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Authors: Han, Xiaoge, Xu, Changchao, Wang, Yutao, Huang, Dan, Fan, Qiang, et al.

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Author for correspondence:
Guorong Xin, Sun Yat-sen University, School of Life Sciences, School of Agriculture, Guangdong Provincial Key Laboratory of Plant Resources, 135 Xingang West Road, Guangzhou 510275, PR China. Email: lssxgr@mail.sysu.edu.cn

*These authors contributed equally to this work.

Dynamics of arbuscular mycorrhizal fungi in relation to root colonization, spore density, and soil properties among different spreading stages of the exotic plant threeflower beggarweed (*Desmodium triflorum*) in a *Zoysia tenuifolia* lawn

Xiaoge Han[1,2,*], Changchao Xu[3,*], Yutao Wang[4], Dan Huang[1], Qiang Fan[5], Guorong Xin[6] and Christoph Müller[7,8]

[1]Graduate Student, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, School of Agriculture, Sun Yat-sen University, Guangzhou, PR China; [2]Postdoctoral Research Associate, Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, PR China; [3]Ph.D, Guangzhou Institute of Forestry and Landscape Architecture, Guangzhou, PR China; [4]Associate Professor, School of Life Sciences, South China Normal University, Guangzhou, PR China; [5]Associate Professor, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, PR China; [6]Professor, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, School of Agriculture, Sun Yat-sen University, Guangzhou, PR China; [7]Professor, Institute of Plant Ecology, Justus Liebig University Giessen, Giessen, Germany and [8]Professor, School of Biology and Environmental Science and Earth Institute, University College Dublin, Belfield, Dublin, Ireland

Abstract

Weed invasion is a prevailing problem in modestly managed lawns. Less attention has been given to the exploration of the role of arbuscular mycorrhizal fungi (AMF) under different invasion pressures from lawn weeds. We conducted a four-season investigation into a *Zoysia tenuifolia* Willd. ex Thiele (native turfgrass)–threeflower beggarweed [*Desmodium triflorum* (L.) DC.] (invasive weed) co-occurring lawn. The root mycorrhizal colonizations of the two plants, the soil AM fungal communities and the spore densities under five different coverage levels of *D. triflorum* were investigated. *Desmodium triflorum* showed significantly higher root hyphal and vesicular colonizations than those of *Z. tenuifolia*, while the root colonizations of both species varied significantly among seasons. The increased coverage of *D. triflorum* resulted in the following effects: (1) the spore density initially correlated with mycorrhizal colonizations of *Z. tenuifolia* but gradually correlated with those of *D. triflorum*. (2) Correlations among soil properties, spore densities, and mycorrhizal colonizations were more pronounced in the higher coverage levels. (3) Soil AMF community compositions and relative abundances of AMF operational taxonomic units changed markedly in response to the increased invasion pressure. The results provide strong evidence that *D. triflorum* possessed a more intense AMF infection than *Z. tenuifolia*, thus giving rise to the altered host contributions to sporulation, soil AMF communities, relations of soil properties, spore densities, and root colonizations of the two plants, all of which are pivotal for the successful invasion of *D. triflorum* in lawns.

Introduction

Arbuscular mycorrhizal fungi (AMF) establish mutualistic symbioses with the majority of terrestrial plants in nature (Smith and Read 2008). This symbiosis contributes to approximately 80% of the nitrogen (N)/phosphorous (P) needs of growing plants (van der Heijden et al. 2015). Furthermore, AMF act as a sensitive indicator of ecological soil quality in diverse ecosystems (Verbruggen et al. 2012). The unique linkage of AMF in soil–plant macronutrient cycles might, therefore, affect the growth and community structure of weeds in variety of ways (Daisog et al. 2012; Helgason et al. 2014; Jordan et al. 2000; Veiga et al. 2011). For instance, AMF could balance the competitive relationship between native and exotic species in many weed invasion events (Hilbig and Allen 2015; Klabi et al. 2014; Madawala 2014; Weber et al. 2015). However, whether the root mycorrhizal infection characteristics differ between native and exotic plants or whether the soil AMF community that supports the coexisting plant system changes during the spreading period of exotic species is still poorly understood (Bunn et al. 2014; Busby et al. 2013; Weber et al. 2015).

