS genes of the representative strains (Table S5), they were divided into 7 families. The dominant family was Didymellaceae (relative abundance = 66.15%), and the numerically dominant genera were *Allophoma* (50.52%), *Alternaria* (26.04%) and *Epicoccum* (5.73%) (Fig. 5a, Table S5). The seedling mortality of these strains exhibited a significant phylogenetic signal (Pagel's λ = 0.82, P = 0.0002). Overall, numerically dominant *Allophoma* (Didymellaceae) and *Alternaria* (Pleosporaceae) showed high seedling mortality (death rate: 54% – 100%) (Fig. 5c, Fig. S4).

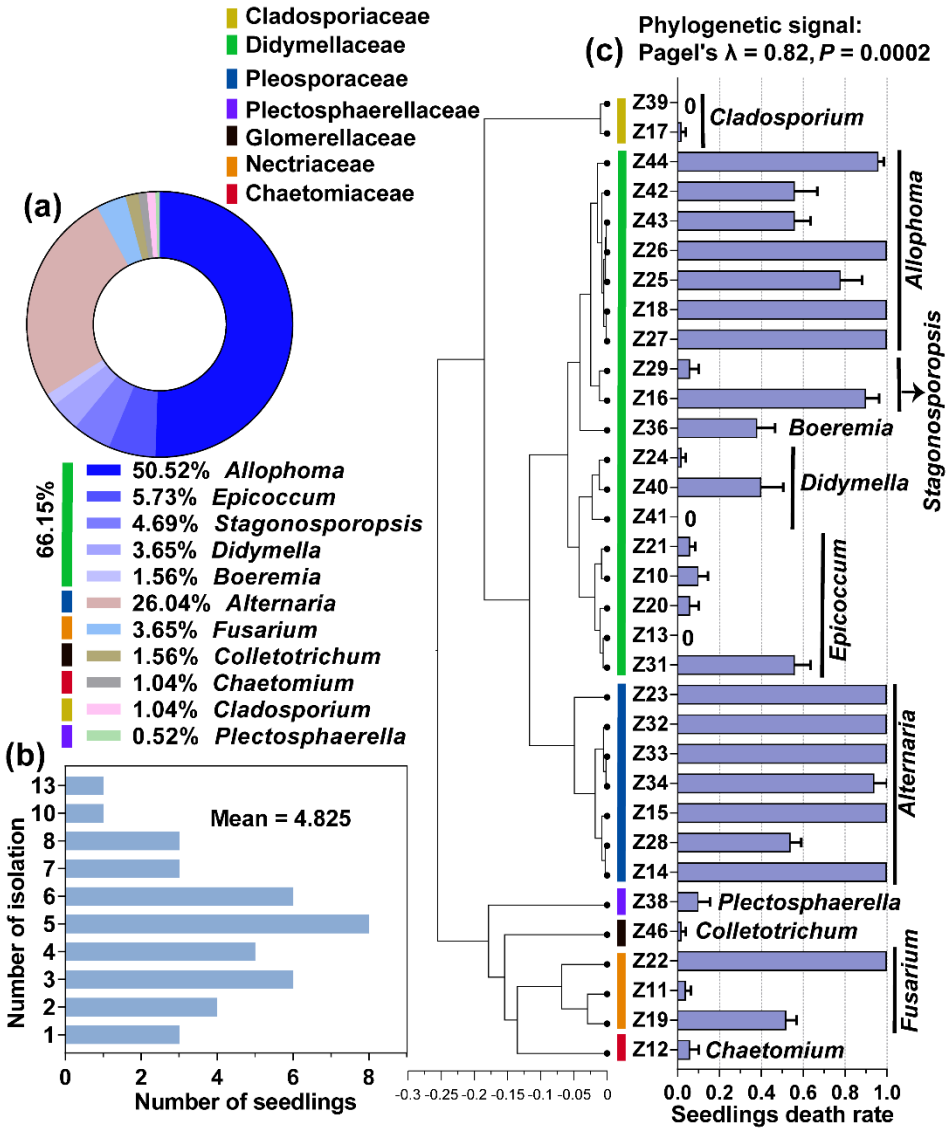

Figure 5. Cultivable fungi associated with dead *A. adenophora* seedlings and their mortality to

seedlings. Cultivable fungal community composition at the genus level (a). Isolation frequency from one dead seedling (b). Death rate of 33 fungal strains to seedlings and their phylogenetic signal (c).

Different feedback, inoculation time, nutrient level and plant compartment affected the seedling bacterial and fungal communities and functional assembly and growth.

The NMDS and PERMANOVA revealed that all four factors significantly affected the bacterial community and functional assembly in seedlings, and the greatest effect was inoculation time and compartment (all $P < 0.05$, R^2: 0.102 – 0.138), followed by feedback and nutrition (all $P < 0.05$, R^2: 0.024 – 0.082, Fig. 6a,b,e,f). Additionally, compartment, feedback and inoculation time significantly affected the fungal community and functional assembly (all $P < 0.05$, R^2: 0.054 – 0.102), but nutrition affected only the fungal community ($P = 0.001$, $R^2 = 0.031$) (Fig. 6c,d,g,h). Further analysis for each inoculation time treatment showed that compartment and feedback mainly affected the microbial community and functional assembly and explained a greater proportion of the variation in bacteria than in fungi and in function than in the community. The nutrient level mainly affected the bacterial community at certain inoculation time ($P < 0.05$ for G0, G21 and G28, Fig. 6i-l, Fig. S5, Table S6-7).

