A parasite indirectly affects nutrient distribution by common mycorrhizal networks between host and neighboring plants

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Abstract. Cascading effects are ubiquitous in nature and can modify ecological processes. Most plants have mutualistic associations with mycorrhizal fungi, and can be connected to neighboring plants through common mycorrhizal networks (CMNs). However, little is known about how the distribution of nutrients by CMNs to the interconnected plants is affected by higher trophic levels, such as parasitic plants. We hypothesized that parasitism would indirectly drive CMNs to allocate more nutrients to the nonparasitized neighboring plants rather than to the parasitized host plants, and that this would result in a negative-feedback effect on the growth of the parasitic plant. To test this, we conducted a container experiment, where each container housed two in-growth cores that isolated the root system of a single *Trifolium pratense* seedling. The formation of CMNs was either prevented or permitted using nylon fabric with a mesh width of 0.5 or 25 μm , respectively. In each container, either both *T. pratense* plants were not parasitized or only one was parasitized by the holoparasite *Cuscuta australis*. To quantify the nutrient distribution by CMNs to the host and neighboring plants, we used ¹⁵N labeling. Growth and ¹⁵N concentrations of *C. australis* and *T. pratense* were measured, as well the arbuscular mycorrhizal fungi–colonization rates of *T. pratense*. We found that parasitism by *C. australis* reduced the biomass of *T. pratense*. In the absence of the parasite, CMNs increased the ¹⁵N concentration of both *T. pratense* plants, but did not affect their biomass. However, with the parasite, the difference between host and neighboring *T. pratense* plants in ¹⁵N concentration and biomass were amplified by CMNs. Furthermore, CMNs decreased the negative effect of *C. australis* on growth of the host *T. pratense* plants. Finally, although CMNs did not influence the ¹⁵N concentration of *C. australis*, they reduced its biomass. Our results indicate that when *T. pratense* was parasitized by *C. australis*, CMNs preferentially distributed more mineral nutrients to the nonparasitized neighboring *T. pratense* plant, and that this had a negative feedback on the growth of the parasite.

Key words: arbuscular mycorrhizal fungi; cascading effect; common mycorrhizal networks; host plant; nitrogen; parasitic plant.

INTRODUCTION

In ecological communities, species are linked to each other in networks of direct and indirect interactions (Hartley et al. 2015, Haan et al. 2018). As a consequence, changes in the presence of some species may have cascading effects at other trophic levels that can indirectly affect ecological processes. Many parasitic plants are generalists that can use many species as hosts, and are known to have profound indirect effects on above- and belowground organisms (e.g., soil microbes, neighboring plants) (Bardgett et al. 2006, March and Watson 2007, Watson 2009, Yang et al. 2019). It has

been shown that they can indirectly affect soil microbes and their activities (Bardgett et al. 2006, March and Watson 2007, Li et al. 2008, Watson 2009, Sui et al. 2019). These indirect effects might benefit the neighboring plants, and thus become visible aboveground (Yang et al. 2019). However, whether and, if so, how parasitic plants trigger such cascading effects from host plants, via soil microbes, on neighboring plants is not known.

Parasitic plants are connected to their host via root-like structures called haustoria (Sui et al. 2014). They extract resources (e.g., water, sugars, nutrients) from the host plants, resulting in depressed growth of the host plant (Sui et al. 2019, Yang et al. 2019). Moreover, the parasite and host can exchange various signals that affect their growth (Westwood and Kim 2017, Banerjee 2020). Because in nature host plants interact with various organisms at other trophic levels, we expect that the

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effect of a parasitic plant on its host indirectly affects the interactions of the host with other organisms, such as soil microbes (Gao et al. 2019, Sui et al. 2019). Most parasitized host plants can still form symbiotic relationships with arbuscular mycorrhizal fungi (AMF), and have common mycorrhizal networks (CMNs) with neighboring plants (Egerton-Warburton et al. 2007, Mikkelsen et al. 2008, Weremijewicz and Janos 2013). CMNs play an important role in plant interactions by regulating the distribution of mineral nutrients between interconnected plant individuals (Egerton-Warburton et al. 2007, Mikkelsen et al. 2008, Weremijewicz and Janos 2013, Workman and Cruzan 2016). However, whether changes in nutrient distribution by CMNs provide a mechanistic link for cascading effects of parasites on neighboring plants of the host plants is not known.