A wealth of studies have revealed that AMF might adopt different strategies in infecting the roots of native and exotic species, thus inducing different invasive results (e.g., Barto et al. 2010;

Endresz et al. 2013; Hilbig and Allen 2015). For example, the AMF infection in invasive species was more intense than in native species, thus directly benefiting the exotic species and contributing to the invasion (Bunn et al. 2014). Moreover, priority effects caused by the order of plant arrival were proven to have strong impacts on the belowground parts of plants, thereby influencing the plant community composition (Fukami 2015; Weidlich et al. 2018). This may have specific implications for the invasion of exotic species, especially when the first exotic plant is mycorrhizal dependent. A meta-analysis showed a positive correlation between the AMF colonization and the growth response in native plants, while this correlation was absent in invasive plants (Bunn et al. 2015). Other studies found that fine-root AMF could cause more positive feedback in the native–exotic plant co-occurrence ecosystems, but the mycorrhizal benefits in the two competitive species were proven to be related to a plant root–based foraging strategy (Herrera et al. 2013; Hilbig and Allen 2015). Nevertheless, AMF induced changes, not only in the relative abundance but also in the community composition of weeds (Jordan et al. 2000; Veiga et al. 2011). Root mycorrhizal colonization is commonly associated with soil AM fungal spore density, while these two indicators are affected by the soil nutrient status (e.g., N and P) and other soil properties (e.g., moisture) (Bunn et al. 2014; Daisog et al. 2012; Liu et al. 2009; Wang et al. 2010, 2016). Moreover, season and phenology are important factors that influence both mycorrhizal colonization and soil sporulation (Santos-Gonzalez et al. 2007; Wang et al. 2015a; Welsh et al. 2010). It is still unknown how AMF balance the relationships of root infection, soil property, soil AMF community, and spore density in response to the changed invasion status during the invasive process of exotic weeds (Bunn et al. 2014).

Weed invasion commonly occurs in modestly managed lawns. Moreover, except for lawn weeds, a certain number of turfgrass species could form mycorrhizal symbioses in undisturbed natural conditions (Koske et al. 1997). However, our knowledge about the mechanisms of weed invasion and distribution and the role of AMF in infecting lawn weeds and native turfgrass plants is still limited (Tanaka et al. 2010; Vogelsang et al. 2006; Wu et al., 2011). Furthermore, several questions remain unresolved, especially regarding co-occurring communities: (1) Is the mycorrhizal infection more prevalent in exotic than in native species during the invasion process? (2) Does the relationship between the root mycorrhizal colonization and the soil properties show interspecific (native and invasive) differences? (3) How do the soil AMF community and the AM fungal spore density change in response to the increasing invasion pressure?

To answer these questions, a four-season investigation in a coexisting turfgrass–weed lawn was conducted in 2012. The lawn initially was established with *Zoysia tenuifolia* Willd. ex Thiele (Poaceae) in 2000 but gradually became occupied by a competitive exotic weed species, threeflower beggarweed [*Desmodium triflorum* (L.) DC.] (Fabaceae), after approximately 12 yr of modest management. Five coverage levels of *D. triflorum*, ranging from level 1 to level 5, with five plots of each as replications, were set based on Braun-Blanquet results (Wikum and Shanholtzer 1978). Plants of the two species and soils that supported the co-occurrence were collected simultaneously for the determination of (1) root AMF colonizations of the two species, (2) soil AM fungal spore densities, (3) soil properties, and (4) soil AMF communities under different coverage levels of *D. triflorum* and in different seasons. The overall goal of this study was to identify the potential mechanisms of AMF in helping *D. triflorum* to spread successfully in the *Z. tenuifolia* lawn from plant (root mycorrhizal colonization), soil (physiochemical property), and microbial (soil AMF community and AM fungal spore density) aspects.

