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Parasitic plants indirectly regulate decomposition of soil organic matter

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Abstract

1. Parasitic plants have been shown to affect soil-organic-matter (SOM) decomposition, but the mechanism is unknown. As arbuscular mycorrhizal fungi (AMF) can affect decomposition and compete with parasitic plants for carbon, we hypothesized that parasitic plants can indirectly regulate SOM decomposition by suppressing the effects of AMF on decomposition.
2. To test this hypothesis, we conducted two container experiments in which the herbaceous plant *Bidens pilosa* was inoculated with the AMF *Rhizophagus intraradices* or not, and *Cuscuta australis* or not. In one experiment, we provided SOM within hyphae-in-growth bags as ^{13}C-/^{15}N-labelled maize leaves and in the other experiment as phytate-P. We assessed growth and nutrient uptake of *B. pilosa*, growth of *C. australis*, the SOM remaining in the hyphae-in-growth bags, and the bacterial communities.
3. Parasitization increased the ^{13}C and decreased the organic P remaining in the bags, but only in the presence of the extraradical AMF hyphae. AMF decreased the ^{13}C and increased the organic P remaining in the absence of the parasite, but not in the presence of the parasite.
4. Our results demonstrate that parasitic plants can regulate the decomposition of organic materials indirectly by suppressing the effect of the extraradical AMF hyphae on decomposition. In other words, parasitic plants can regulate SOM decomposition indirectly via a multitrophic cascading effect. Our study helps to unravel the mechanisms of a sophisticated hidden ecological process, and is an important step forward in elucidating the roles of parasitic plants in soil nutrient cycling.

KEYWORDS
arbuscular mycorrhizal fungi, cascading effect, decomposition, multi-trophic interactions, parasitic plant, plant–soil interactions, soil microbes

1 | INTRODUCTION

Trophic cascades and top-down control, in which organisms at higher trophic levels regulate organisms at lower trophic levels, are important drivers of ecosystem processes. Great attention has been directed toward predator-based food webs (Estes et al., 2011; Ripple et al., 2014), but few studies have explored the role of top-down cascades in regulating decomposition of organic matter and nutrient

cycling. Parasitic plants obtain part or all of their resources from their hosts, thereby reducing host productivity (Bardgett et al., 2006; Sui et al., 2019; Yuan et al., 2021). Parasitic plants are also known to cause top-down cascading effects on the decomposition of soil organic matter (SOM; Bardgett et al., 2006; Di et al., 2017; Li et al., 2008). For example, Bardgett et al. (2006) showed that the hemiparasite *Rhinanthus minor* L. indirectly promotes nitrogen (N) mineralization and thereby modifies the availability of mineral N relative to dissolved organic N. However, the mechanism underlying the cascading effects of parasitic plants on decomposition of organic matter remains unknown.

Increasing numbers of studies show that parasitic plants can affect soil nutrient cycling (Ameloot et al., 2008; March & Watson, 2010; Ndagurwa et al., 2014, 2015; Quested, 2008; Spasojevic & Suding, 2011). For example, the concentrations of nutrients, like N, phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), in the soil beneath parasitized trees differed significantly from those beneath nonparasitized trees (Muvengwi et al., 2015; Ndagurwa et al., 2016). Possible explanations for this may be the litter pathway or parasitism pathway. The litter pathway refers to the direct input of nutrient-rich litter of the parasitic plant into the soil, or the accelerated soil nutrient turnover of parasitic litter (Demey et al., 2014; Ndagurwa et al., 2020; Spasojevic & Suding, 2011). The parasitism pathway refers to the direct negative effect of the parasite on the host plant and the subsequent cascading effects on soil microbes and soil nutrient cycling (Bardgett et al., 2006; Press & Phoenix, 2005; Yuan et al., 2021). For example, parasitism on host plants can indirectly affect the microbial communities, including arbuscular mycorrhizal fungi (AMF; Brunel et al., 2020), which may affect nutrient cycling.

AMF form symbiotic associations with the roots of about 72% of all plant species (Brundrett & Tedersoo, 2018). AMF acquire soil nutrients, especially N and P, with their mycelium and pass these on to their host plants in return for carbon (Jiang et al., 2017; Luginbuehl et al., 2017). Previous studies suggested that AMF can also affect nutrient availability by promoting (Cheng et al., 2012; Hodge et al., 2001) or inhibiting (Carrillo et al., 2016; Leifheit et al., 2015) decomposition of SOM. However, the underlying mechanism is still unclear. One hypothesis is that AMF do not have any saprotrophic capability themselves, but that they influence associated microbial decomposers (Cheng et al., 2012; Hodge et al., 2001; Schäfer et al., 2019; Verbruggen et al., 2016). For example, AMF could carry and enrich microbial decomposers, and affect their activity by providing them with carbon (C; Jansa & Hodge, 2021; Jiang et al., 2021; Zhang, Xu, et al., 2016) or by changing the physicochemical environment (Ding et al., 2014; Wang et al., 2013). In line with this hypothesis, Zhang, Xu, et al. (2016) found that AMF can provide C for the growth and activity of phosphate-solubilizing bacteria. On the other hand, Jannoura et al. (2012) reported that in N-deficient soil, the presence of AMF can decrease N supply to microbial decomposers, and thereby reduce SOM decomposition.

Several studies have shown that AMF can not only affect the strength of the direct negative effect of parasitic plants on their host plants (Li et al., 2013; Sui et al., 2019), but that they could also cascade effects of parasitic plants to other trophic levels (Yuan et al., 2021). For example, the stem holoparasite *Cuscuta australis* R.Br. can indirectly affect growth of neighbouring unparasitized plants via the belowground hyphal bridges connecting host and neighbouring plants (Yuan et al., 2021). Because both parasitic plants and AMF obtain C from their host plants (Bardgett et al., 2006; Brundrett & Tedersoo, 2018), the C obtained by parasitic plants may decrease the C available to AMF, and thereby suppress AMF growth. Whether this is the case and could explain the effects of parasitic plants on SOM decomposition is not known yet.