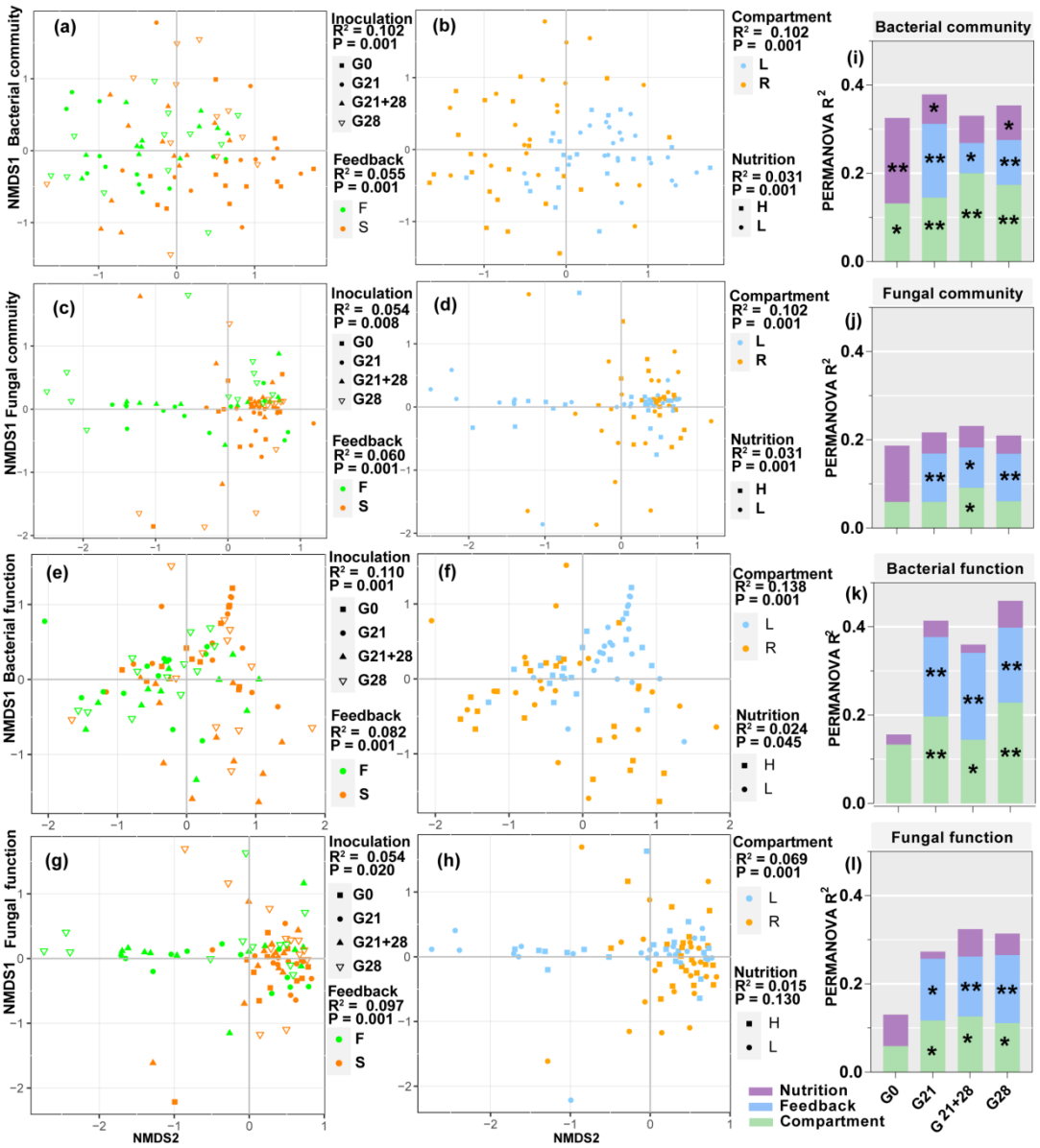

Figure 6. Bacterial and fungal communities and functions enrichment in *A. adenophora* seedlings under different treatments. Nonmetric multidimensional scaling (NMDS) ordinations of Bray–Curtis dissimilarity matrices with permutational analysis of variance (PERMANOVA) of bacterial and fungal communities and function (a-h). Contribution of compartment, feedback and nutrient level to the variation in bacterial and fungal communities and function at each inoculation time based on PERMANOVA (i-l). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. In the figures, F and S represent foliage and soil feedback; L and R represent leaves and roots for different compartments; and H and L represent high and low nutrient levels, respectively.

We further analysed the correlation between microbial abundance and putative functions enriched by seedlings and seedling growth (RI). We identified 47 root

endophytic genera that were significantly correlated with *A. adenophora* growth. Among them, seven negative genera were less abundant in seedlings treated by litter inoculation than in those treated by soil inoculation but were similar in abundance in seedlings treated by different inoculation time, and in seedlings grown at different nutrient levels. In contrast, forty positive genera, e.g., the fungi *Duganella and Mortierella* and the bacteria *Massilia, Pseudomonas, and Sphingomonas*, were more abundant in seedlings treated by litter inoculation than by soil inoculation but lower abundance in seedlings inoculated at G0 than at the other three inoculation times (Fig. 7a). Eighteen leaf endophytic genera with significant correlation with RI were identified, of which three negative genera, including bacteria *Tardiphaga*, *Brevundimonas* and fungi *Microsphaera*, were more abundant in seedlings inoculated at G0 than at other three inoculation times and slightly higher in seedlings treated by soil inoculation than litter inoculation; in contrast, fifteen positive genera, e.g., fungi *Hypocrea*, Pleosporales_unclassified, were more abundant in seedlings by litter inoculation than by soil inoculation, and higher in seedlings inoculated at G21 than three inoculation time treatment (Fig. 7b).

We identified several bacterial functions in the roots and fungal function guilds enriched in the roots and leaves of *A. adenophora* seedlings that were significantly correlated with the RI (Fig. 7c-e). Two bacterial functions involved in the N cycle (nitrate ammonification and nitrite ammonification) in roots showed a significantly positive correlation with the RI (Fig. 7c). The fungal guild Ectomycorrhizal showed a significantly positive correlation. Unexpectedly, the putative Plant Pathogen guild showed a significantly positive correlation with seedling growth, while the Arbuscular Mycorrhizal guild showed a negative correlation (Fig. 7d,e). Those positive bacterial functions in roots and fungal guilds in leaves had greater relative abundances in the seedlings after litter inoculation than after soil inoculation (Fig. 7c,e); additionally, the positive fungal guilds in roots and leaves had significantly greater abundances in the seedlings of G21 than in those of the other three inoculation time points. Surprisingly,

there was no difference in the relative abundance of microbes or functions involved in seedling growth under different nutrient levels (Fig 7a-e).

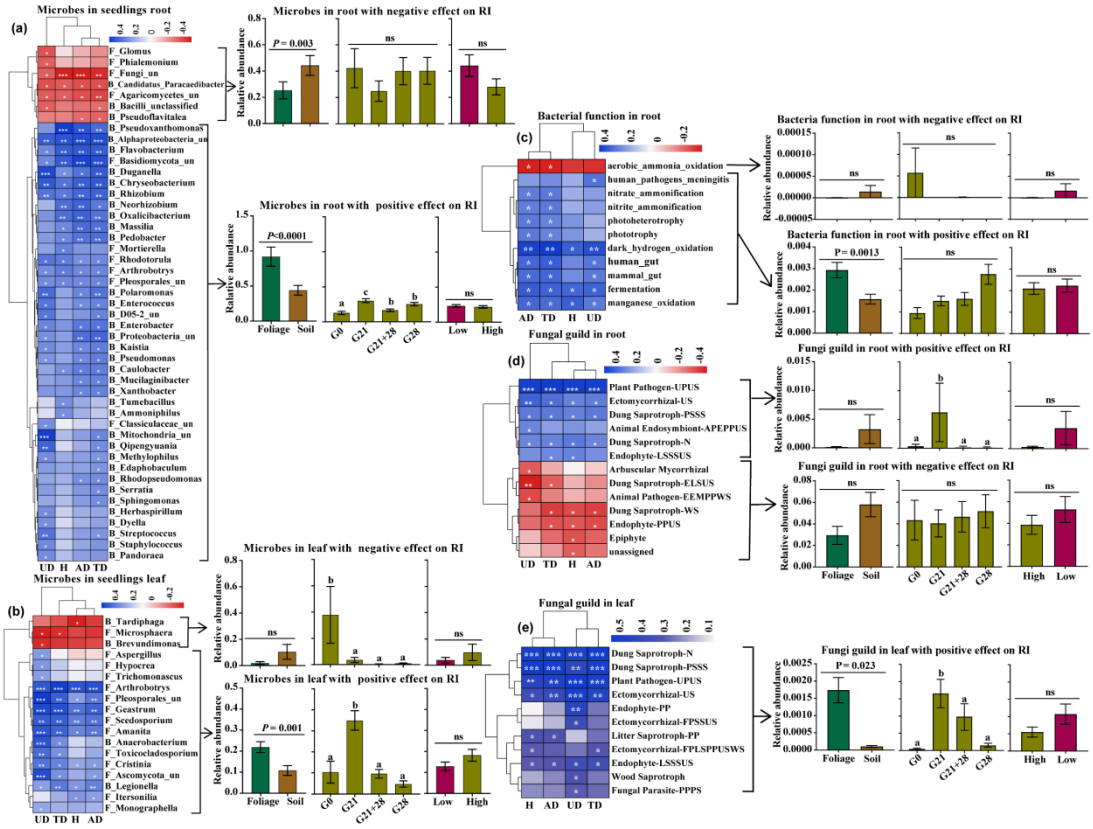

Figure 7. Correlations between the enriched microbial community, functions and seedling growth

These genera accounted for more than 1% of the total relative abundance in seedling roots or leaves. (a) Correlations between 47 out of 214 genera enriched in roots with RI (left) and the relative abundances of genera under different treatments, i.e., different feedbacks, inoculation time and nutrient levels (right). (b) Correlations between 18 out of 184 genera enriched in leaves with RI (left) and the relative abundances of genera under different treatments (right). (c) Correlations between putative bacterial functions enriched in roots with RI (left) and the relative abundances of functions with negative and positive effects under different treatments (right). (d) Correlations between fungal guilds enriched in roots with RI (left) and the relative abundances of guilds with negative and positive effects under different treatments. (e) Correlations between fungal guilds in leaves with RI (left) and the relative abundances of guilds with positive effects under different treatments (right). No bacterial functions in the leaves showed a significant correlation with seedling growth. “F_” represents fungal genera, “B_” represents bacterial genera, “un” represents unclassified; H: RI of seedling height; AD: RI of aboveground dry biomass; UD:

RI of underground dry biomass; TD: RI of total dry biomass. Red and blue represent negative and positive Spearman's coefficients, respectively. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For abbreviations of fungal function guilds, please see Table S8.

Discussion

Leaf litter microbes had more adverse effects on *A. adenophora* seed germination and seedling survival than soil microbes

Supporting our first expectation, leaf litter had more adverse effects on seed germination and seedling survival than soil (Fig. 1). Leaf litter has been previously reported to have adverse effects on seedling emergence and population establishment (Abbas et al., 2023; Möhler et al., 2021; Zhang et al., 2022) by reducing light and physical barriers and releasing allelochemicals (Abbas et al., 2023; Gross, 1984; Möhler et al., 2018; Zhang et al., 2019). Although our data also indicated that leaf litter can produce allelochemicals to delay seed germination time because sterile leaves cause longer germination time than non–sterile leaves, our study emphasized the adverse microbial role of leaf litter in seedling mortality (Fig. 1). Indeed, we found that leaf litter harbored more abundant bacterial and fungal genera associated with seedling mortality, as well as a greater proportion of plant pathogens than soil (Fig. 4). The results implied that litter harboured many plant pathogens and thus played an essential role in mediating *A. adenophora* population density by killing conspecific seedlings.