Materials and Methods

Site Description

The studied lawn is located on the Zhuhai Campus of Sun Yat-sen University, Zhuhai City (21°48′N to 22°27′N, 113°03′E to 114°19′E), Guangdong Province, China. The climate of Zhuhai is characterized as subtropical oceanic, with an average annual temperature of 22.3 C and a minimum temperature of 2.5 C during the year. The rainfall is abundant but is unevenly distributed throughout the year, with an annual precipitation of 1,700 to 2,200 mm. The rainy season runs from April to September and accounts for 84% of the total annual precipitation. The lawn was established in 2000 as a *Z. tenuifolia* monoculture. The soil under the turf was an artificial mix of materials often prepared for turf areas. Moreover, the lawn was managed modestly after it was established, with occasional mowing and irrigation, infrequent dethatching, and no fertilization or liming. During approximately 12 yr of management, plants of many weed species (Supplementary Table S1) invaded and established in the original *Z. tenuifolia* lawn. In particular, *D. triflorum* became the most aggressive species among these exotic weeds. Due to minimal weeding activity and the height of the *D. triflorum* plants (usually shorter than *Z. tenuifolia* plants), the spread of *D. triflorum* increased year by year compared with previous observations of the lawn (Supplementary Figure S1). *Desmodium triflorum* is a perennial herb that is widely distributed in tropical and subtropical regions of China and commonly grows in patches in natural environments, with a natural growth height of 1.5 to 2.5 cm. *Desmodium triflorum* also has a well-developed root system, including a taproot (approximately 82 cm for the ripe taproot), a few subroots, and many fibers growing on the taproot (Ma and Yang 2002; Ma et al. 2003). The stem organs of the plant include the erect stems and the stolons. The stolons originate from the mother plant and grow radially, while the interwoven growth of the stolons increases the distribution of this plant (Ma and Yang 2002; Ma et al. 2003). For a better understanding the spread of *D. triflorum* in the *Z. tenuifolia* lawn, coverage was used to describe the quantitative characteristics of *D. triflorum* in the native plant–exotic weed community. Moreover, the Braun-Blanquet coverage classification, one of the most widely used methods for characterizing the coverage of a specific species in plant communities, was applied in this study. The modified Braun-Blanquet coverage classification includes five levels: level 1 (coverage < 5%), level 2 (5% < coverage < 25%), level 3 (25% < coverage < 50%), level 4 (50% < coverage < 75%), and level 5 (75% < coverage < 100%) (Wikum and Shanholtzer 1978). In the studied lawn, *D. triflorum* and *Z. tenuifolia* were growing together, while other weeds represented only very small coverage in the lawn. Therefore, *D. triflorum* coverage in different areas of the lawn could represent different degrees of *D. triflorum* spread. Based on the definition of the Braun-Blanquet coverage classification, the spreading status of *D. triflorum* plants in the lawn was artificially divided into five levels: level 1, level 2, level 3, level 4, and level 5, corresponding to a coverage of <5%, 5% to 25%, 25% to 50%, 50% to 75%, and 75% to 100% for *D. triflorum*, respectively. The change of the coverage from level 1 to level 5 indicated an increase in the plant

Table 1. Definition of the coverage levels of *Desmodium triflorum* based on the Braun-Blanquet coverage classification (modified method from Wikum and Shanholtzer 1978) and the corresponding coverage of *Zoysia tenuifolia* and other weed species in each level.

Coverage level of *D. triflorum*	Level 1	Level 2	Level 3	Level 4	Level 5
Coverage of *D. triflorum* (%)	<5	5–25	25–50	50–75	75–100
Coverage of *Z. tenuifolia* and other weeds (%)	>95	75–95	50–75	25–50	0–25

density of *D. triflorum* but a decrease in the plant density of *Z. tenuifolia* and other weeds in the lawn (Table 1).

Sample Collection

Plant and soil samples were collected for each coverage level of *D. triflorum* in April, July, September, and December of 2012, corresponding to spring, summer, autumn, and winter, respectively. For each coverage level of *D. triflorum* in a certain season, five 1 m by 1 m replicated plots, with a randomly selected 20 cm by 20 cm smaller plot in each, were chosen as representatives of that level in the lawn. Moreover, the four seasonal samples were collected from different smaller plots of the same 1 m by 1 m plot, and the smaller plots were marked using ropes and tags after sample collection to avoid resampling an area in the following season. An intact sample, including all the growing plants (*Z. tenuifolia*, *D. triflorum*, and other weed species) and the soil below the plants (from 0- to 10-cm deep), was collected from each smaller plot using a shovel. In this way, the aboveground parts (e.g., stems, leaves, and flowers) and the belowground parts (roots) of all the plants were still connected biologically, and only the roots clearly attached to the target plants were collected and used as root samples. The holes in the smaller plots were back filled after sample collections using 0 to 10 cm of soils collected from a nearby site outside the 1 m by 1 m plots. After collection, the plants and soil for each intact sample were separated into a plant sample and a soil sample. Within each plant sample, *Z. tenuifolia* and *D. triflorum* (intact plants, including aboveground and belowground parts) were carefully separated for subsequent root dyeing and AMF colonization studies. The soil samples were air-dried and prepared for AM fungal spore density quantification, physiochemical property determination, and mycorrhizal fungal community analyses.