Here, we tested whether and, if so, how the parasitic plant *Cuscuta australis* has a top-down cascading effect on SOM decomposition via one of its host plants, *Bidens pilosa* L., and the extraradical hyphae of an AMF, *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler. As the C allocated to the parasitic plant may reduce the amount of C distributed to the AMF, we hypothesized that the parasitic plant can indirectly regulate SOM decomposition by suppressing the effect of the AMF on decomposition. As these effects might depend on the type of SOM, we assessed the effects of the parasitic plant and the AMF on the decomposition of two types of organic materials, maize leaves and phytate (the most abundant inositol phosphate in the soil; Turner et al., 2002; Wang et al., 2017), which have frequently been used in previous studies testing the effects of AMF on decomposition (Griffiths et al., 2012; Wang et al., 2013). Our study aimed to unravel the possible mechanism by which parasitic plants affect soil nutrient cycling and to improve our understanding of the ecological roles of parasitic plants in ecosystems.

2 | MATERIALS AND METHODS

2.1 | Study species

The annual herb *Bidens pilosa* L. was used as the host plant, and the herbaceous holoparasite *Cuscuta australis* R.Br. was used as the parasite. *Cuscuta australis* acquires water, carbon and other nutrients from its host and can parasitize a wide range of herbaceous species, including *B. pilosa* (Li et al., 2015; Zhang et al., 2012). *Cuscuta australis* was chosen because it is an obligate shoot parasite (i.e. has no roots) and thus cannot have any direct effect on SOM decomposition. *Bidens pilosa* was chosen because it can be parasitized by *C. australis*, and because it can form symbiosis with AMF (Wei et al., 2012).

2.2 | Substrate, containers and seed germination

The substrate used in our study consisted of a 1:1 mixture of field soil and sand (v:v). The field soil was sandy, with a pH of 6.69, 12.8 $g\,kg^{-1}$ organic matter, 0.648 $g\,kg^{-1}$ total N and 0.413 $g\,kg^{-1}$ total P, and was obtained from a riverside in the Jiaojiang District, Taizhou, China. When collecting field soil, we removed the

surface soil and collected 120 kg soil from five randomly chosen points within a 10 m^2 area. To kill all microbes, all substrate was autoclaved at 121°C for 2 h. We filled 64 plastic containers (length × width × height: 20 cm × 10 cm × 11.5 cm) with 1.3 kg autoclaved substrate each. One plastic core (height × diameter: 8.5 cm × 10 cm), filled with 500 g substrate, was dug into the substrate on one side of each container (Figure S1). The plastic core had a lateral opening accounting for a quarter of its total surface area, which was directed toward the other side of the container (Awaydul et al., 2019). The opening was covered with a 25-μm nylon mesh that allowed penetration by AMF hyphae but not by plant roots (Figure S1; Cheng et al., 2012). The plastic core thus divided the container into separate root+hyphae and hyphae-only compartments.

After sterilization in 10% sodium hypochlorite, the seeds of *B. pilosa* were sown into a plastic plate filled with autoclaved peat, and placed in a greenhouse on June 12, 2020. On June 27, 2020, when seedlings of aboveground parts were approximately 2 cm tall (Yuan et al., 2013), we planted a single seedling of *B. pilosa* into the plastic core of each container (Figure S1).

2.3 | Experimental design

We did two experiments that only differed from each other in the organic material used. Experiment 1 used maize leaves and Experiment 2 used calcium phytate. Each experiment had two factors: (1) with or without AMF inoculum (+AMF or −AMF); (2) with or without *C. australis* parasitization of the host plant (+P or −P). Each of the four treatment combinations had eight replicate containers, resulting in 32 containers per experiment, and 64 containers in total.

For the +AMF treatment, each plastic core received 50 g of AMF inoculum. The inoculum was derived from pot cultures of *Sorghum bicolor* (L.) Moench grown in coarse sand, and consisted of root fragments and soil colonized by the AMF *Rhizophagus intraradices*, and its spores. This AMF species was chosen because it is widespread in natural ecosystems (Moora et al., 2011), and can form symbiosis with *B. pilosa* (Zhang, 2015). We mixed the inoculum into the substrate before seedling transplantation. To correct for possible differences in microbial communities of the +AMF and −AMF treatments, each plastic core of the −AMF containers received 50 g of autoclaved inoculum and 100 ml of a filtered solution, made from live AMF inoculum, and from which AMF spores had been excluded (Cheng et al., 2012).

Four weeks after transplantation, one 10-cm long stem piece of *C. australis* was wound around the stems of the *B. pilosa* plants to cause parasitization. This was done in half of the containers, in the other half we had nonparasitized *B. pilosa* as controls (Li et al., 2014). The stems of *C. australis* had been collected from a field population near Taizhou University.

Immediately after addition of the parasite, we buried one hyphae-in-growth bag (length × width: 6.5 cm × 5.5 cm), made of 25-μm nylon mesh, into the container at a distance of 7 cm from the plastic core (Figure S1). For Experiment 1, each bag contained 50 g autoclaved coarse sand and 1.0 g of oven-dried ^{15}N and ^{13}C labelled maize leaves. The grain size of the coarse sand was between 0.425 and 2 mm (Awaydul et al., 2019). The maize leaves were cut into 1 mm pieces and evenly mixed into the sand. Because maize is a C_4 plant, which naturally has a higher $^{13}C/^{12}C$ ratio ($\delta^{13}C$ is −13.6‰) than C_3 plants (Smith & Epstein, 1971), its leaves can be treated as being naturally labelled with the ^{13}C stable isotope. For ^{15}N labelling, we had grown the maize plants on soil supplemented with ^{15}N-$(NH_4)_2SO_4$ (with 99 atom% of enrichment). For Experiment 2, each bag contained 50 g sand and 0.5 g calcium phytate (P0410, Tokyo Chemical Industry), which was not enriched with ^{13}C and ^{15}N.

To assure that SOM decomposing microorganisms would be present in the containers, we added, immediately after insertion of the hyphae-in-growth bags, 50 ml field-soil filtrate to each container. The filtrate was obtained by adding 250 g of unsterilized field soil to 1 L of sterilized water. This was mixed thoroughly and then passed through a 38-μm sieve (Xu et al., 2018), which removes the larger spores and hyphae, such as those of AMF, but allows most other components of the microbial community to pass (Wagg et al., 2014).