These findings provide novel insights for understanding plant invasion. Invasive plants are commonly characterized by ruderal species, with rapid growth and a high yield of seeds (Baker, 1974; Parsons WT, 2001; Zheng et al., 2009). These traits are beneficial for rapid population establishment and range expansion at newly invaded sites. However, these invasive plant species commonly form high-density monocultures once the population is established, e.g., *A. adenophora*. In such a situation, high-density seedlings may exacerbate intraspecific competition. Self-limiting of the population

elicited by leaf microbes may in turn help *A. adenophora* maintain monocultures at established sites. Thus, it is highly interesting to determine whether leaf microbe-mediated self-limitation at an early life stage is common and important in other invasive systems.

Peripheral microbial sources produce more adverse effects on seedling survival and growth when inoculated at the early growth stage than at the later stage

Supporting our second expectation, inoculation time significantly affected seedling survival and growth; in particular, seedling mortality was higher and seedlings grew more adversely when inoculated at G0 than at later growth stage (Fig. 1c,g, Fig. 2c). The plant growth stage could change the impact of plant host-associated microbes, and such an impact is always strongest in early plant growth stages (Bagchi et al., 2014; Jevon et al., 2020). One potential reason is that small seedlings usually allocate most of their resources to survive and grow, while older seedlings have relatively more resources to defend against pathogen infection (Geisen et al., 2021; Schloter & Matyssek, 2009). For example, smaller seedlings were more sensitive to inoculated individual fungi, soil microbiota or litter addition than older seedlings due to fewer defense resources and little chance of recovering from biomass loss (Geisen et al., 2021; Jevon et al., 2020; Zhang et al., 2022). Another possible reason is related to the interaction between seed-borne microbes and peripheral microbial sources in young seedlings. There is evidence that seed–borne endophytes are likely to be beneficial for seedling growth and stress resistance (Herrera et al., 2016; Wang & Zhang, 2023). These seed endophytes might be inhibited or even excluded from young seedlings by external sources of microbes inoculated at G0, when seedlings are highly sensitive to inoculated microbes (Geisen et al., 2021; Jevon et al., 2020). Therefore, it is very valuable to determine how peripheral microbial sources interact with seed–borne endophytes in seedlings across ontogeny.

We did not observe an adverse effect of leaf litter microbes on *A. adenophora*

growth, as observed previously by Kai Fang et al. (2019), who inoculated *A. adenophora* at G0 (sowing seeds) because all seedlings by leaf litter inoculation at G0 died after transplantation into soils in this study. In contrast, both the microbial community and function were significantly positively correlated with seedling growth and had a greater relative abundance in seedlings inoculated with leaf litter than in those inoculated with soil, while those with a negative correlation showed the opposite trend (Fig. 7). This fact suggested that leaf litter microbes might have a more positive effect on *A. adenophora* growth than soil microbes if inoculated during the latter growth stage (Fig. 2a). Interestingly, we found that N circle-related bacterial functions in seedling roots were positively correlated with seedling growth (Fig. 7c). Similarly, Zhao et al. (2019) showed that *A. adenophora* invasion increased N circle-related bacterial functional genes in soil and subsequently directly promoted plant growth and invasion. K. Fang et al. (2019) also reported that several root endophytic nitrogen–fixing bacteria of *A. adenophora* could significantly promote its growth. Interestingly, the abundance of these N circle–related bacterial functions was greater in seedling roots inoculated with leaf litter than in those inoculated with soil (Fig. 7c), which suggests a possible way in which some N circle–related bacteria associated with leaf litter may migrate from leaves into roots after leaf litter inoculation.

Nutrient levels affect seedling mortality but not microbe-mediated *A. adenophora* growth

Nutrient addition could promote more severe invasion, as invasive plants often have higher performance under high nutrition than native plants. (Shan et al., 2023; Slate et al., 2022). However, it is unclear whether such an advantage involved in a changed microbial community-driven host growth effect. We also found that *A. adenophora* grew bigger and more rapidly in the high-nutrient treatment than in the low-nutrient treatment (Fig. S7); moreover, the nutrient level significantly changed the microbial community and bacterial function (Fig. 6). However, in contrast to our third expectation, nutrient level negligently affected microbe-mediated *A. adenophora*

growth, and the relative abundance of microbes and functions involved in seedling growth (Fig. 2b,7a-e). Previously, Ai et al. (2018) reported that nutrient additions cause crops to enrich some bacteria and fungi from soil and increase yield; however, there is no evidence that the increased yield effect is due to enriched microbial communities. Our data indicated that the invasion advantage driven by high nutrient availability may be driven primarily by plant physiological traits, such as rapid nutrient absorption and growth strategies, rather than by enriched microbes. Alternatively, it is possible that our delayed harvest of seedlings under low nutrition level may cover the distinct microbial role in seedling growth between the two nutrient levels (see Method).

However, there was an interaction effect between nutrient level and inoculation period (Fig. S3). For example, high nutrient level resulted in a more significant positive microbial effect on seedling growth than low nutrient level when inoculated at G21, regardless of leaf litter or soil inoculation. It is unclear whether, during the first 21 days before inoculation, more beneficial seed endophytes are enriched to produce a more positive effect on seedling growth under high-nutrition conditions than under low-nutrition conditions, as seed endophytes can facilitate nutrient acquisition and subsequently promote plant growth (Khalaf & Raizada, 2016; Sanchez-Lopez et al., 2018; Shao et al., 2021). Nonetheless, seedlings grown in high-nutrient soil had greater mortality than those grown in low-nutrient soil (Fig. 1f). Similarly, Jessen et al. (2023) also reported that fertilization, independent of litter removal, significantly decreased the total number and richness of seedlings, likely because higher ammonium levels in fertilized plots could create a toxic environment for seedlings (Britto & Kronzucker, 2002).

The same microbial genera have distinct effects on *A. adenophora* seedling survival versus growth

Correlation analysis of the microbial community and function with seedling survival and growth revealed several genera with distinct effects on seedling survival versus growth. For example, the bacterial genera *Pseudomonas*, *Sphingomonas* and

Massilia are adverse to seedling survival but beneficial for later seedling growth (Figs. 4,7). Many strains belonging to these genera have been reported to promote the growth of many plant species (Jimenez et al., 2020; Luo et al., 2019; Qiao et al., 2019), including *A. adenophora* (Chen et al., 2019; K. Fang et al., 2019), because they are commonly involved in N_2 fixation (Albino et al., 2006). However, some microbes, e.g., the fungi *Mortierella* and *Hypocrea*, were consistently beneficial to *A. adenophora* by reducing early seedling mortality and promoting later seedling growth (Figs. 4,7). These fungi, as plant growth-promoting fungi (PGPFs), have been widely reported (Contreras-Cornejo et al., 2009; Ozimek & Hanaka, 2021; Wani et al., 2017). These findings suggested that microbial interactions are highly complicated during the early life stage of *A. adeonophora*. On the one hand, there may be sequential effects for some plant growth-promoting microbial groups. For example, bacteria *Massilia, Pseudomonas* and *Sphingomonas* may negatively affect seedling growth and even kill seedlings if the arrival time is too early after germination. On the other hand, such distinct effects of these bacterial groups on seedling survival versus growth may result from different species from the same genus or even from genetically distinct strains from one species. Interestingly, we found that most *Pseudomonas* and *Sphingomonas* ASVs enriched in seedlings (>80%) had no share with the inoculum sources. This suggested that most of the bacterial ASVs with beneficial growth effects were from seeds rather than from the inocula (Fig. S9).

Surprisingly, related plant pathogen guilds showed a positive correlation with *A. adenophora* seedling growth (Fig. 7). Because these putative plant pathogens were classified as plant pathogens based on the current database, it remains to be determined whether such putative plant pathogens for most native plant species are not detrimental to invader *A. adenophora* growth. Indeed, the pathogenicity of plant pathogens is often dependent on host plant identity, and it could switch from a beneficial endophyte to a pathogen or vice versa among host plant species (Delaye et al., 2013; Tian et al., 2020).