Mycorrhizal Colonization and Spore Density Determination

Fine roots of *Z. tenuifolia* and *D. triflorum* were thoroughly washed and cut into 1-cm-long segments. The root segments were stained using the 10% KOH clearing–Trypan blue dyeing method (Philips and Hayman 1970) and were prepared for microscopic observation (Nikon, Eclipse E400, Tokyo, Japan). The mycorrhizal structures, mainly entry points, hyphae, and vesicles were observed under an optical microscope at 400× magnification. The root mycorrhizal colonization of the two species was then determined and calculated using the grid counting method (Giovannetti and Mosse 1980). The AM fungal spores from each air-dried soil sample were sieved using the wet sieving-sucrose centrifugation method (An et al. 1990) and then were observed under a dissecting microscope (Zeiss, Stemi DV4, Oberkochen, Germany). The occurrence of AM fungal spores was recorded during microscopic observation by using a counter. The spore density was then calculated and expressed in units per gram of dry soil.

Soil Physiochemical Property Determination

The soil electrical conductivity (EC) was determined in the centrifuged supernatant of a soil–water mixture (200 g L^{-1}) by using a conductivity meter (DDS-307, Fangzhou, Chengdu, China). The soil pH was determined in a mixture of soil–water (400 g L^{-1}) by using a portable pH detector (PHS-3C, Leici, Shanghai, China), while the total organic C (TOC) was determined by the H_2SO_4-$K_2Cr_2O_7$ heating method. The soil available phosphorus (AP) was determined by using the 0.05 M HCl-1/2 H_2SO_4 method, while the total phosphorus (TP) was determined by using the $HClO_4$-H_2SO_4 method. The soil available nitrogen (AN) and total nitrogen (TN) (digested with H_2SO_4) were determined by titration of the distillates after Kjeldahl sample preparation and analyses. All determinations were performed using standard methods (Bao 2000; Lu 2000).

Molecular AMF Identification

One gram of soil was taken from each air-dried soil sample and mixed into a composite sample with soils at the same coverage level of *D. triflorum*, that is, every composite sample was a mixture of soils from four seasons with the same *D. triflorum* coverage levels. Thus, five composite soil samples were prepared to represent corresponding *D. triflorum* coverage levels for subsequent AMF community analyses. The total DNA of the composite soil samples was extracted using the PowerSoil DNA Isolation Kit (MoBio, Anbiosci Tech, Carlsbad, CA) according to the manufacturer's instructions. The extracted DNA samples were then checked for quantity and quality using an ultramicrospectrophotometer (NanoDrop 2000, NanoDrop Technologies, Wilmington, DE) and were also checked for integrity using 1% agar gel electrophoresis (5 V cm^{-1}, 30 min). A PCR was then performed by using fungi universal primers (ITS1F-CTTGGTCATTTAGAGGAAGTAA/ITS2-2043R-GCTGCGTTCTTCATCGATGC) (De Beeck et al. 2014). Reactions were performed with a 60-ng template (extracted DNA), 1 µl of forward and reverse primers (10 µM), and 25 µl of premix taq (EX Taq™ Version 2.0 plus dye, Takara, Tokyo, Japan) in a final reaction volume of 50 µl. The PCR program was 94 C for 5 min, followed by 30 cycles (94 C for 30 s, 52 C for 30 s, and 72 C for 30 s), and a final extension step of 72 C for 10 min. DNA sequences were analyzed with the Illumina Miseq sequencing platform (Magigen Biotech Company, Guangzhou, China) and were compared with the public database using the BLAST sequence similarity search tool (GenBank). All AMF sequences were grouped into operational taxonomic units (OTUs) with sequence similarities ≧97%, using the Mothur program (Schloss et al. 2009). The relative abundance of each AMF OTU was calculated after learning the relative abundance of the Glomeromycota within the whole soil fungal community.

Statistical Analyses

Two-way ANOVA followed by LSD tests were conducted to compare the soil properties, root mycorrhizal colonizations, and spore densities within *D. triflorum* coverage levels and seasons. Three-way ANOVA followed by mean separation using Fisher's protected LSD was conducted to compare the root colonizations within different *D. triflorum* coverage levels and seasons and between species (*Z. tenuifolia* and *D. triflorum*). Simultaneously, Pearson correlation analyses were carried out to explore the relationships among the total colonization (TC), hyphal colonization (HC), and vesicular colonization (VC) of *Z. tenuifolia*/*D. triflorum* roots and the