The experiments were conducted in a greenhouse at Taizhou University, China, with a daily photoperiod of 16 h, and day and night temperatures of 22°C and 18°C, respectively. Tap water was sprayed evenly onto the soil of each container every day.

2.4 | Harvest and sample preparation

We harvested the experiments 7 weeks after adding the parasite. All hyphae-in-growth bags were carefully removed, and 5 g of soil from each bag was immediately transferred into a −80°C freezer for later DNA extraction and enzyme-activity measurements. The remaining soil from each bag was air-dried and then ground to a fine powder in a ball mill (Retsch Technology GmbH) for nutrient analysis.

The *C. australis* plants (i.e. the parasites) were harvested by detaching them from the *B. pilosa* host plants. The *B. pilosa* plants were separated into shoots and roots. The plant materials were dried in a drying oven at 65°C for 72 h and then weighed. After that, the shoots of *B. pilosa* were ground into powder by using a ball mill for analysis of shoot N and P concentrations using the H_2SO_4-H_2O_2 digestion method (Li et al., 2008). AMF-colonization rate of *B. pilosa* roots was assessed using the gridline-intersection method (Giovannetti & Mosse, 1980). No AMF colonization was found in the −AMF treatment.

2.5 | Measurement of ^{15}N and ^{13}C

In Experiment 1, the ^{15}N and ^{13}C stable isotope fractions in the hyphae-in-growth bags and in the shoot of *B. pilosa* were determined using a continuous-flow isotope-ratio mass spectrometer (CF-IRMS, Thermo

Finnigan DELTA Plus). The $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) values were converted to absolute isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$). The ^{15}N and ^{13}C concentrations of the sample were then calculated from fractional abundances ($^{15}N/[^{15}N+^{14}N]$ and $^{13}C/[^{13}C+^{12}C]$) and the total N and C concentration, respectively, of the sample (Cheng et al., 2012; Yuan et al., 2021). Then, the percentages of ^{15}N and ^{13}C remaining within the hyphae-in-growth bags were calculated as the total amounts of N and C, respectively, in the samples at the end of the experiment divided by the initial values (i.e. when we added the bags to the containers).

2.6 | Measurements of enzyme activities and remaining organic P

In Experiment 2, because phytase and acid phosphatase play key roles in decomposition of phytate (Ding et al., 2014; Wang et al., 2013), we tested their activities using the soil phytase ELISA kit (Jiangsu Yutong Biotechnology Co., Ltd) and the soil phosphatase Assay kit (Jiangsu Yutong Biotechnology Co., Ltd), according to the manufacturer's protocols. The remaining organic P concentration of the substrate in the hyphae-in-growth bags was also determined, as it reflects the decomposition of phytate (Ding et al., 2014). It was calculated by deducting the inorganic P concentration from the total P concentration (Ding et al., 2014). The total P concentration in the bags was determined using *a continuous flow analyser*, and the inorganic P concentration was determined using the Olsen method (Jackson, 1973).

2.7 | Analysis of bacterial communities

Three hyphae-in-growth bags per treatment in each experiment (i.e. 24 bags in total) were randomly selected for analysis of the bacterial communities using the 16 S amplicon-sequencing method (for details, see Method S1). As measures of microbial alpha diversity, we calculated the Shannon, Simpson and Pielou's evenness indices. As measure of beta diversity, we calculated dissimilarities among bacterial communities by using non-metric multidimensional scaling (NMDS) at the OTU level based on the unweighted UniFrac distance metric. In addition, permutational analysis of variance (PERMANOVA) was used to test for effects of AMF inoculation, parasitization, and their interaction on bacterial community composition. PERMANOVA was also used to test for differences in bacterial community composition between hyphae-in-growth bags with maize leaves and those with phytate. These analyses were performed in R (version 3.1.2; R Core Team, 2014, http://www.r-project.org/) using the VEGAN package version 3.6 (Oksanen et al., 2011).

2.8 | Statistical analysis

Two-way ANOVAs were used to test the effects of parasitization and AMF inoculation on the remaining amounts of ^{15}N, ^{13}C and organic P, and on enzyme activities in the hyphae-in-growth bags, as well as on the growth and nutrient uptake of *B. pilosa*. We checked for normality of the residuals using the Shapiro–Wilk test. The independent-sample t test was used to test the effect of parasitization on AMF colonization of *B. pilosa* in +AMF containers (there was no colonization in the −AMF containers), and the effect of AMF inoculation on biomass of *C. australis*. Pearson's correlation analysis was performed to determine the association between shoot ^{15}N and shoot P concentrations in Experiment 1. Pearson's correlation analysis was also performed to determine the associations between acid phosphatase activity in the hyphae-in-growth bags and shoot N concentration with shoot P concentration in Experiment 2. Data were transformed if necessary to improve normality. Specifically, we used sin-square-root for % ^{15}N remaining in the hyphae-in-growth bags, square for AMF colonization rate in Experiment 1, sine for shoot P content in Experiment 1, and natural logarithm ($\log_e$) for shoot P concentration and for biomass of *C. australis* in both experiments. Differences between treatment combinations were compared using LSD at the 5% significance level. The Shapiro–Wilk tests were run in R, version 3.5.0 using the shapiro.test function, and all other analyses were performed using the Statistical Product and Service Solution (SPSS) software (version 16.0; SPSS Inc.).

3 | RESULTS

3.1 | Effects of the parasite and AMF on decomposition and enzymatic activities

In Experiment 1 (with maize leaves), the percentage of ^{15}N remaining in the hyphae-in-growth bags was not significantly affected by parasitization and AMF inoculation (Table 1; Figure 1a). However, the percentage of ^{13}C remaining in the bags was significantly lower in the +AMF/−P treatment combination than in the other three treatment combinations (significant AMF × parasitism interaction in Table 1; $F_{1,26} = 6.373$, $P = 0.018$; Figure 1b).