Seedling-killing microbes are those associated with leaf litter

We found that most seedling-killing microbes isolated from dead seedlings were previously reported as leaf spot pathogens. For example, *Alternaria* (Pleosporaceae) and several genera belonging to the family Didymellaceae, such as *Allophoma*, *Stagonosporopsis*, *Didymella*, *Boeremia*, and *Epicoccum*, showed high seedling mortality (Fig. 5). *Alternaria* sp. is often pathogenic to a large variety of plants, such as stem cancer, leaf blight or leaf spot (Leiminger et al., 2015; Thomma, 2003; Vergnes et al., 2006), and members of *Allophoma* have also been reported as pathogens for dieback (Babaahmadi et al., 2018) and leaf spot (Garibaldi et al., 2012). All these fungi are leaf spot pathogens of *A. adenophora* and its neighboring native plant (Chen et al., 2022; Kai Fang et al., 2021). In particular, the numerically dominant *Allophoma* strains obtained in this study had the same ITS genes as the leaf endophyte and leaf spot pathogen *Allophoma* associated with *A. adenophora* (Chen et al., 2022; Kai Fang et al., 2021; Yang et al., 2023). Recently, these strains were identified as the novel species *Mesophoma speciosa* (Chen et al., 2022; Yang et al., 2023). We did not isolate fungi from healthy seedlings in this study. However, a previous report revealed that the dominant genera in healthy seedlings inoculated with leaf litter were *Didymella* and *Alternaria* (Kai Fang et al., 2019). Based on these results, these fungal genera likely exist in *A. adenophora* by a lifestyle switch from endophytic to pathogenic. The virulence of these strains for seedling survival under certain conditions may play an essential role in limiting the population density of *A. adenophora* monocultures.

Implications for developing biocontrol for *A. adenophora* invasion

Our data also have implications for the development of biocontrol agents for *A. adenophora* invasion. Currently, several leaf spot fungi, such as the leaf spot fungus *Phaeoramularia* sp., which is released against *A. adenophora* (Kluge, 1991); the white smut fungus *Entyloma ageratinae* against *A. riparia* (Barton et al., 2007); the rust fungus *Uromycladium tepperianum* against the weed *Acacia saligna* (Wood, 2012); and

the rust fungus *Puccinia spegazzinii* against *Mikania micrantha* (Day & Riding, 2019), have been used as biological agents for the control of plant invasion. These agents mainly control weeds by damaging the leaves, stems, and petioles and reducing growth rates, flowering, percentage cover and population density. In this study, the strains associated with leaf litter, such as *Allophoma* sp. and *Alternaria* sp., caused high seedling mortality and thus could control *A. adenophora* invasion at the seedling establishment stage. On the other hand, we found that an external source of microbes had a greater adverse effect on seedling survival and growth when inoculated at G0 than at the later growth stage. Therefore, prevention and control measures by microbial agents taken at the early seedling stage of invasive plants may be more effective than at the mature stage.

Materials and Methods

Sample collection and preparation

All seeds, rhizosphere soil (AAS) and leaf litter (AAL) of *A. adenophora* were collected from Xishan Forest Park, Kunming city, Yunnan Province (25°55′34″N; 102°38′30″E, 1890 m), on 9th April 2022. We collected dead leaves (litters) from the stems as inoculated leaves to avoid contamination by soil microbes; moreover, dead leaves could better represent litter in natural systems than fresh leaves. All leaf litter and soil samples were collected from five *A. adenophora* populations ~200 m away from each other and treated as independent biological replicates. These *A. adenophora* plants grow in monoculture for more than 10 years, thus their rhizosphere soils and litters were used in our feedback experiment rather than via a typical two-phase approach (the first conditioning phase vs the second testing phase) (Brinkman et al., 2010). The collected soil and litter samples were naturally dried in a clean room and weighed. The soil was ground to a 2 mm sieve before weighing. For convenience in the inoculation application, we prepared these samples in leaf litter bags (each containing

2 g of leaf litter) (Zaret et al., 2021) and soil bags (each containing 5 g of soil) as well as 0.1 g of soil or litter (cut into smaller than 2×2 mm) in centrifuge tubes. All sample bags and tubes were divided into nonsterile and sterile groups and stored at 4°C until inoculation. The sterile groups (soils or litters) were sterilized by gamma irradiation (30 kGy, 30 h, Huayuan Nuclear Radiation Technology Co., Ltd., Kunming, China), which can kill all microorganisms because no colonies were formed after 7 days of inoculation on PDA media for gamma irradiation samples (see Fig. S6); moreover, there was no evidence that this irradiation method changed the chemistry of the samples. The non–sterile groups (soil or litter) were natural samples containing live microorganisms. The natural soil or litter (0.3 g) was weighed into tubes and placed at –80°C until DNA extraction.

Experimental design

The experimental design is shown in Fig. 8: (1) We inoculated *A. adenophora* with non–sterile rhizosphere soil and leaf litter of *A. adenophora* and sterile groups as control groups to distinguish the effects of aboveground from belowground microbes. (2) We inoculated soils or leaf litter of *A. adenophora* at three growth stages (0 d, 21 d, 28 d) to explore seedling susceptibility to microbial infection and growth effects. We performed inoculation on the day of sowing seeds on water agar plates (containing 5 g of agar and 500 mL of water) for germination, named G0 inoculation; on the 21st day after seedling growth, named G21; and on the 28th day after seedling growth, named G28. We further performed a combination inoculation, that is, inoculation on both the 21st and 28th days after seedling growth, named G21+28 inoculation. Therefore, we had four treatments for inoculation time. (3) We grew all the inoculated seedlings in background soil (made of Pindstrup substrate, pearlite and vermiculite at a volume ratio of 8:1:1, and nutrient content of Pindstrup substrate see Table S9) by adding the same volume of Hoagland nutrient solution (high nutrient level) or only water (low nutrient level) to the soil. In total, our experimental design included 4 inoculation time (G0, G21, G28, G21+28) × 2 inoculum sources (leaf litter, rhizosphere soil) × 2 microbial

treatments (sterile, non–sterile) × 2 nutrient levels (high, low) × 5 replicates = 160 cups. All the cups were randomly placed in the growth chamber and rearranged randomly every week to mitigate potential positional effects. Seedlings were harvested after 8 weeks of growth under high nutrient conditions. We had to grow seedlings under low nutritional condition for another 4 weeks to harvest because they were too small to harvest, while those under high nutritional conditions grew too fast and up to the cup PTFE cover (see Fig. S7). No seedlings survived at G0 inoculation of non–sterile leaf litters when harvested. Stem height, dry aboveground biomass and underground biomass were measured at harvest. Fresh seedling leaves and roots (0.3 g) from three seedlings per treatment as three replicates when harvested were surface-sterilized for microbial community detection. The aboveground and underground dry biomasses for seedlings with less than 0.3 g fresh weight were obtained by linear regression (see Fig. S8). Seed germination and inoculation manipulation for each inoculation treatment are detailed in supplementary method 1 (also see Fig. 8).