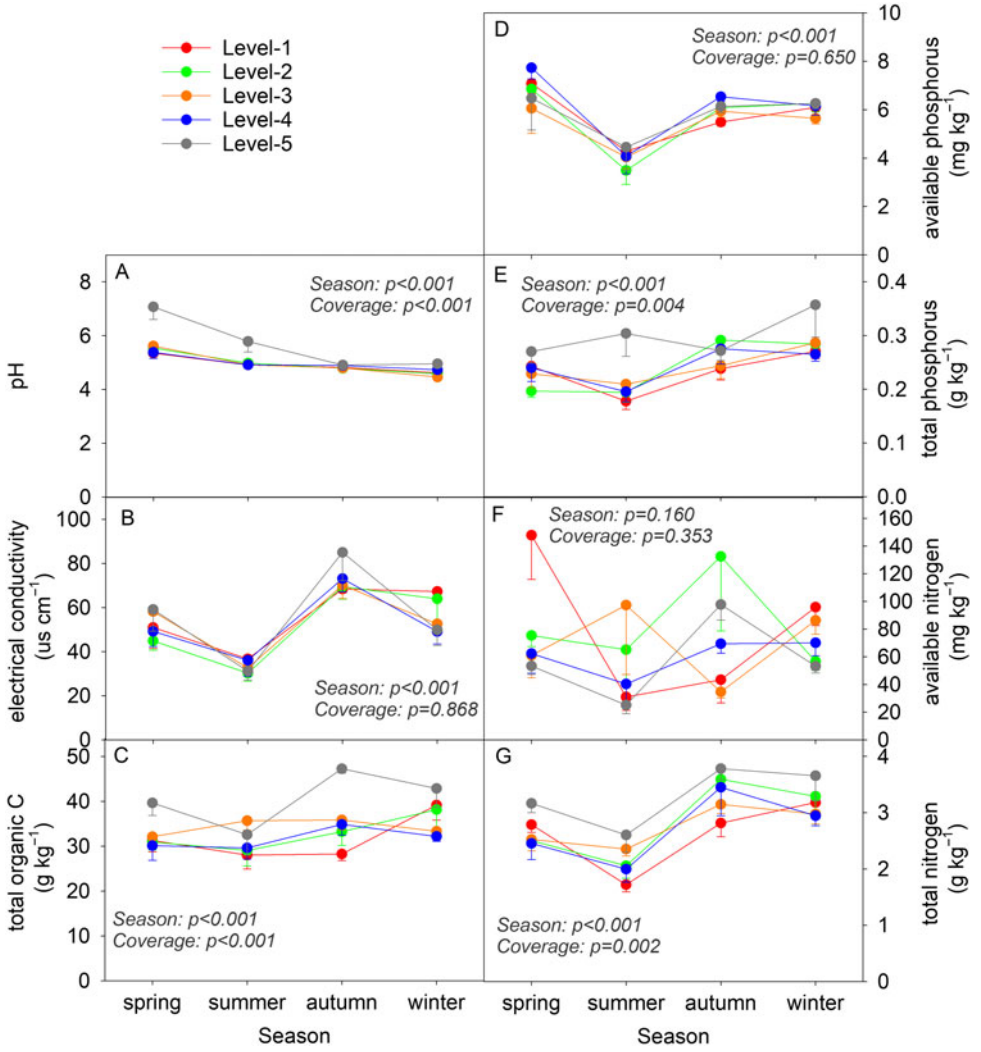

Figure 1. Dynamics of the soil physiochemical properties (average ± SE, $n = 5$) within different *Desmodium triflorum* coverage levels and seasons. "Season" and "Coverage" indicate ANOVA results of each indicator among seasons and *D. triflorum* coverage levels, respectively. Level 1, level 2, level 3, level 4, and level 5 indicate the coverage levels of *D. triflorum* in the *Zoysia tenuifolia* lawn, respectively, in this and all following figures.

interactive relationship between the two species' root mycorrhizal colonizations. Correlation analyses were also performed to test the relationships among the mycorrhizal colonizations, spore densities, and soil properties in different *D. triflorum* coverage levels. All of the statistical analyses were performed using SPSS v. 17.0 (IBM, Armonk, NY, USA). The statistically significant difference was analyzed at the $P < 0.05$ level, unless otherwise stated.

Results and Discussion

Dynamics of the Soil Properties within Seasons and Desmodium Triflorum Coverage Levels

Except for the AN concentration, concentrations of other soil property indicators, including the pH, EC, TOC, AP, TP, and TN, showed significant differences ($P < 0.001$) among the four seasons during the 1-yr investigation (Figure 1). The lawn soil underwent an acidification process, with the pH decreasing from 5.8 in spring to 4.7 in winter. A decrease from spring to summer followed by an increase from summer to winter was observed in the soil TOC, AP, and TP concentrations. Significant differences among the five coverage levels of *D. triflorum* were observed in the pH and TOC, TP, and TN concentrations (Figure 1A, C, E, and G). Moreover, the level 5 soil with the highest *D. triflorum* coverage in the lawn showed relatively higher pH, EC, and TOC, TP, and TN concentrations compared with soils of the other coverage levels (Figure 1B, C, E, and G). In contrast, the level 1 soil with the lowest *D. triflorum* coverage showed relatively lower TOC, TP, and TN concentrations in summer and autumn in comparison with soils of the other coverage levels (Figure 1C, E, and G).