There are three possible patterns (or hypotheses) with regard to how CMNs distribute nutrients among different plants. The first one is that nutrient distribution by CMNs is more or less random and not correlated with size or competitive ability of the plants. Indeed, Chiariello et al. (1982) found that mineral nutrients taken up by CMNs were “shared” among interconnected plants with no clear pattern as to which individuals received the most mineral nutrients. The second hypothesis is that CMNs preferentially transfer more mineral nutrients to plants suppressed by, for example, shading, thus alleviating the plants from competitive pressures of surrounding individuals (Grime et al. 1987). However, there is little evidence in support of this hypothesis. The third hypothesis is that CMNs transfer mineral nutrients preferentially to large host individuals, which are likely to be strong carbon suppliers, rather than to suppressed individuals that are minor carbon suppliers (Lekberg et al. 2010, Merrild et al. 2013). In line with this idea, Merrild et al. (2013) found that CMNs allocated more phosphorus to large established plants than to small seedlings, thus amplifying nutrient competition between the connected plants. Similarly, Weremijewicz et al. (2016) showed that CMNs preferentially distributed more mineral nutrients to large, unshaded individuals than to connected small, shaded individuals. The third hypothesis thus seems to have most support.

One possible mechanism by which CMNs may preferentially allocate more nutrients to large, strong plants might be through a “reciprocal rewards” mechanism (Kiers et al. 2011). Kiers et al. (2011) proposed this mechanism based on in vitro research with root-organ cultures, which showed that plants rewarded the most beneficial fungal partners with more resources, such as carbohydrates. In turn, their fungal partners reinforced this cooperation by increasing nutrient transfer to those roots that provided more carbohydrates. Similarly, CMNs might reward the interconnected plants that supply the most resources with more mineral nutrients, so that these plants would continue to provide more carbohydrates to the CMNs.

Parasitic plants solely or partly depend on host plants for carbon. When parasitized host plants are connected with their nonparasitized neighbors via CMNs, it is likely that they cannot provide as much carbohydrate to CMNs as their nonparasitized conspecific neighbors. This is because host plants have already lost part of their photosynthetic products to the parasitic plants. If the “reciprocal rewards” mechanism applies, CMNs might thus preferentially distribute more mineral nutrients to the nonparasitized conspecific neighbors than to the parasitized hosts. Consequently, as growth of the parasitic plant depends on the growth of its host (Zhang et al. 2013), it is likely that the unequal mineral nutrient distribution by CMNs will ultimately be disadvantageous to the growth of the parasitic plant.

In this study, we tested whether there are cascading effects of a parasitic plant on its host plant, and via CMNs on the neighboring plants. Specifically, we assessed the effect of the parasitic plant *Cuscuta australis* on nutrient distribution by CMNs between parasitized *Trifolium pratense* and nonparasitized *T. pratense*. We also assessed the potential feedback of the cascading effects on growth of the parasitic plant. We hypothesized that the parasitic plant would indirectly cause CMNs to allocate more mineral nutrients to nonparasitized neighboring plants than to parasitized host plants, and thereby lead to a negative feedback on growth of the parasitic plant.

METHODS

Study species

Cuscuta australis was used as the parasitic plant species. *Cuscuta australis* is an obligate shoot parasite, which acquires water, carbon, and nutrients from its host, and thereby inhibits the growth of the host. *Cuscuta australis* can parasitize a wide range of herbaceous species. As host and neighboring plant species, we chose *T. pratense*, a herbaceous legume, because it is known that *C. australis* heavily suppresses the growth of *Trifolium* species in nature (Shi et al. 2007), and because *T. pratense* is widely parasitized by *C. australis* in the field.