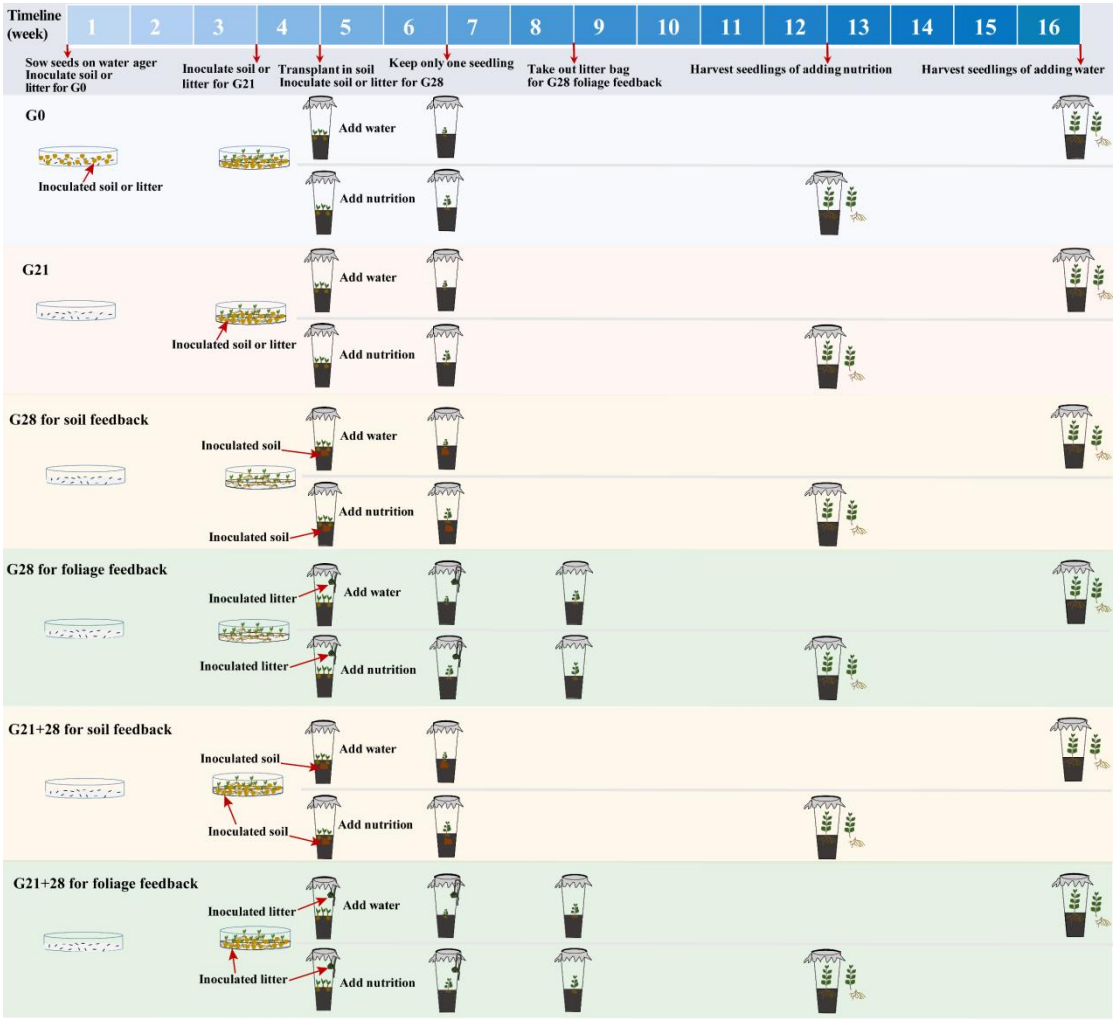

Figure 8. **Schematic diagram of soil or litter inoculation at different growth stages.** The abbreviations G0, G21, G28, and G21+28 are shown in Fig. 1.

Molecular sequencing of the microbial community

To link microbial sources (leaf litter and soil) with seed germination, seedling mortality and subsequent seedling biomass, we sequenced the microbial community associated with inoculum samples (natural *A. adenophora* soils (AAS) and leaf litter (AAL)), as well as fresh leaves and roots of *A. adenophora* seedlings grown in the non–sterile treatments. Fresh leaves and roots (0.3 g) of each seedling at harvest were chosen for surface sterilization and then stored at –80°C until total DNA was extracted. For DNA extraction, target–gene amplification and sequencing steps, see Supplementary Method 2.

Seedling-killing fungus experiment

No dead seedlings were observed from Petri dishes inoculated with non–sterile soils at G0. Thus, we used 40 dead seedlings obtained from Petri dishes inoculated with non–sterile leaf litter at G0 to isolate fungi. Each dead seedling was cut into 1 mm × 1 mm pieces, and three tissues were placed on each PDA Petri dish and incubated at ambient temperature (20–25°C) for 6–8 days or until mycelia grew. Hyphal tip cultures were subsequently transferred onto new PDA plates and incubated until pure colonies appeared. The DNA of these purified fungal strains was extracted and identified by sequencing the ITS region (for details, see Supplementary Method 2).

To test the mortality of the fungal strains to seedlings, sixteen surface-sterilized *A. adenophora* seeds were sown in a water ager plate in a Petri dish. Ten similar-sized seedlings in one petri dish 21 days after sowing were selected for fungal inoculation. Five Petri dishes were used as five replicates for each strain. Fungi were grown on PDA for 7 days in an incubator at 25°C, after which 6 mm diameter agar discs with fungal mycelia were inoculated into seedlings by touching the leaves or stems (see Fig. S4). Seedlings were regarded as dead when the leaf and stem became brown and rotten. We recorded the number of dead seedlings after 14 days of inoculation of agar discs and then calculated the death rate (DR = the number of dead seedlings/10).

Statistical analysis

It is unreasonable to directly compare seedling biomass among treatments because of different seedling growth time under high or low soil nutrition condition (see method description above), the response index (RI) was calculated to evaluate the feedback intensity (or direction) of microbes in inocula soil or leaf: RI = ($variable_{non\text{-}sterile}$ − $variable_{sterile}$)/$variable_{sterile}$) (Bruce Williamson & Richardson, 1988), then a one–sample T test was used to define the significance between the RI value and zero, where RI > 0 and < 0 represent microbes to promote or inhibit seedling growth, respectively. Generalized linear models (GLMs) with Gaussian error distributions (identity link)

generated by the “lme4” package were used to identify the effects of feedback, microbe, inoculation time, nutrient level treatments and their interaction on seedling mortality as well as the effects of feedback, inoculation time, nutrient level treatments and their interaction on the RI value. The R^2 values of the models were obtained by the “piecewiseSEM” package, and P values were estimated using the Anova function via chi-squared ($\chi 2$) tests in GLMs. The nonparametric Mann–Whitney U test was used to perform all two-group comparisons, and the Kruskal–Wallis test was performed to compare the differences in RI or microbial relative abundances among the four inoculation time treatments.

Nonparametric Mann–Whitney U tests, Kruskal–Wallis tests and one–sample T tests were executed using SPSS v. 22.0 (SPSS, Inc., Chicago, IL, USA). Nonmetric multidimensional scaling (NMDS) analysis was used to visualize the similarities in bacterial and fungal composition and function among the treatments. Permutational analysis of variance (PERMANOVA) was performed with the ADONIS function in the R (v. 4.2.0) package “vegan” to test the differences in the bacterial and fungal communities and functions among the treatment groups. Bacterial functional profiles were predicted using functional annotation of prokaryotic taxa (FAPROTAX) (Louca et al., 2016). Fungal functional guilds were inferred using the program FUNGuild, and guild assignments with confidence rankings of “Highly probable” and “Probable” were retained (Nguyen et al., 2016). The core microbial taxa were primarily selected from the ASVs that appeared (100% prevalence) among all the samples. Spearman’s correlation analysis was used to link microbial communities in inoculation sources (leaf litter and soils) with seed germination and seedling mortality in petri dishes of the non-sterile G0 treatment, as well as to link seedling growth with microbial communities or functions enriched by seedling leaves or roots based on all treatments. A correlation was considered significant when $P < 0.05$. Heatmap plotting was performed in R 4.2.0 with the “pheatmap” package. To examine the phylogenetic signal of the seedling death rate to fungal strains, we calculated Pagel's λ with the R package “phytools”, which

measures the distribution of a trait across a phylogeny. A Pagel's λ was closer to 1, indicating a stronger phylogenetic signal (Pagel, 1999). The remaining figures were visualized in GraphPad Prism v7.0 (GraphPad Software, Inc., San Diego, CA, USA).

Data availability

The raw bacterial and fungal sequence data are archived in GenBank under accession numbers PRJNA1008375 and PRJNA1008403, respectively. Fungal ITS sequences could be obtained from GenBank under accession numbers OR473386-OR473418.

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Author contributions

Zhao-ying Zeng: conceptualization, formal analysis, investigation, methodology, visualization, writing – original draft, writing – review & editing. Jun-rong Huang, Zi-qing Liu, Ai-ling Yang, Yu-xuan Li and Yong-lan Wang: investigation. Han-bo Zhang: conceptualization, writing – review & editing, supervision, funding acquisition, project administration.

Additional files

Method S1: Detailed seed germination and inoculation manipulation for each inoculation time treatment.

Method S2: DNA extraction, target–gene amplification and sequencing.

Figure S1. Germination and dead seedlings of *A. adenophora* inoculated soil (upper) or litter (bottom) in Petri dishes.

Figure S2. GLM analysis (a) and comparison (b) of seedling death rate after two weeks when seedlings were transplanted into soil for sterile and non-sterile soil- and foliage-feedback under high and low nutrient levels.

Figure S3. GLM analysis (a) and comparison (b) of microbial role in seedling growth based on total dry biomass (RI) between foliage- and soil-feedback under low and high nutrition level.

Figure S4. Death rate of seedlings caused by several representative strains.

Figure S5. NMDS results for the bacterial and fungal communities and function in each inoculation time treatment.

Figure S6. Gamma irradiation was effective at killing all the microorganisms.

Figure S7. Seedlings were grown for 8 weeks under low (adding water, left) or high (adding Hoagland nutrient solution, right) nutrient levels.

Figure S8. Linear regression between the fresh and dry weights of aboveground biomass (a) and underground biomass (b).

Figure S9 Number of shared ASVs between seedlings and inoculum source (soil and litter) of several genera with significant effects on seedling survival and growth.