Characteristics of Plant Root Mycorrhizal Colonizations within Seasons and Desmodium Triflorum Coverage Levels

Hyphae and vesicle structures were commonly present, while the arbuscular structure was not observed in the plant roots of both species (Supplementary Figure S2). The TC, HC, and VC of *D. triflorum* were significantly higher than those of *Z. tenuifolia* ($P < 0.001$) (Figure 2), implying that as the invasive species in the lawn, *D. triflorum* had a greater advantage in mycorrhizal infection than the native species, *Z. tenuifolia*. Moreover, the mycorrhizal colonizations of both *Z. tenuifolia* and *D. triflorum* showed consistently changing trends within seasons, that is, an

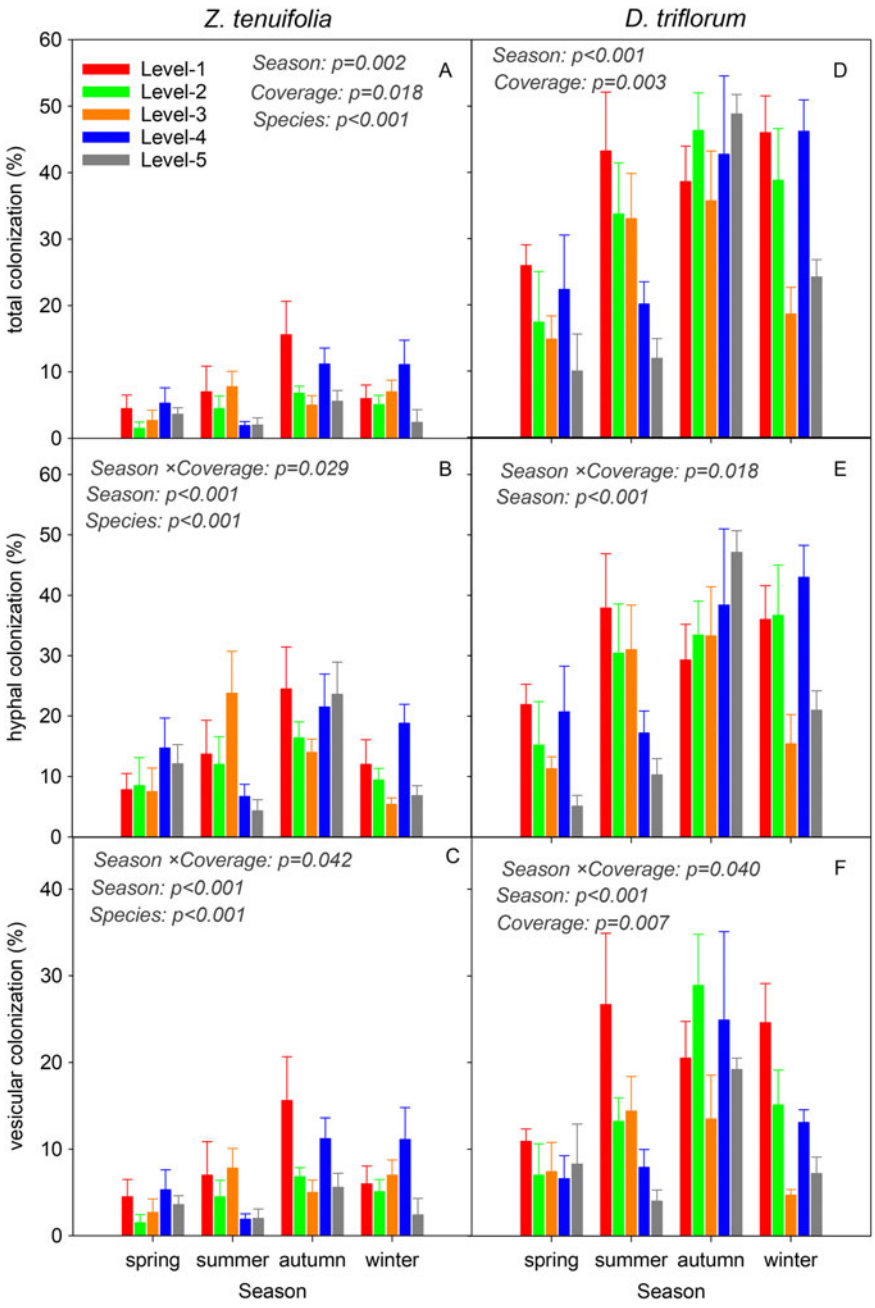

Figure 2. Dynamics of the total, hyphal, and vesicular colonizations of *Zoysia tenuifolia* and *Desmodium triflorum* among different *D. triflorum* coverage levels and seasons. "Season," "Coverage," and "Species" indicate ANOVA results of each indicator among seasons and *D. triflorum* coverage levels and between the two plants, respectively.