In Experiment 2 (with phytate), the organic P remaining in the hyphae-in-growth bags was higher in the +AMF than in the −AMF treatment, and higher in the −P than in the +P treatment (Table 1; Figure 1c). Although the effect of the AMF treatment was stronger for −P than for +P (Figure 1c), the AMF × parasitism interaction was not statistically significant (Table 1). Furthermore, AMF inoculation significantly decreased the phytase activity, whereas parasitization did not (Figure 1d). Parasitization tended to slightly increase the acid phosphatase activity, although this effect was only visible for +AMF (Table 1; Figure 1e).

3.2 | Effects of parasite on AMF-colonization rates of *Bidens pilosa*

Parasitization significantly decreased the AMF-colonization rate of *B. pilosa* in Experiment 1 ($t = 2.519$, $P = 0.025$; Figure 2a), but not in Experiment 2 ($t = 1.084$, $p = 0.297$; Figure 2b).

TABLE 1 Effects of AMF inoculation and parasitization by *Cuscuta australis* on ^{15}N and ^{13}C remaining in hyphae-in-growth bags, on ^{15}N uptake by *Bidens pilosa* in Experiment 1, and on the organic P remaining and the activities of the enzymes phytase and acid phosphatase in the hyphae-in-growth bags in Experiment 2. Significant differences ($p < 0.05$) are marked in bold

	Variables	Source of variation	df	*F*	*p*
Experiment 1	^{15}N remaining	AMF	1, 26	2.152	0.154
		parasitism	1, 26	1.301	0.264
		AMF × parasitism	1, 26	0.728	0.401
	^{13}C remaining	AMF	1, 26	0.862	0.362
		parasitism	1, 26	1.933	0.176
		AMF × parasitism	1, 26	6.373	**0.018**
	Shoot ^{15}N concentration	AMF	1, 28	0.484	0.492
		parasitism	1, 28	7.620	**0.010**
		AMF × parasitism	1, 28	2.694	0.112
Experiment 2	Organic P remaining	AMF	1, 25	4.428	**0.046**
		parasitism	1, 25	4.767	**0.039**
		AMF × parasitism	1, 25	1.282	0.268
	Phytase activity	AMF	1, 28	9.709	**0.004**
		parasitism	1, 28	<0.001	0.995
		AMF × parasitism	1, 28	0.005	0.945
	Phosphatase activity	AMF	1, 28	0.939	0.341
		parasitism	1, 28	2.990	0.095
		AMF × parasitism	1, 28	1.520	0.228

3.3 | Effects of the parasite and AMF on bacterial communities

In Experiment 1, a total of 1,258,474 16S reads, belonging to 27 bacterial phyla, were detected. The bacterial communities were dominated by the phyla Firmicutes (~50%), Proteobacteria (~29%), and Actinobacteria (~12%; Figure S2a; Table S1), and by the genera *Bacillus* (~36%), *Devosia* (~3%) and *Pseudomonas* (~2%; Figure S3a; Table S3). In the presence of AMF, parasitization significantly increased the abundance of the phylum Gemmatimonadetes ($F_{1,4} = 8.65$, $p = 0.042$; Table S1). Most of the dominant bacterial genera did not show significant differences among the treatment combinations, with the exception that for +AMF, parasitization significantly increased the abundance of *Bacillus* ($F_{1,4} = 22.24$, $P = 0.009$; Table S3). Furthermore, AMF inoculation significantly decreased the abundance of the genus *Paenibacillus* ($F_{1,10} = 10.52$; $P = 0.009$; Table S3). For the three measures of alpha diversity of the bacterial communities, there was only a significant effect for the Simpson index: parasitization significantly decreased it for +AMF ($F_{1,4} = 34.26$; $P = 0.004$; Figure S4c). PERMANOVA showed that the bacterial community composition was not significantly affected by AMF inoculation ($r^2 = 0.101$, $p = 0.234$), parasitization ($r^2 = 0.072$, $p = 0.937$) and their interaction ($r^2 = 0.109$, $p = 0.143$; Figure 3a).

In Experiment 2, a total of 1,209,931 16S reads, belonging to 32 bacterial phyla, were detected. The bacterial communities were dominated by the phyla Proteobacteria (~44%), Actinobacteria (~26%) and Firmicutes (~12%; Figure S2b; Table S2), and by the genera *TRA3-20* (~4%), *Pseudomonas* (~4%) and *Streptomyces* (~3%; Figure S3b; Table S4). AMF inoculation significantly decreased the abundance of the dominant phyla Firmicutes ($F_{1,10} = 8.77$; $p = 0.014$; Table S2) and Cyanobacteria ($F_{1,10} = 5.12$; $p = 0.047$; Table S2). Most bacterial genera did not show significant differences among the treatment combinations, except for +AMF, where parasitization significantly decreased the abundance of the genus *Streptomyces* ($F_{1,4} = 28.07$; $p = 0.006$; Table S4). The alpha diversity of the bacterial community was not significantly affected by AMF inoculation and parasitization (Figure S4). PERMANOVA showed that the bacterial composition was significantly affected by AMF inoculation ($r^2 = 0.118$, $p = 0.038$), but not by parasitization ($r^2 = 0.082$, $p = 0.643$) and their interaction ($r^2 = 0.085$, $p = 0.590$; Figure 3b).

3.4 | Effects on growth and nutrient uptake of *Bidens pilosa* and *Cuscuta australis*

In both experiments, parasitization significantly decreased biomass of *B. pilosa*, but this effect was only significant for +AMF (Figure S6a,c). The root weight ratio of *B. pilosa* tended to be lower for parasitized plants, but this effect was not statistically significant (Figure S6b,d). In both experiments, AMF inoculation did not significantly affect biomass of *C. australis* (Figure S7).

In Experiment 1, parasitization significantly increased shoot ^{15}N concentration of *B. pilosa*, but this was only statistically significant for +AMF (Figure 4a). Moreover, for +AMF, shoot ^{15}N and P concentration of *B. pilosa* was significantly positively correlated (Figure 4b), suggesting that the uptake of both nutrients was linked.