Table S1 Potential functions of the core bacteria in *A. adenophora* rhizosphere soils.

Table S2 Potential functions of the core bacteria in *A. adenophora* litter.

Table S3 Potential functional guilds of core fungi in *A. adenophora* rhizosphere soils.

Table S4 Potential functional guilds of the core fungi in *A. adenophora* litter.

Table S5 Taxonomy information of 33 representative strains isolated from 40 dead seedlings.

Table S6 PERMANOVA of bacterial communities and function at each inoculation time.

Table S7 PERMANOVA of fungal communities and function at each inoculation time.

Table S8 Information about the abbreviations for the fungal functional guilds shown in Fig. 7D-E.

Table S9 Fertilizer content in the Pindstrup substrate.

References

Abbas, A. M., Alomran, M. M., Alharbi, N. K., & Novak, S. J. (2023). Suppression of Seedling Survival and Recruitment of the Invasive Tree Prosopis juliflora in Saudi Arabia through Its Own Leaf Litter: Greenhouse and Field Assessments. *Plants (Basel)*, *12*(4). https://doi.org/10.3390/plants12040959

Ai, C., Zhang, S. Q., Zhang, X., Guo, D. D., Zhou, W., & Huang, S. M. (2018). Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma*, *319*, 156-166. https://doi.org/10.1016/j.geoderma.2018.01.010

Albino, U., Saridakis, D. P., Ferreira, M. C., Hungria, M., Vinuesa, P., & Andrade, G. (2006). High diversity of diazotrophic bacteria associated with the carnivorous plant Drosera villosa var. villosa growing in oligotrophic habitats in Brazil. *Plant and Soil*, *287*(1-2), 199-207. https://doi.org/10.1007/s11104-006-9066-7

Babaahmadi, G., Mehrabi-Koushki, M., & Hayati, J. (2018). Allophoma hayatii sp nov., an undescribed pathogenic fungus causing dieback of Lantana camara in Iran. *Mycological Progress*, *17*(3), 365-379. https://doi.org/10.1007/s11557-017-1360-7

Bagchi, R., Gallery, R. E., Gripenberg, S., Gurr, S. J., Narayan, L., Addis, C. E., Freckleton, R. P., & Lewis, O. T. (2014). Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature*, *506*(7486), 85-+. https://doi.org/10.1038/nature12911

Baker, H. G. (1974). The Evolution of Weeds. *Annual Review of Ecology and Systematics*, *5*(1), 1-24. https://doi.org/10.1146/annurev.es.05.110174.000245

Barton, J., Fowler, S. V., Gianotti, A. F., Winks, C. J., de Beurs, M., Arnold, G. C., & Forrester, G. (2007). Successful biological control of mist flower (Ageratina riparia) in New Zealand: Agent establishment, impact and benefits to the native flora. *Biological Control*, *40*(3), 370-385. https://doi.org/10.1016/j.biocontrol.2006.09.010

Bauer, J. T., Blumenthal, N., Miller, A. J., Ferguson, J. K., & Reynolds, H. L. (2017). Effects of between-

site variation in soil microbial communities and plant-soil feedbacks on the productivity and composition of plant communities. *Journal of Applied Ecology*, *54*(4), 1028-1039. https://doi.org/10.1111/1365-2664.12937

Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the Soil Community into Plant Population Dynamics: The Utility of the Feedback Approach. *Journal of Ecology*, *85*(5), 561-573. https://doi.org/10.2307/2960528

Brinkman, E. P., Van der Putten, W. H., Bakker, E. J., & Verhoeven, K. J. F. (2010). Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology*, *98*(5), 1063-1073. https://doi.org/10.1111/j.1365-2745.2010.01695.x

Britto, D. T., & Kronzucker, H. J. (2002). NH4+ toxicity in higher plants: a critical review. *Journal of Plant Physiology*, *159*(6), 567-584. https://doi.org/10.1078/0176-1617-0774

Bruce Williamson, G., & Richardson, D. (1988). Bioassays for allelopathy: Measuring treatment responses with independent controls. *Journal of Chemical Ecology*, *14*(1), 181-187. https://doi.org/10.1007/BF01022540

Bunyoo, C., Roongsattham, P., Khumwan, S., Phonmakham, J., Wonnapinij, P., & Thamchaipenet, A. (2022). Dynamic Alteration of Microbial Communities of Duckweeds from Nature to Nutrient-Deficient Condition. *Plants-Basel*, *11*(21), Article 2915. https://doi.org/10.3390/plants11212915

Callaway, R. M., Montesinos, D., Williams, K., & Maron, J. L. (2013). Native congeners provide biotic resistance to invasive Potentilla through soil biota. *Ecology*, *94*(6), 1223-1229. https://doi.org/10.1890/12-1875.1

Chen, L., Fang, K., Zhou, J., Yang, Z. P., Dong, X. F., Dai, G. H., & Zhang, H. B. (2019). Enrichment of soil rare bacteria in root by an invasive plant Ageratina adenophora. *Science of the Total Environment*, *683*, 202-209. https://doi.org/10.1016/j.scitotenv.2019.05.220

Chen, L., Yang, A.-L., Li, Y.-X., & Zhang, H.-B. (2022). Virulence and Host Range of Fungi Associated With the Invasive Plant Ageratina adenophora [Original Research]. *Front Microbiol*, *13*. https://doi.org/10.3389/fmicb.2022.857796

Contreras-Cornejo, H. A., Macías-Rodríguez, L., Cortés-Penagos, C., & López-Bucio, J. (2009). Trichoderma virens, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in Arabidopsis. *Plant Physiology*, *149*(3), 1579-1592. https://doi.org/10.1104/pp.108.130369 %J Plant Physiology

Day, M. D., & Riding, N. (2019). Host specificity of Puccinia spegazzinii (Pucciniales: pucciniaceae), a biological control agent for Mikania micrantha (Asteraceae) in Australia. *Biocontrol Science and Technology*, *29*(1), 19-27. https://doi.org/10.1080/09583157.2018.1520807

Delaye, L., Garcia-Guzman, G., & Heil, M. (2013). Endophytes versus biotrophic and necrotrophic pathogens-are fungal lifestyles evolutionarily stable traits? *Fungal Diversity*, *60*(1), 125-135. https://doi.org/10.1007/s13225-013-0240-y

Demey, A., Staelens, J., Baeten, L., Boeckx, P., Hermy, M., Kattge, J., & Verheyen, K. (2013). Nutrient input from hemiparasitic litter favors plant species with a fast-growth strategy. *Plant and Soil*, *371*(1-2), 53-66. https://doi.org/10.1007/s11104-013-1658-4

Dostál, P. (2021). The temporal development of plant-soil feedback is contingent on competition and nutrient availability contexts. *Oecologia*, *196*(1), 185-194. https://doi.org/10.1007/s00442-021-

04919-6

Fang, K., Bao, Z. S. N., Chen, L., Zhou, J., Yang, Z. P., Dong, X. F., & Zhang, H. B. (2019). Growth-promoting characteristics of potential nitrogen-fixing bacteria in the root of an invasive plant Ageratina adenophora. *Peerj*, *7*, Article e7099. https://doi.org/10.7717/peerj.7099

Fang, K., Chen, L., Zhou, J., Yang, Z.-P., Dong, X.-F., & Zhang, H.-B. (2019). Plant-soil-foliage feedbacks on seed germination and seedling growth of the invasive plant Ageratina adenophora. *Proceedings of the Royal Society B-Biological Sciences*, *286*(1917), Article 20191520. https://doi.org/10.1098/rspb.2019.1520

Fang, K., Chen, L. M., & Zhang, H. B. (2021). Evaluation of foliar fungus-mediated interactions with below and aboveground enemies of the invasive plant Ageratina adenophora. *ECOLOGY AND EVOLUTION*, *11*(1), 526-535. https://doi.org/10.1002/ece3.7072

Fang, K., Zhou, J., Chen, L., Li, Y.-X., Yang, A.-L., Dong, X.-F., & Zhang, H.-B. (2021). Virulence and community dynamics of fungal species with vertical and horizontal transmission on a plant with multiple infections. *PLOS Pathogens*, *17*(7), e1009769. https://doi.org/10.1371/journal.ppat.1009769

Flory, S. L., & Clay, K. (2013). Pathogen accumulation and long-term dynamics of plant invasions. *Journal of Ecology*, *101*(3), 607-613. https://doi.org/10.1111/1365-2745.12078

Garibaldi, A., Gilardi, G., Ortu, G., & Gullino, M. L. (2012). First Report of Leaf Spot of Lettuce (Lactuca sativa L.) Caused by Phoma tropica in Italy. *Plant Disease*, *96*(9), 1380-1380. https://doi.org/10.1094/pdis-04-12-0394-pdn