increase from spring to autumn followed by a decrease to winter (Figure 2), coinciding with seasonal dynamics of the AM fungal infection in the two species. During the whole investigation period, the TC of *D. triflorum* was in the range of 10.0% to 48.8%, while that of *Z. tenuifolia* was in the range of 5.2% to 28.0% (Figure 2A and D). The TC of *Z. tenuifolia* showed a "decrease–increase–decrease" variation tendency with the coverage level of the *D. triflorum* increase in the lawn (from level 1 to level 5) in all four seasons (Figure 2A). The TC of *D. triflorum* in spring and winter showed similar change trends, with the coverage level of *D. triflorum* increasing in the lawn, while the TC of *D. triflorum* showed an opposite trend (increase–decrease–increase) in autumn but an obviously decreasing trend in summer (Figure 1D). The HC of *Z. tenuifolia* was in the range of 4.3% to 24.5%, which was also lower than that of *D. triflorum* (5.1% to 47.1%) (Figure 2B and E). With the coverage level of *D. triflorum* increasing in the lawn, the HC of *Z. tenuifolia* showed a change trend similar to that of the TC, while the HC of *D. triflorum* showed a decreasing trend in summer but an increasing trend in autumn (Figure 2B and E). The VC of *Z. tenuifolia* was in the range of 1.5% to 15.6%, while that of *D. triflorum* was in the range of 4.7% to 28.9%, respectively (Figure 2C and F). In addition to the significant seasonal variances of VC, a significant coverage by season interaction effect was also observed in the VC of the two plants (Figure 2C and F).

The seasonal dynamics of plant root mycorrhizal colonization are thought to be linked to soil properties and spore density

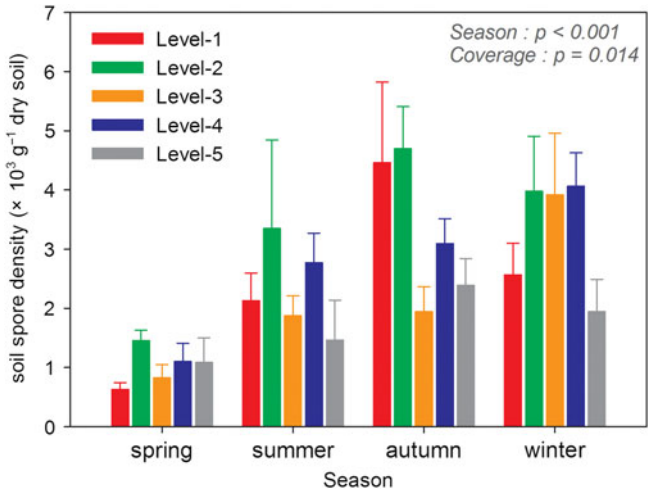

Figure 3. Dynamics of the soil arbuscular mycorrhizal fungal spore density within *Desmodium triflorum* coverage levels and seasons.

(Verbruggen et al. 2013) and vary among plant species, mycorrhizal structures, and AM fungal communities (Bencherif et al. 2016; Smith 1980; Wang et al. 2015b). For example, root colonization of saltmeadow cordgrass [*Spartina patens* (Aiton) Muhl.] was high in the growth period and decreased in the reproductive and aging periods, showing growth-period specificity (Welsh et al. 2010). Therefore, the seasonal dynamics of the two plants' root colonizations in the present study could be explained by the growth season aspects: the lower soil temperature, dormant AMF spores, and weakly growing plants in the early spring induced a low root colonization; the increased soil temperature from spring to summer benefited not only spore germination and mycorrhizal formation but also plant photosynthesis and root growth, which further promoted mycorrhizal infection in the roots of the two plants, while the root colonizations were highest in the late growth period (autumn); furthermore, the decreased soil temperature in autumn and winter depressed plant growth and mycorrhizal infection, therefore inducing a decline of root colonization (Figure 2). Moreover, the commonly observed hyphae and vesicle structures and the rarely seen arbuscular structure of the two plants might be related to the traits of the two host plants and the host specificity of soil AMF taxa (Opik and Moora 2012; Verbruggen et al. 2013).