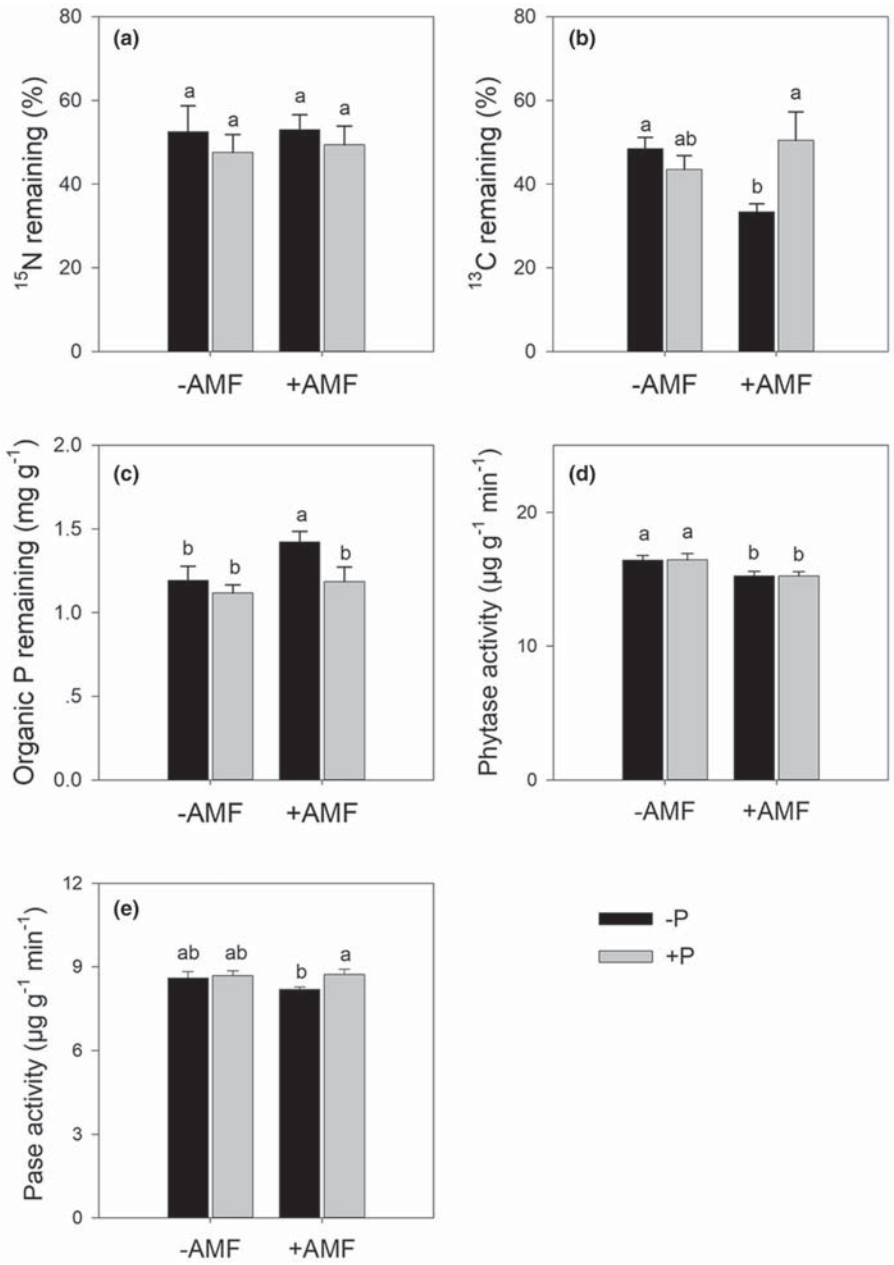

FIGURE 1 Organic materials remaining in the hyphae-in-growth bags and enzyme activities. ^{15}N (a) and ^{13}C (b) remaining in the bags of Experiment 1, and organic P remaining (c), phytase activity (d), and acid phosphatase (Pase) activity (e) in the bags of Experiment 2. −P: *Bidens pilosa* not parasitized by *Cuscuta australis*; +P: *B. pilosa* parasitized by *C. australis*; −AMF: without AMF inoculation; +AMF: with AMF inoculation. Values are means ± SE. Different lowercase letters indicated a significant difference ($p < 0.05$) between treatment combinations.

In Experiment 2, the acid phosphatase activity in hyphae-in-growth bags was significantly positively correlated with the shoot P concentration of *B. pilosa* for +AMF (Figure 4c). For +AMF, the shoot N concentration of *B. pilosa* was significantly positively correlated with the shoot P concentration (Figure 4d).

4 | DISCUSSION

The results of our two experiments (summarized in Figure 5) provide insights into the cascading effects of parasitic plants on SOM decomposition and soil-nutrient cycling. We found that the parasite decreased the decomposition of maize leaves and increased the decomposition of phytate, but only in the presence of AMF. We also found that AMF increased the decomposition of maize leaves, whereas it decreased the decomposition of phytate, but only in the absence of the parasite. So, the parasite neutralized the effect of the AMF on SOM decomposition. In other words, the results support our hypothesis that parasitic plants can indirectly regulate SOM dynamics by suppressing the effect of AMF on decomposition.

Changes in the amounts of organic material remaining in the hyphae-in-growth bags in the two experiments suggest that AMF

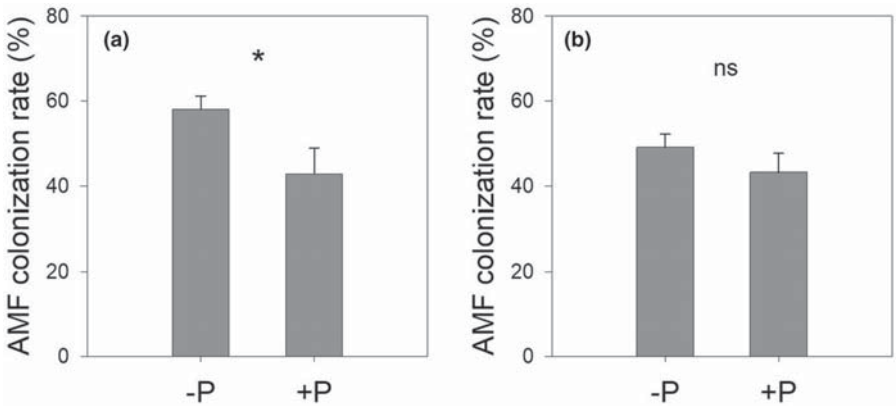

FIGURE 2 AMF-colonization rate of *Bidens pilosa* in Experiment 1 (a) and Experiment 2 (b). −P: *B. pilosa* not parasitized by *Cuscuta australis*; +P: *B. pilosa* parasitized by *C. australis*. Values are means ± SE. *Indicates that means are significantly different; ns indicates that means are not significantly different.