Geisen, S., ten Hooven, F. C., Kostenko, O., Snoek, L. B., & van der Putten, W. H. (2021). Fungal root endophytes influence plants in a species-specific manner that depends on plant's growth stage. *JOURNAL OF ECOLOGY*, *109*(4), 1618-1632. https://doi.org/10.1111/1365-2745.13584

Gross, K. L. (1984). Effects of seed size and growth form on seedling establishment of 6 monocarpic perennial plants. *Journal of Ecology*, *72*(2), 369-387. https://doi.org/10.2307/2260053

Gu, C., Tu, Y., Liu, L., Wei, B., Zhang, Y., Yu, H., Wang, X., Yangjin, Z., Zhang, B., & Cui, B. (2021). Predicting the potential global distribution of Ageratina adenophora under current and future climate change scenarios. *ECOLOGY AND EVOLUTION*, *11*(17), 12092-12113. https://doi.org/10.1002/ece3.7974

Gustafson, D. J., & Casper, B. B. (2004). Nutrient addition affects AM fungal performance and expression of plant/fungal feedback in three serpentine grasses. *PLANT AND SOIL*, *259*(1-2), 9-17. https://doi.org/10.1023/B:PLSO.0000020936.56786.a4

Herrera, S. D., Grossi, C., Zawoznik, M., & Groppa, M. D. (2016). Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of Fusarium graminearum. *Microbiol Res*, *186*, 37-43. https://doi.org/10.1016/j.micres.2016.03.002

Jessen, M.-T., Auge, H., Harpole, W. S., & Eskelinen, A. (2023). Litter accumulation, not light limitation, drives early plant recruitment. *Journal of Ecology*, *111*(6), 1174-1187. https://doi.org/https://doi.org/10.1111/1365-2745.14099

Jevon, F. V., Record, S., Grady, J., Lang, A. K., Orwig, D. A., Ayres, M. P., & Matthes, J. H. (2020). Seedling survival declines with increasing conspecific density in a common temperate tree. *Ecosphere*, *11*(11), e03292. https://doi.org/https://doi.org/10.1002/ecs2.3292

Jimenez, J. A., Novinscak, A., & Filion, M. (2020). Pseudomonas fluorescens LBUM677 differentially

increases plant biomass, total oil content and lipid composition in three oilseed crops. *Journal of Applied Microbiology*, *128*(4), 1119-1127. https://doi.org/10.1111/jam.14536

Julien, M. H., & Griffiths, M. W. (1998). *Biological control of weeds: a world catalogue of agents and their target weeds.. ed. 4*. C.A.B. International.

Kardol, P., Cornips, N. J., van Kempen, M. M. L., Bakx-Schotman, J. M. T., & van der Putten, W. H. (2007). Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs*, *77*(2), 147-162. https://doi.org/10.1890/06-0502

Khalaf, E. M., & Raizada, M. N. (2016). Taxonomic and functional diversity of cultured seed associated microbes of the cucurbit family. *BMC microbiology*, *16*, Article 131. https://doi.org/10.1186/s12866-016-0743-2

Kluge, R. L. (1991). Biological control of crofton weed, Ageratina adenophora (Asteraceae), in South Africa. *Agriculture, Ecosystems & Environment*, *37*(1), 187-191. https://doi.org/https://doi.org/10.1016/0167-8809(91)90146-O

Lamb, E. G. (2008). Direct and indirect control of grassland community structure by litter, resources, and biomass. *Ecology*, *89*(1), 216-225. https://doi.org/10.1890/07-0393.1

Leiminger, J., Bassler, E., Knappe, C., Bahnweg, G., & Hausladen, H. (2015). Quantification of disease progression of Alternaria spp. on potato using real-time PCR. *European Journal of Plant Pathology*, *141*(2), 295-309. https://doi.org/10.1007/s10658-014-0542-2

Liu, B., Daryanto, S., Wang, L. X., Li, Y. J., Liu, Q. Q., Zhao, C., & Wang, Z. N. (2017). Excessive Accumulation of Chinese Fir Litter Inhibits Its Own Seedling Emergence and Early Growth-A Greenhouse Perspective. *Forests*, *8*(9), Article 341. https://doi.org/10.3390/f8090341

Liu, Y., Zheng, Y. L., Jahn, L. V., & Burns, J. H. (2023). Invaders responded more positively to soil biota than native or noninvasive introduced species, consistent with enemy escape. *Biological Invasions*, *25*(2), 351-364. https://doi.org/10.1007/s10530-022-02919-y

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *SCIENCE*, *353*(6305), 1272-1277. https://doi.org/10.1126/science.aaf4507

Loydi, A., Eckstein, R. L., Otte, A., & Donath, T. W. (2013). Effects of litter on seedling establishment in natural and semi-natural grasslands: a meta-analysis. *Journal of Ecology*, *101*(2), 454-464. https://doi.org/https://doi.org/10.1111/1365-2745.12033

Lu, P., Ma, K., & Sang, W. (2006). Effects of environmental factors on germination and emergence of Crofton weed (Eupatorium adenophorum). *Weed Science*, *54*(3), 452-457. https://doi.org/10.1614/WS-05-174R1.1

Luo, Y., Wang, F., Huang, Y. L., Zhou, M., Gao, J. L., Yan, T. Z., Sheng, H. M., & An, L. Z. (2019). Sphingomonas sp. Cra20 Increases Plant Growth Rate and Alters Rhizosphere Microbial Community Structure of Arabidopsis thaliana Under Drought Stress. *Front Microbiol*, *10*, Article 1221. https://doi.org/10.3389/fmicb.2019.01221

Ma, Z. W., Wang, Y. X., Gu, Y. C., Bowatte, S., Zhou, Q. P., & Hou, F. J. (2020). Effects of Litter Leachate on Plant Community Characteristics of Alpine Grassland in Qinghai Tibetan Plateau. *Rangeland Ecology & Management*, *73*(1), 147-155. https://doi.org/10.1016/j.rama.2019.10.003

Mitchell, C. E., & Power, A. G. (2003). Release of invasive plants from fungal and viral pathogens [Article]. *Nature*, *421*(6923), 625-627. https://doi.org/10.1038/nature01317

Möhler, H., Diekötter, T., Bauer, G. M., & Donath, T. W. (2021). Conspecific and heterospecific grass litter effects on seedling emergence and growth in ragwort (Jacobaea vulgaris). *PLoS One*, *16*(2), e0246459. https://doi.org/10.1371/journal.pone.0246459

Möhler, H., Diekötter, T., Herrmann, J. D., & Donath, T. W. (2018). Allelopathic vs. autotoxic potential of a grassland weed—evidence from a seed germination experiment. *Plant Ecology & Diversity*, *11*(4), 539-549. https://doi.org/10.1080/17550874.2018.1541487

Nguyen, N. H., Song, Z. W., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *FUNGAL ECOLOGY*, *20*, 241-248. https://doi.org/10.1016/j.funeco.2015.06.006

Niu, H. B., Liu, W. X., Wan, F. H., & Liu, B. (2007). An invasive aster (Ageratina adenophora) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant and Soil*, *294*(1-2), 73-85. https://doi.org/10.1007/s11104-007-9230-8

Olson, B. E., & Wallander, R. T. (2002). Effects of invasive forb litter on seed germination, seedling growth and survival. *Basic and Applied Ecology*, *3*(4), 309-317. https://doi.org/10.1078/1439-1791-00127

Ozimek, E., & Hanaka, A. (2021). Mortierella Species as the Plant Growth-Promoting Fungi Present in the Agricultural Soils. *Agriculture-Basel*, *11*(1), 7. https://www.mdpi.com/2077-0472/11/1/7

Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, *401*(6756), 877-884. https://doi.org/10.1038/44766

Parsons WT, C. E. (2001). *Noxious weeds of Australia*. CSIRO Publishing.