Experimental containers

Each of 20 experimental containers (26 × 12 × 12 cm for length × width × height) were filled with 2.5 kg of sterilized substrate consisting of a 6:3:1 mixture of peat, sand and vermiculite (v:v:v). The peat had a pH of 6.2, and contained 635 g/kg organic matter, 8.43 g/kg total nitrogen, and 0.604 g/kg total phosphorus. Two in-growth cores, each filled with 0.5 kg of the same sterilized substrate, were inoculated with AMF as described below, and dug into the substrate of each container (Fig. 1). The in-growth cores were 9 cm apart, and were fabricated using 5.5 × 12 cm (diameter × height) PVC cups with openings on the side that

accounted for a quarter of the total lateral surface area (see Awaydul et al. 2018). Each in-growth core contained either a parasitized or a nonparasitized *T. pratense* plant (Fig. 1). The lateral openings were covered with a nylon fabric with a mesh width of either 0.5 or 25 μm (Jiuding High-Tech Filtration Equipment Co., Ltd, Beijing, China). The 0.5- μm nylon mesh prevents the penetration by both AMF hypha and plant roots, and the 25- μm nylon mesh allows penetration by hyphae but not by roots.

Experimental design

In each container, one of the two *T. pratense* plants in an in-growth core was designated to be the host plant, and the other *T. pratense* plant was designated as nonparasitized neighboring plant. The experiment had three factors: (1) the presence or absence of common mycorrhizal networks (CMN+, CMN–); (2) the presence or absence of *C. australis* parasitism on the host plant (P+, P–); (3) plant status: host or neighboring *T. pratense* (note that we also refer to one of the *T. trifolium* plants as host when both plants in a container were nonparasitized). Each treatment combination had five replicate containers.

AMF inoculum

We conducted a pilot experiment to test whether the experimental methods described by Awaydul et al. (2018) resulted in a CMN between the two *T. pratense* plants (Appendix S1). In this pilot experiment, we inoculated one of the two plants in each container and examined whether the mycelium colonized the uninoculated plant on the other side of the container. We indeed found that this was the case, no matter whether we inoculated the parasitized host or nonparasitized neighboring plant alone (Appendix S1). To further increase the likelihood that CMNs would establish in the main experiment, we inoculated both *T. pratense* plants in each container. For each in-growth core, 10 g of an AMF inoculum was mixed into the substrate. The inoculum had been purchased from the Institute of Microbiology, Guangxi Academy of Agricultural Sciences, China, and was derived from pot cultures of *Sorghum bicolor* (L.) Moench grown in coarse sand. The inoculum consisted of colonized root fragments of *S. bicolor*, soil, and spores of the AMF *Rhizophagus intraradices*. This is a widespread generalist AMF species (Moora et al. 2011). The spore density of the AMF inoculum was 168 spores per 10 g.

FIG. 1. Diagram showing the experimental design. The paired host and neighboring *Trifolium pratense* plants colonized by arbuscular mycorrhizal fungi (AMF) were either interlinked through formation of a common mycorrhizal network (CMN) or not, as allowed by lateral side openings in the PVC in-growth cores covered with either a 25- μm (permitting) or 0.5- μm (preventing) nylon mesh. (a) Absence of CMNs and the parasite, (b) presence of CMNs in the absence of the parasite, (c) absence of CMNs in the presence of the parasite, (d) presence of both CMNs and the parasite. CMN–: CMN absent, CMN+: CMN present, P–: host *T. pratense* not parasitized by *Cuscuta australis*, P+: host *T. pratense* parasitized by *C. australis*.

Planting, parasitism, and growth conditions

Seeds of *T. pratense* had been purchased from the Beijing Golden Earth Agricultural Technology Research Institute. On 23 June 2018, seeds of *T. pratense* were surface sterilized for 10 min in 5% sodium hypochlorite and germinated in a mixture of vermiculite and peat moss in the greenhouse facilities of Taizhou University. When seedlings were approximately 2 cm tall, seedlings of similar size were selected and transplanted individually into the two in-growth cores of each container. Each in-growth core had been inoculated with AMF on the same day that the seedlings were planted.