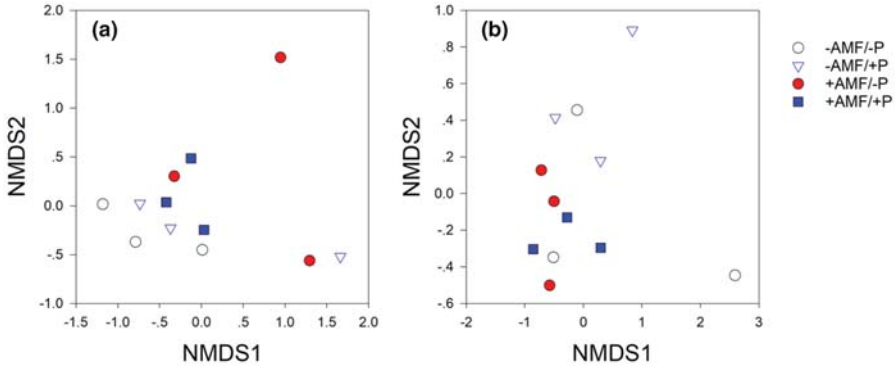

FIGURE 3 Differences in composition of the bacterial communities in the hyphae-in-growth bags are shown as Nonmetric Multidimensional Scaling (NMDS) plots for Experiment 1 (a) and Experiment 2 (b). −AMF/−P: without AMF inoculation and without parasite; −AMF/+P: without AMF inoculation and with parasite; +AMF/−P: with AMF inoculation and without parasite; +AMF/+P: with AMF inoculation and with parasite.

had opposite effects on the decomposition of maize leaves and phytate. The reason for this could be differences in nutrient release from these two organic materials, especially the P availability (Figures S5 and S10). It has been shown that nutrient release from SOM can directly shape the microbial community (Abril et al., 2021; Ferreira & Graça, 2016). However, the presence of AMF may have further changed the composition of the microbial communities or the activities of the microbial decomposers, and therefore the final decomposition of maize leaves and phytate by AMF after 2 months may have been different, or even opposite. On the other hand, it could be that the nutrients released from maize leaves and phytate may affect competition between AMF and the bacterial decomposers differently. Previous studies have shown that high soil-P availability can exacerbate N competition between AMF and decomposers (Jannoura et al., 2012; Xu et al., 2018). When P availability is relatively low (i.e. when there is a high N:P ratio), AMF may promote decomposition, whereas under high soil-P availability—as would result from decomposition of phytate—AMF may suppress the decomposition of organic matter (Xu et al., 2018). Therefore, we speculate that the high P availability in the hyphae-in-growth bags with phytate may make the bacterial decomposers suffer from stronger N competition with AMF, and this may reduce the bacterial production of the enzyme phytase. Consequently, AMF was more likely to promote the decomposition of maize leaves, whereas the opposite was true for phytate. However, how the bacterial community and nutrients released from maize leaves and phytate differently affect the relationship between AMF and SOM decomposition needs further study.

Irrespective of the opposing AMF effects on decomposition of the two organic materials, the presence of the parasite suppressed or offset the effect of AMF on decomposition. A reason for this could be that the parasite decreased C allocation from the host to AMF (Sui et al., 2019). This is supported by our finding that parasitization decreased AMF colonization of *B. pilosa* (Figure 2), most likely as a consequence of competition for C. Although AMF provide N and P to the host plant, they rely, just like the holoparasite, for C on the host. It has been estimated that 10%–23% of the photosynthates of the host can be transferred to their AMF (Jakobsen &