Qiao, C. C., Penton, C. R., Xiong, W., Liu, C., Wang, R. F., Liu, Z. Y., Xu, X., Li, R., & Shen, Q. R. (2019). Reshaping the rhizosphere microbiome by bio-organic amendment to enhance crop yield in a maize-cabbage rotation system. *APPLIED SOIL ECOLOGY*, *142*, 136-146. https://doi.org/10.1016/j.apsoil.2019.04.014

Sanchez-Lopez, A. S., Thijs, S., Beckers, B., Gonzalez-Chavez, M. C., Weyens, N., Carrillo-Gonzalez, R., & Vangronsveld, J. (2018). Community structure and diversity of endophytic bacteria in seeds of three consecutive generations of Crotalaria pumila growing on metal mine residues. *Plant and Soil*, *422*(1-2), 51-66. https://doi.org/10.1007/s11104-017-3176-2

Schloter, M., & Matyssek, R. (2009). Tuning growth versus defence–belowground interactions and plant resource allocation. *Plant and Soil*, *323*(1), 1-5. https://doi.org/10.1007/s11104-009-0070-6

Shan, L. P., Oduor, A. M. O., Huang, W., & Liu, Y. J. (2023). Nutrient enrichment promotes invasion success of alien plants via increased growth and suppression of chemical defenses. *Ecological Applications*. https://doi.org/10.1002/eap.2791

Shao, J. H., Miao, Y. Z., Liu, K. M., Ren, Y., Xu, Z. H., Zhang, N., Feng, H. C., Shen, Q. R., Zhang, R. F., & Xun, W. B. (2021). Rhizosphere microbiome assembly involves seed-borne bacteria in compensatory phosphate solubilization. *SOIL BIOLOGY & BIOCHEMISTRY*, *159*, Article 108273. https://doi.org/10.1016/j.soilbio.2021.108273

Slate, M. L., Matallana-Mejia, N., Aromin, A., & Callaway, R. M. (2022). Nitrogen addition, but not pulse frequency, shifts competitive interactions in favor of exotic invasive plant species. *Biological Invasions*, *24*(10), 3109-3118. https://doi.org/10.1007/s10530-022-02833-3

Teste, F. P., Kardol, P., Turner, B. L., Wardle, D. A., Zemunik, G., Renton, M., & Laliberte, E. (2017). Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *SCIENCE*, *355*(6321), 173-+. https://doi.org/10.1126/science.aai8291

Thomma, B. P. (2003). Alternaria spp.: from general saprophyte to specific parasite. *Mol Plant Pathol*, *4*(4), 225-236. https://doi.org/10.1046/j.1364-3703.2003.00173.x

Tian, B., Xie, J., Fu, Y., Cheng, J., Li, B., Chen, T., Zhao, Y., Gao, Z., Yang, P., Barbetti, M. J., Tyler, B. M., & Jiang, D. (2020). A cosmopolitan fungal pathogen of dicots adopts an endophytic lifestyle on cereal crops and protects them from major fungal diseases. *Isme j*, *14*(12), 3120-3135. https://doi.org/10.1038/s41396-020-00744-6

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N., Van de Voorde, T. F. J., & Wardle, D. A. (2013). Plant-soil feedbacks: the past, the present and future challenges. *JOURNAL OF ECOLOGY*, *101*(2), 265-276. https://doi.org/10.1111/1365-2745.12054

Vergnes, D. M., Renard, M. E., Duveiller, E., & Maraite, H. (2006). Identification of Alternaria spp. on wheat by pathogenicity assays and sequencing. *Plant Pathology*, *55*(4), 485-493. https://doi.org/10.1111/j.1365-3059.2006.01391.x

Wang, Y.-L., & Zhang, H.-B. (2023). Assembly and Function of Seed Endophytes in Response to Environmental Stress. *Journal of microbiology and biotechnology*, *33*(9), 1-11. https://doi.org/10.4014/jmb.2303.03004

Wang, Z. N., Wang, D. Y., Liu, Q. Q., Xing, X. S., Liu, B., Jin, S. F., & Tigabu, M. (2022). Meta-Analysis of Effects of Forest Litter on Seedling Establishment. *Forests*, *13*(5), Article 644. https://doi.org/10.3390/f13050644

Wani, Z. A., Kumar, A., Sultan, P., Bindu, K., Riyaz-Ul-Hassan, S., & Ashraf, N. (2017). Mortierella alpina CS10E4, an oleaginous fungal endophyte of Crocus sativus L. enhances apocarotenoid biosynthesis and stress tolerance in the host plant. *Scientific Reports*, *7*, Article 8598. https://doi.org/10.1038/s41598-017-08974-z

Whitaker, B. K., Bauer, J. T., Bever, J. D., & Clay, K. (2017). Negative plant-phyllosphere feedbacks in native Asteraceae hosts - a novel extension of the plant-soil feedback framework. *Ecol Lett*, *20*(8), 1064-1073. https://doi.org/10.1111/ele.12805

Widdig, M., Heintz-Buschart, A., Schleuss, P. M., Guhr, A., Borer, E. T., Seabloom, E. W., & Spohn, M. (2020). Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *SOIL BIOLOGY & BIOCHEMISTRY*, *151*, Article 108041. https://doi.org/10.1016/j.soilbio.2020.108041

Wood, A. R. (2012). Uromycladium tepperianum (a gall-forming rust fungus) causes a sustained epidemic on the weed Acacia saligna in South Africa. *Australasian Plant Pathology*, *41*(3), 255-261. https://doi.org/10.1007/s13313-012-0126-6

Xiong, S., & Nilsson, C. (1999). The effects of plant litter on vegetation: a meta-analysis. *Journal of Ecology*, *87*(6), 984-994. https://doi.org/https://doi.org/10.1046/j.1365-2745.1999.00414.x

Xu, C. W., Yang, M. Z., Chen, Y. J., Chen, L. M., Zhang, D. Z., Mei, L., Shi, Y. T., & Zhang, H. B. (2012). Changes in non-symbiotic nitrogen-fixing bacteria inhabiting rhizosphere soils of an invasive plant Ageratina adenophora. *APPLIED SOIL ECOLOGY*, *54*, 32-38. https://doi.org/10.1016/j.apsoil.2011.10.021

Xu, L., Naylor, D., Dong, Z. B., Simmons, T., Pierroz, G., Hixson, K. K., Kim, Y. M., Zink, E. M., Engbrecht, K. M., Wang, Y., Gao, C., DeGraaf, S., Madera, M. A., Sievert, J. A., Hollingsworth, J., Birdseye, D., Scheller, H. V., Hutmacher, R., Dahlberg, J., . . . Coleman-Derr, D. (2018). Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(18), E4284-E4293. https://doi.org/10.1073/pnas.1717308115

Yang, A.-L., Chen, L., Cheng, L., Li, J.-P., Zeng, Z.-Y., & Zhang, H.-B. (2023). Two Novel Species of Mesophoma gen. nov. from China. *Current Microbiology*, *80*(4), 129. https://doi.org/10.1007/s00284-023-03238-8

Zaret, M. M., Bauer, J. T., Clay, K., & Whitaker, B. K. (2021). Conspecific leaf litter induces negative feedbacks in Asteraceae seedlings. *Ecology*, *102*(12), e03557. https://doi.org/https://doi.org/10.1002/ecy.3557

Zhang, A., Wang, D., & Wan, S. Q. (2019). Litter addition decreases plant diversity by suppressing seeding in a semiarid grassland, Northern China. *Ecology and Evolution*, *9*(17), 9907-9915. https://doi.org/10.1002/ece3.5532

Zhang, R., Hu, X., Baskin, J. M., Baskin, C. C., & Wang, Y. (2017). Effects of Litter on Seedling Emergence and Seed Persistence of Three Common Species on the Loess Plateau in Northwestern China. *Front Plant Sci*, *8*, 103. https://doi.org/10.3389/fpls.2017.00103

Zhang, X., Ni, X., Hedĕnec, P., Yue, K., Wei, X., Yang, J., & Wu, F. (2022). Litter facilitates plant development but restricts seedling establishment during vegetation regeneration. *Functional Ecology*, *36*(12), 3134-3147. https://doi.org/https://doi.org/10.1111/1365-2435.14200

Zhang, Z. J., Liu, Y. J., Brunel, C., & van Kleunen, M. (2020). Evidence for Elton's diversity-invasibility hypothesis from belowground. *Ecology*, *101*(12). https://doi.org/10.1002/ecy.3187

Zhao, M. X., Lu, X. F., Zhao, H. X., Yang, Y. F., Hale, L., Gao, Q., Liu, W. X., Guo, J. Y., Li, Q., Zhou, J. Z., & Wan, F. H. (2019). Ageratina adenophora invasions are associated with microbially mediated differences in biogeochemical cycles. *Science of the Total Environment*, *677*, 47-56. https://doi.org/10.1016/j.scitotenv.2019.04.330

Zheng, Y. L., Feng, Y. L., Liu, W. X., & Liao, Z. Y. (2009). Growth, biomass allocation, morphology, and photosynthesis of invasive Eupatorium adenophorum and its native congeners grown at four irradiances. *Plant Ecology*, *203*(2), 263-271. https://doi.org/10.1007/s11258-008-9544-5