Four weeks after transplantation, one 10-cm-long stem piece of *C. australis* was wound counterclockwise around the stems of the host *T. pratense* plants to allow the parasite to infect the plants. Nonparasitized *T. pratense* plants were used as controls (Li et al. 2014). The stems of *C. australis* had been collected from a field population near Taizhou University.

The experiments were conducted in a greenhouse at Taizhou University with a photoperiod of 16 h and 8 h of dark at 22°C (day) and 18°C (night), respectively. Water was sprayed evenly onto the soil of each container every day. We added 20 ml Hoagland nutrient solution to each in-growth core on 13 July 2018.

¹⁵N labeling

To test the effect of CMNs on nitrogen distribution between host and neighboring *T. pratense* plants, ¹⁵N tracer was introduced 2 wk before the plants were harvested. We dissolved 5 mg ¹⁵N-enriched mineral (¹⁵NH₄)₂SO₄ (99.7% atom ¹⁵N; from Shanghai Research Institute of Chemical Industry, Shanghai, China) in 6 ml of distilled water, and injected it with a syringe into the substrate in the middle of each container at a rate of 2 ml per day for 3 d.

Harvest and measurement

Seventy-seven days after transplantation, the *C. australis* plants were harvested by detaching them from the host plants. After that, the *T. pratense* plants were separated into shoots and roots by cutting the shoot at a distance of 2 cm above the soil surface (Göransson et al. 2016). The aboveground biomass was oven dried at 65°C for 72 h. The roots were washed to remove soil and carefully checked for the presence of nodules. We found very few nodules, indicating that there was very little contamination with N-fixing rhizobia, which might otherwise have affected our nitrogen concentration results. Half of each root sample, by fresh weight, was then oven dried at 65°C for 72 h to calculate the total root dry biomass. The remaining half of each root sample was used for quantification of AMF colonization using the gridline-intersection method (Giovannetti and Mosse 1980) under an SMZ-168 light microscope (×20 magnification,

Motic China Group Co. Ltd., Xiamen, China). A total of 200 1-cm root segments were used for each replicate.

The dried shoot samples were ground in a PM400 high-energy ball mill (Retsch Technology GmbH, Haan, Germany) for plant nutrient analysis. Stable N isotope ratios were determined using a continuous-flow isotope-ratio mass spectrometer (CF-IRMS, Thermo Finnigan DELTA Plus, Waltham, Massachusetts, USA). The δ¹⁵N (‰) was converted to the absolute isotope ratio (¹⁵N/¹⁴N) of the sample based on the atomic ratio of atmospheric nitrogen. The ¹⁵N concentration of the sample was then calculated from fractional abundances (¹⁵N/(¹⁵N + ¹⁴N)) and the total N concentration of the sample (Hu et al. 2001).

Statistical analyses

The effects of plant status (host vs. neighbor), CMN (present vs. absent) and parasite (present vs. absent), as well as their interactions on aboveground biomass, belowground biomass, AMF-colonization rate, and ¹⁵N concentration of *T. pratense*, were analyzed using mixed-effect models. We accounted for nonindependence of the two *T. pratense* plants in a container by including container identity as the random factor. The effects of CMNs on biomass and ¹⁵N concentration of *C. australis* were analyzed with a one-way analysis of variance (ANOVA). Assumptions of normality and homogeneity of variance were assessed based on standardized residual analysis. Data were transformed as necessary; specifically, a reciprocal transformation for ¹⁵N concentration was conducted. Differences between treatments were compared using the least-significant difference (LSD) at the 5% significance level. In addition, the relationships of ¹⁵N concentration to aboveground biomass and to AMF-colonization rate were determined using Pearson correlations. The linear mixed-effect models were run in R (3.5.0, R Development Core Team 2018) using the lme function of the nlme package (Pinheiro et al. 2018), and all other analyses were performed using the Statistical Product and Service Solution (SPSS) software (version 16.0; SPSS Inc., Chicago, Illinois, USA).