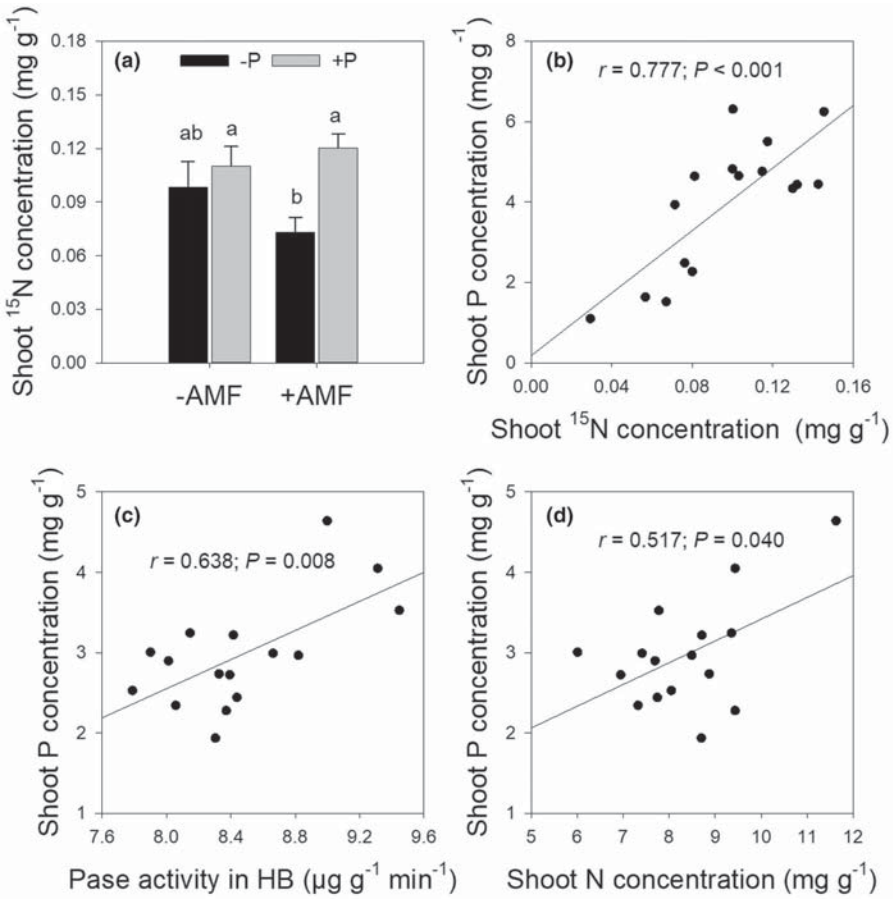

FIGURE 4 The shoot ^{15}N concentration (a), and the correlation between shoot ^{15}N concentration and shoot P concentration in the presence of AMF (b) in Experiment 1. And the correlation between acid phosphatase activity in the hyphae-in-growth bags and shoot P concentration of *B. pilosa* (c), and the correlation between shoot N concentration and shoot P concentration (d) in the presence of AMF in Experiment 2. −P: *Bidens pilosa* not parasitized by *Cuscuta australis*; +P: *B. pilosa* parasitized by *C. australis*. −AMF: without AMF inoculation; +AMF: with AMF inoculation. Values are means ± SE. Different lowercase letters indicated a significant difference ($p < 0.05$) between treatment combinations.

Rosendahl, 1990; Kucey & Paul, 1982). Direct competition for C between AMF and the parasite may thus have limited AMF growth and thereby suppressed the effect of AMF on decomposition (Figure 5). Another possible explanation for the suppressive effect of the parasite on the effect of AMF could be that parasitization of the host plant changed the chemicals (like secondary metabolites, hormonome) released from its roots (Mishev et al., 2021; Shen et al., 2020), and that this influenced AMF growth. For example, parasitization of plants by *Phelipanche* spp. led to the changes in the production of cytokinins, which can influence AMF development (Mishev et al., 2021; Pons et al., 2020). However, which mechanism plays the main role needs further testing. It should also be noted that our results are only a snapshot after 2 months of plant growth. Therefore, whether the effects of parasitization and AMF on decomposition changes with time needs further study.

AMF have been considered to affect SOM decomposition by influencing the bacterial community (Nuccio et al., 2013; Xu et al., 2018). We found that AMF inoculation significantly affected the bacterial community in the hyphae-in-growth bags with phytate (Experiment 2). Because AMF have no known saprotrophic capability, that is they lack the ability to secrete enzymes like phosphatases (Tisserant et al., 2013; Zhang, Cao, et al., 2016), it is likely that changes in the bacterial community caused by AMF altered the secretion of enzymes by microbial decomposers, which then changed the decomposition of phytate. Interestingly, AMF inoculation did not significantly affect the diversity and composition of the bacterial community in the hyphae-in-growth bags with maize leaves. Nevertheless, AMF significantly affected the abundance of some genera, like *Paenibacillus* (Figure S2). As studies have shown that *Paenibacillus* are associated with AMF and components of the soil-nutrient cycle, like nitrogen fixation and phosphorus solubilization (Coelho et al., 2003; Larsen et al., 2009), it is likely that the decreased abundance of the genus *Paenibacillus* may affect decomposition.

Parasitization of *B. pilosa* by *C. australis* did not significantly change the composition of the bacterial community in both experiments. Nevertheless, parasitization decreased the Simpson diversity index and increased the abundance of the genus *Bacillus* in Experiment 1, and decreased the abundance of the genus *Streptomyces* in Experiment 2. Previous work has shown that

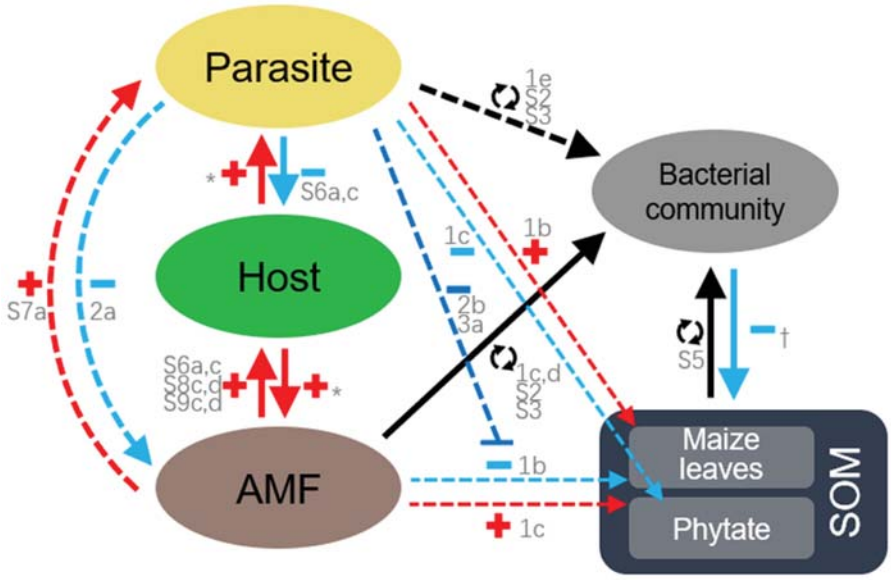

FIGURE 5 A conceptual framework of the cascading effect of parasitic plants on the decomposition of soil organic matter (SOM) via extraradical hyphae of AMF. Overall, the parasitic plant directly suppressed the host plant growth, which in turn indirectly suppressed AMF growth. The characteristics of SOM modified the composition of the decomposing bacteria or differently changed the physicochemical environment for decomposers. This drove the AMF to change their influence on the decomposing bacteria, which then promoted or suppressed decomposition. Therefore, the presence of the parasite either indirectly decreased SOM decomposition (like found for maize leaves in Experiment 1) or increased SOM decomposition (like found for phytate in Experiment 2). Solid and dashed lines indicate direct and indirect effects, respectively. Red and light blue arrows, accompanied by + and − symbols, indicate positive and negative effects, respectively. Black arrows, accompanied by a circular arrows symbol, indicate a change in the bacterial community (which cannot be quantified as positive or negative). The dark blue dashed line with a T-heading indicates that the presence of the parasite neutralized the effect of AMF on SOM. The numbers of the figures that show the indicated effects are given in grey font. *: the positive effect of the host plant on the parasite and AMF is given, †: the negative effect of decomposing bacteria on SOM is given.

both *Bacillus* and *Streptomyces* are associated with decomposition (Chater, 1993; Nalini et al., 2020; Nuccio et al., 2013). In particular, *Bacillus* species have been shown to be associated with decomposing hyphae (Artursson & Jansson, 2003; Toljander et al., 2006), while *Streptomyces* and other *Actinobacteria* have been shown to be major contributors to biological buffering of soils and play important roles in SOM decomposition (Chater, 1993; Nalini et al., 2020). Therefore, the effect of the parasite on these groups may be relevant for decomposition. Although more studies are needed to test the role of the bacterial community in SOM decomposition, our results indicate that the genera *Bacillus* and *Streptomyces* are involved in the regulation of the decomposition by AMF and the parasitic plant.