RESULTS

Effects of CMNs, the parasite and plant status on Trifolium pratense biomass

We found that the aboveground biomass of *T. pratense* was significantly affected by plant status ($P = 0.005$) and CMN presence ($P = 0.046$; Table 1). Furthermore, although the parasite did not significantly alter the aboveground biomass on average ($P = 0.528$), there was a significant interactive effect between plant status and infection by the parasite ($P = 0.007$; Table 1). When the designated host *T. pratense* plant was not parasitized by *C. australis*, there was no significant difference in the aboveground biomass between the two

TABLE 1. Effect of plant status, formation of common mycorrhizal networks (CMNs), and infection by the *Cuscuta australis* parasite on the aboveground biomass, belowground biomass, arbuscular mycorrhizal fungi (AMF) colonization rate, and ^{15}N concentration in *Trifolium pratense*.

Effect	df	F	P
Aboveground biomass			
Plant status	1	10.516	0.005
CMN	1	4.704	0.046
Parasite	1	0.416	0.528
Plant status $\times$ CMN	1	0.560	0.465
Plant status $\times$ parasite	1	9.585	0.007
CMN $\times$ parasite	1	3.536	0.078
Plant status $\times$ CMN $\times$ parasite	1	1.075	0.315
Denominator	16		
Belowground biomass			
Plant status	1	4.814	0.043
CMN	1	0.560	0.465
Parasite	1	0.658	0.429
Plant status $\times$ CMN	1	0.590	0.454
Plant status $\times$ parasite	1	8.035	0.012
CMN $\times$ parasite	1	2.265	0.152
Plant status $\times$ CMN $\times$ parasite	1	0.353	0.561
Denominator	16		
AMF colonization rate			
Plant status	1	0.653	0.431
CMN	1	0.032	0.861
Parasite	1	0.009	0.925
Plant status $\times$ CMN	1	1.881	0.189
Plant status $\times$ parasite	1	5.000	0.040
CMN $\times$ parasite	1	0.002	0.968
Plant status $\times$ CMN $\times$ parasite	1	21.889	0.000
Denominator	16		
^{15}N concentration			
Plant status	1	4.288	0.055
CMN	1	220.665	0.000
Parasite	1	0.412	0.530
Plant status $\times$ CMN	1	8.350	0.011
Plant status $\times$ parasite	1	0.648	0.433
CMN $\times$ parasite	1	5.481	0.033
Plant status $\times$ CMN $\times$ parasite	1	8.080	0.012
Denominator	16		

Bold P values indicate $P < 0.05$.

T. pratense plants, and the presence of CMNs did not significantly affect the aboveground biomass of either plants (Fig. 2a). However, when the designated host plants were actually parasitized by *C. australis*, the aboveground biomass of the neighboring plants was significantly higher than that of the host plant (Fig. 2a). Moreover, although the presence of CMNs did not significantly affect the host aboveground biomass, they did significantly increase the aboveground biomass of the neighboring plant (Fig. 2a). In the absence of CMNs, the parasite significantly decreased the aboveground biomass of the host *T. pratense* plants, but had no effect on the neighboring *T. pratense* plants (Fig. 2a). On the other hand, in the presence of CMNs, the parasite did not significantly affect the aboveground biomass of the

host *T. pratense* plants, but significantly increased the aboveground biomass of neighboring *T. pratense* plants (Fig. 2a).

Similarly, the belowground biomass of *T. pratense* was significantly affected by plant status ($P = 0.043$), and there was a significant interactive effect between plant status and the presence of the parasite ($P = 0.012$) (Table 1). However, the presence of CMNs and their interactions with plant status, or parasite presence, did not significantly affect the belowground biomass (Table 1). The post hoc test revealed that in the absence of CMNs, the parasite significantly decreased the belowground biomass of the host *T. pratense* plants, but did not significantly affect neighboring *T. pratense* plants (Fig. 2b). In the absence of CMNs, the parasite did not significantly affect belowground biomass of both the host and neighboring *T. pratense* plants (Fig. 2b). However, in the presence of both CMNs and the parasite, there was a significant difference in belowground biomass between the host and neighboring *T. pratense* plants (Fig. 2b).