Based on the different characteristics of maize leaves and phytate, we measured different variables in the two experiments to evaluate the effect of the parasite and AMF on decomposition and on nutrient uptake by AMF hyphae. In Experiment 1, we used the amounts of ^{13}C and ^{15}N remaining in the hyphae-in-growth bags to quantify the amount of SOM that had been decomposed. This approach has also been used in previous studies (Qiu et al., 2016; Verbruggen et al., 2016; Xu et al., 2018). We found that the ^{13}C remaining in the hyphae-in-growth bags was significantly affected by AMF and the parasite, but that the ^{15}N remaining in the bags was unaffected. This is similar to the results of a study by Verbruggen et al. (2016) showing that AMF hypha increased ^{13}C loss in hyphae-in-growth cores, whereas ^{15}N content was unaffected. Both the results of Verbruggen et al. (2016) and our results indicate that litter-derived ^{13}C was lost as CO_2 from the soil. This indicates that ^{13}C may be a better indicator for decomposition than ^{15}N, because the $^{13}CO_2$ that is produced in decomposition is removed whereas the inorganic ^{15}N that is produced might remain in the soil.

In Experiment 2, as the activities of phytase and acid phosphatase have been reported to be correlated with the decomposition of phytate (Ding et al., 2014; Wang et al., 2013), we tested their activities to assess the effect of the parasite and AMF on decomposition. We found that AMF, but not the parasite, significantly decreased phytase activity, while the parasite, but not AMF, significantly increased the acid phosphatase activity. These results indicate that the parasite did not simply reduce the effect of AMF on the activity of phytase, but may induce a new pathway via AMF to change the activity of acid phosphatase to regulate SOM decomposition.

Previous studies have shown that the effect of AMF on SOM decomposition sometimes affects (Xu et al., 2018) and sometimes does not affect (Hodge et al., 2001) the uptake of nutrients released from organic matter. Our ^{15}N tracing results showed that the decomposition of maize leaves did not increase the N uptake by *B. pilosa* (Figure 4a). However, the significant correlation between shoot P concentration and acid phosphatase activity in the hyphae-in-growth bags showed that the decomposition of organic P may increase P uptake from decomposed organic matter (Figure 4c). As in both experiments shoot N and P concentrations were positively

correlated (Figure 4b,d), it is likely that the N and P uptake of host plants was jointly regulated by plant intrinsic properties. This might be, because host plants need to maintain a certain N:P stoichiometric balance to function properly (Fujita et al., 2010).

Soil organic C decomposition can also be affected by other factors than AMF, such as by soil nutrient availability, soil moisture content and detritivore activity (Ge et al., 2018; Tonin et al., 2018; Xu et al., 2018). For example, Ge et al. (2018) found that drought decreased carbon storage in the upper 60 cm of the soil by 12.2%. Xu et al. (2018) found that without AMF, the ^{13}C remaining in litter bags was ~5% lower at high than at low soil P concentration, whereas with AMF, the ^{13}C remaining in litter bags was ~6% higher at high than at low soil P concentration. In our experiments, AMF decreased the ^{13}C remaining in the hyphae-in-growth bags by ~31% in the absence of the parasite, whereas it increased the remaining ^{13}C remaining by ~16% in the presence of the parasite. So, the magnitude of the effect of AMF on SOM decomposition appears to be higher than the effects of other factors, which indicates the important role of AMF in soil nutrient cycling.

In this study, we only used one AMF species (*R. intraradices*), one parasitic plant species (*C. australis*), one host plant species (*B. pilosa*) and one field soil location. This means that the results cannot be generalized yet. However, as *R. intraradices* is a widespread AMF species (Moora et al., 2011), which has been used as a representative AMF species in many studies (Rodriguez-Caballero et al., 2017; Wu et al., 2015), it is highly likely that *R. intraradices* is one of the main AMF species through which *C. australis* and other parasites can indirectly affect SOM decomposition in the field. Furthermore, based on the observed intermediate growth of the host plants, we judge that the field soil we had collected was neither extremely nutrient-rich nor extremely nutrient-poor, and thus representative for many soils. Although we cannot yet generalize our findings, we believe our study paves the way for further studies with more AMF species, plant species and field soil origins.

The roles of different plant species and functional groups in determining ecosystem processes is a major focus of ecology. Parasitic plants are one group of plants which are known to influence soil nutrient cycling, but the mechanism has rarely been explored. Our results show for the first time that a parasitic plant can indirectly regulate SOM decomposition by suppressing the effect of AMF on decomposition (Figure 5). Our study provides an example of how a parasitic plant can trigger a series of cascading effects between above-and belowground processes. Such trophic cascades have been frequently reported in studies on herbivory (van Dam & Heil, 2011; Zhuang et al., 2018), but less so in studies on parasitic plants. Our results highlight the importance of cascading effects in a community context to unravel the mechanisms underlying sophisticated hidden ecological processes, and represents an important step forward in elucidating the roles of parasitic plants in soil nutrient cycling. Our study indicates that future work needs to take AMF into account when exploring the mechanisms that underly the effect of parasitic plants on SOM decomposition, and more work is needed to test the interactions of microbial decomposers with AMF and with parasitic plants.

AUTHOR CONTRIBUTIONS

Yongge Yuan and Junmin Li conceived the ideas and designed the experiments. Yongge Yuan, Xinru Lin, and Gelv Chen performed the experiments. Junmin Li and Yongge Yuan analysed the data. Junmin Li and Yongge Yuan prepared the manuscript. Junmin Li, Yongge Yuan and Mark van Kleunen improved the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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