Effects of CMNs, the parasite, and plant status on AMF colonization of *Trifolium pratense*

For AMF-colonization rate, the main effects of plant status, CMN presence, and parasite presence were not significant ($P > 0.05$), but there was a significant three-way interaction (Table 1). When the designated host *T. pratense* was not parasitized by *C. australis*, there was no significant difference in AMF-colonization rate of the two *T. pratense* plants, regardless of the presence of CMNs between the plants (Fig. 2c). However, when the designated host plants were parasitized by *C. australis* in the presence of CMNs, the AMF-colonization rate of the neighboring *T. pratense* plants was significantly higher than that of the host plants (Fig. 2c).

Effects of CMNs, the parasite, and plant status on ^{15}N concentration of *Trifolium pratense*

The ^{15}N concentration of *T. pratense* shoots was significantly affected by the presence of CMNs ($P < 0.001$) and their interaction with plant status ($P = 0.011$; Table 1). Although the parasite did not significantly affect the ^{15}N concentration of *T. pratense* ($P = 0.530$), there was a significant interactive effect between presence of CMNs and the presence of the parasite ($P = 0.033$), and a significant three-way interactive effect of plant status, presence of CMNs and presence of the parasite ($P = 0.012$; Table 1). When the designated host *T. pratense* plant was not parasitized by *C. australis*, there was no significant difference in the ^{15}N concentration between the two *T. pratense* plants (Fig. 2d). Presence of CMNs significantly increased the ^{15}N concentration in both host and neighboring plants (Fig. 2d). However, when host plants were parasitized by *C. australis* in the presence of CMNs, the ^{15}N concentration of the neighboring *T. pratense* plants was

FIG. 2. Effects of plant status (host vs. neighbor), common mycorrhizal networks (CMNs), and *Cuscuta australis* parasitism on (a) aboveground biomass, (b) belowground biomass, (c) arbuscular mycorrhizal fungi (AMF) –colonization rate, and (d) ¹⁵N concentration of *Trifolium pratense*. Bars with the same letter do not differ significantly according to a least significant difference (LSD) post hoc test at the 5% level. Values are means ($n = 5$) $\pm$ SE. CMN–: CMN absent, CMN+: CMN present, P–: host *T. pratense* not parasitized by *C. australis*, P+: host *T. pratense* parasitized by *C. australis*.

significantly higher than for the host *T. pratense* plant (Fig. 2d). Moreover, the presence of CMNs significantly increased the ¹⁵N concentration of the neighboring plants, but had no significant impact on the ¹⁵N concentration of the host plants (Fig. 2d).

In the presence of CMNs, the aboveground biomass was significantly correlated with the ¹⁵N concentration of *T. pratense* ($r = 0.547$, $P = 0.013$; Appendix S1: Fig. S1a). The ¹⁵N concentration of *T. pratense* was not significantly correlated with the AMF-colonization rate ($r = 0.227$, $P = 0.336$; Appendix S1: Fig. S1b).

Effect of CMNs on biomass and ¹⁵N concentration of *Cuscuta australis*

The presence of CMNs significantly decreased the biomass of the parasitic plant *C. australis* (Fig. 3a), but did not significantly affect its ¹⁵N concentration (Fig. 3b).

DISCUSSION

Our study is, to the best of our knowledge, the first to demonstrate a multitrophic cascading effect triggered by

a parasitic plant. By using a stem parasite and isolated root systems, it was possible to assess the effects of the parasite on the host plant, on the CMNs, and ultimately on the neighboring plant. We found that with *C. australis* parasitism, the biomass and ¹⁵N concentration of the neighboring *T. pratense* plants were significantly higher than those of the host *T. pratense* plants. Furthermore, we found that the presence of CMNs decreased the suppression of the host *T. pratense* biomass by *C. australis* parasitism, and also decreased the biomass of the parasite. Our results provide evidence for the idea that nutrients in CMNs are preferentially transported to larger, more robust plants rather than to smaller, less robust plants (Merrild et al. 2013, Awaydul et al. 2018). Our results also provide evidence that *C. australis* parasitism can affect plant–plant interactions and create competitive hierarchies between host and neighboring plants (Watson 2009, Poulin 2010). Thus, our results support our hypothesis that *C. australis* parasitism indirectly triggers the CMNs to allocate more mineral nutrients to the nonparasitized neighboring *T. pratense* plants than to parasitized host *T. pratense* plants. This unequal nutrient distribution also resulted in a negative

FIG. 3. Effects of common mycorrhizal networks (CMNs) on (a) biomass and (b) ^{15}N concentration of the parasitic plant *Cuscuta australis*. Bars with the same letter do not differ significantly according to a least significant difference (LSD) post hoc test at the 5% level. Values are means ($n = 5$) $\pm$ SE. CMN-: CMN absent, CMN+: CMN present.

feedback on the growth of the *C. australis* parasitic plant.

Indirect effect of *Cuscuta australis* parasitism on nutrient distribution of CMNs

Our results show that in the presence of CMNs and the parasitic plant *C. australis*, the ^{15}N concentration and biomass of the nonparasitized neighboring *T. pratense* plants was significantly higher than for the parasitized host *T. pratense* plants (Fig. 2; Appendix S1: Fig. S2). The AMF-colonization rate of neighboring *T. pratense* plants was also higher than for the host *T. pratense* plants. However, the AMF-colonization rate did not induce the higher ^{15}N concentration in the neighboring *T. pratense* plants, as it was not significantly correlated with ^{15}N concentration in *T. pratense* plants (Appendix S1: Fig. S1).

Cuscuta australis parasitism affected the biomass, AMF colonization, and ^{15}N concentration of the neighboring *T. pratense* plants only when the CMNs were present. This shows that CMNs are a potential mediator of the indirect interactions between plants. Because *C. australis* is an obligate shoot parasite, it was not in direct

contact with the neighboring *T. pratense* plants. This means that the CMNs formed by the AMF-hyphal bridges between the parasitized host and neighboring *T. pratense* plants were the only possible links for their interaction. Therefore, we conclude that the higher ^{15}N concentration found in neighboring *T. pratense* compared with the parasitized host *T. pratense* plants in the presence of both CMNs and *C. australis* was mediated through the AMF mycelia that connected the two plants.

Furthermore, our results indicate that CMNs preferentially allocated more nutrients to nonparasitized neighbors than to parasitized host *T. pratense* plants. Such unequal nutrient distribution between plants connected by CMNs has also been reported previously (Walder et al. 2012, Weremijewicz and Janos 2013, Weremijewicz et al. 2016, 2018). There is increasing evidence that the observed unequal nutrient distribution by CMNs does not occur randomly, but rather that CMNs preferentially allocate more nutrients to the most robust plants (Weremijewicz et al. 2016, Awaydul et al. 2018). Consistent with this, our study demonstrates that CMNs preferentially allocated more nitrogen to the larger, nonparasitized neighboring *T. pratense* plants than to the smaller, parasitized *T. pratense* plants, a situation likely driven by the proposed “reciprocal rewards” mechanism (Weremijewicz et al. 2016). In other words, CMNs might allocate more nutrients to nonparasitized plants, thereby most likely enabling the fungus to receive more carbohydrates from these plants in return (Kiers et al. 2011, Jiang et al. 2017). However, whether CMNs are indeed rewarded with more carbohydrates by nonparasitized individuals still needs to be verified. Further studies using ^{13}C labeling to trace the distribution of carbohydrates through the AMF-plant networks are necessary.

Although our pilot experiment indicates that our experimental setup allowed for the establishment of CMNs (Appendix S1), our setup may have resulted in an unintended side effect. Namely, the 0.5- μm nylon mesh used to prevent CMNs also prevented the plants' AMF from fully accessing the soil compartment. Indeed, the overall low retrieval of ^{15}N in all plants in the CMN treatment indicates that those plants (and their AMF) had limited access to the soil nutrients. Therefore, an alternative explanation for our findings could be that *C. australis* parasitism decreased nutrient uptake by the AMF-hyphal system of host *T. pratense* plants, and that as consequence there were more nutrients available to the AMF-hyphal system of neighboring *T. pratense* plants. Indeed, our results showed that the dominance of the neighboring plants became manifest only in the parasitism treatment with CMNs (i.e., in which the AMF also had full access to the nutrients in the soil compartment). However, we did not find that parasitism significantly reduced the biomass and ^{15}N concentration of the host plants in the presence of CMNs. The latter suggests that parasitism had no or only a minor effect on the ability of the host plant's AMF to compete for nutrients. It also suggests that the increased biomass and ^{15}N concentration of the

neighboring plants in the same containers were unlikely a result of the increased nutrient availability to the AMF-hyphal system of neighboring *T. pratense* plants. Therefore, even though the unintended side effect of the increased nutrient availability to the AMF-hyphal system in the presence of CMN may have contributed to our findings, it is unlikely to be the main cause.

Effect of CMNs on Cuscuta australis parasitism

A limited number of studies have reported that AMF can alleviate damage to host plants by suppressing the growth of parasitic plants, because AMF competes with the parasitic plants for host-derived resources (Tesitel et al. 2011, Li et al. 2012, Sui et al. 2019), especially carbon (Li et al. 2019). To the best of our knowledge, our study provides the first direct evidence that the growth of parasitic plants is also inhibited by the presence of CMNs. We found that although CMNs did not significantly affect the ^{15}N concentration in the parasite *C. australis*, they significantly decreased the *C. australis* biomass. Because *C. australis* is an obligate shoot parasite, the feedback inhibitory effect on the biomass of *C. australis* mediated by the CMNs could only be induced by the host *T. pratense* plant. However, in the presence of the parasite, CMNs did not have a significant positive impact on the biomass and ^{15}N concentration of the parasitized host. Although this could be because of the relatively low statistical power of this study, there are also two potential biological reasons that could explain this observation. First, the CMNs probably decreased the mineral nutrient distribution to the parasitized host *T. pratense* plants, which had a negative effect on the growth of the hosts. Second, it might be directly related to the effect of the parasite *C. australis*. Studies have shown that the growth of parasitic plants is generally positively correlated with the growth of the host plant (Zhang et al. 2013). The negative reallocation of resources by CMNs to host plants probably suppressed the growth of *C. australis*, which might trigger a feedback loop that has a positive effect on the growth of *T. pratense*. Therefore, we speculate that the direction and strength of the cascading effects triggered by stem parasitic plants and the feedback loop might not be fixed but change over time. More experiments over longer time periods should be done to test how changes in nutrient distribution by CMNs are triggered by parasitic plants, including tests of how nutrient distribution between nonconspecific plants is affected by different parasitism levels.

Effect of Cuscuta australis parasitism on plant interactions

Although an increasing number of studies have reported that parasitic plants might change vegetation structure by affecting plant interactions and creating competitive hierarchies between host and neighboring plants (Watson 2009, Poulin 2010, Yang et al. 2019), few

of them have revealed the underlying mechanism. Our study provides evidence that parasitic plants might mediate plant interactions between hosts and neighbors by indirectly affecting the nutrient distribution of CMNs. We found that the presence of CMNs amplified the difference in biomass between the parasitized host and nonparasitized neighbor. This result is consistent with those of a previous study showing that parasitism by *Cuscuta* species strongly affects the composition of soil microbes and resulted in different feedbacks on host and nonhost neighboring plants, thereby shifting competitive hierarchies (Yang et al. 2019). As most plants in nature are likely to be connected by hyphal networks, parasitic plants might shape vegetation structure by the cascading effects mediated by CMNs.

CONCLUSIONS

In summary, we found that growth suppression of the host by the parasite resulted in the CMNs distributing more of the mineral nutrients to neighboring plants and less to host plants. This change in mineral nutrient distribution in turn resulted in a negative feedback effect on growth of the parasitic plant. Although there are more and more studies indicating that parasitic plants have major indirect effects on the structure and functioning of above- and belowground ecosystems, few studies have revealed the mechanisms. Our results show for the first time how a parasitic plant indirectly affects the nutrient distribution by common mycelial networks between the parasitized host and non-parasitized neighboring plants. We thus provide an example of how a parasitic plant can trigger a series of cascading effects between the above- and belowground ecosystems.

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