The *D. triflorum* roots showed significantly higher mycorrhizal colonizations (total, hyphal and vesicular) compared with *Z. tenuifolia* roots in all five coverage levels of *D. triflorum* (Figure 3). This is contrary to the prevailing viewpoint that exotic plants with lower dependences on AMF symbiosis have greater chances to invade a new community compared with those with strong AMF associations (Endresz et al. 2013; Pringle et al. 2009). Nevertheless, the more intense AMF infections in the invasive species (*D. triflorum*) than in the native species (*Z. tenuifolia*) in the present study are supported by other studies of symbiotic communities (Bunn et al. 2015; Greipsson and DiTommaso 2006). This might be partly due to the fact that *D. triflorum* is a legume. Previous studies have demonstrated that the mycorrhization in legume roots facilitates the nodule symbiotic efficiency and further promotes N_2 fixation and plant growth (Bournaud et al. 2018; de Oliveira et al. 2017). In a symbiotic community with a leguminous species and a non-leguminous species, the non-leguminous plant would receive multiple benefits owing to the nutrient transfer via extraradical hyphae of the neighboring leguminous plants (Temperton et al. 2007). Considering that *D. triflorum* was much more dominant than the other weed species in the lawn, we speculated that *D. triflorum* might be the first species invading the lawn and then inhibited the development of the following species because of the priority effects aboveground and belowground (Weidlich et al. 2017, 2018). Thus, the priority effects and the superior competitive ability of *D. triflorum* arguably contributed to a high root mycorrhizal colonization of its own, and that *D. triflorum* overcame *Z. tenuifolia* as well as other weed species in the lawn. Furthermore, the AM fungal colonization of native plants would decrease when grown with/after invasive plants (Bunn et al. 2015; Stinson et al. 2006). But this is inconsistent with our results, because there was no signal demonstrating a significant decline in the root mycorrhizal colonizations of *Z. tenuifolia* with the coverage level of *D. triflorum* increase in the lawn, possibly because *Z. tenuifolia* is also AMF dependent and could form mycorrhizal structures of its own. On the other hand, the relatively higher mycorrhizal infections in *D. triflorum* imply that *D. triflorum* may exploit the AM symbiosis more efficiently than the native species *Z. tenuifolia*, absorbing soil nutrients such as phosphorus from a wider area (Walling and Zabinski 2004). In addition in the symbiotic community dominated by *Z. tenuifolia* and *D. triflorum*, the long taproots of the *D. triflorum* plants (Ma and Yang 2002) could reach much deeper in soil than those of *Z. tenuifolia*, facilitating additional nutrient and water uptake via extraradical hyphae. However, to confirm this, additional data, including plant/soil nutrient concentrations, extraradical hyphae of the two species, and the relationships of the root mycorrhizal colonizations and plant growth responses are needed. Therefore, *D. triflorum*'s more efficient symbiosis (reflected in the number of mycorrhizal infections) and greater nutrient/water absorption might contribute to *D. triflorum* outcompeting *Z. tenuifolia*, leading to its successful spread in the lawn.

Characteristics of the Soil AMF Spore Densities within Seasons and Desmodium Triflorum Coverage Levels

The soil AM fungal spore density varied significantly ($P < 0.05$) within not only the four seasons but among the five *D. triflorum* coverage levels (Figure 3). The soil spore density was low in spring, increased in summer, reached its highest level in autumn, and then decreased in winter. Similar change trends were observed in spring and summer as the coverage level of *D. triflorum* increased in the lawn; that is, the AM fungal spore density was lower in the level 1 soil and increased in the level 2 soil, but decreased in the soils of higher *D. triflorum* coverage levels (levels 3, 4, and 5) (Figure 3). Whereas the level 1 and level 2 soils in autumn and the level 2, level 3 and level 4 soils in winter showed relatively higher AM fungal spore densities than the soils of the other coverage levels (Figure 3). Previous studies suggested that the seasonal dynamics of the soil AM fungal spore density differed depending upon soil types and host plant species (Cuenca and Lovera 2010; Liu et al. 2013; Verbruggen et al. 2013; Wang et al. 2015b; Xin et al. 2012). In the *Z. tenuifolia–D. triflorum* co-occurring lawn soil, the seasonal change trends of the AM fungal spore density are consistent with the seasonal dynamics of the spore densities in the rhizosphere soils of three turfgrass plants (Koske et al. 1997). These special dynamics might be partially due to the soil temperature dependency of AM fungal spore germination and growth (Xin et al. 2012): after a cold winter, a lower number of active spores remained in the soil. The AM fungal spores began to germinate and promote infections when the climate became

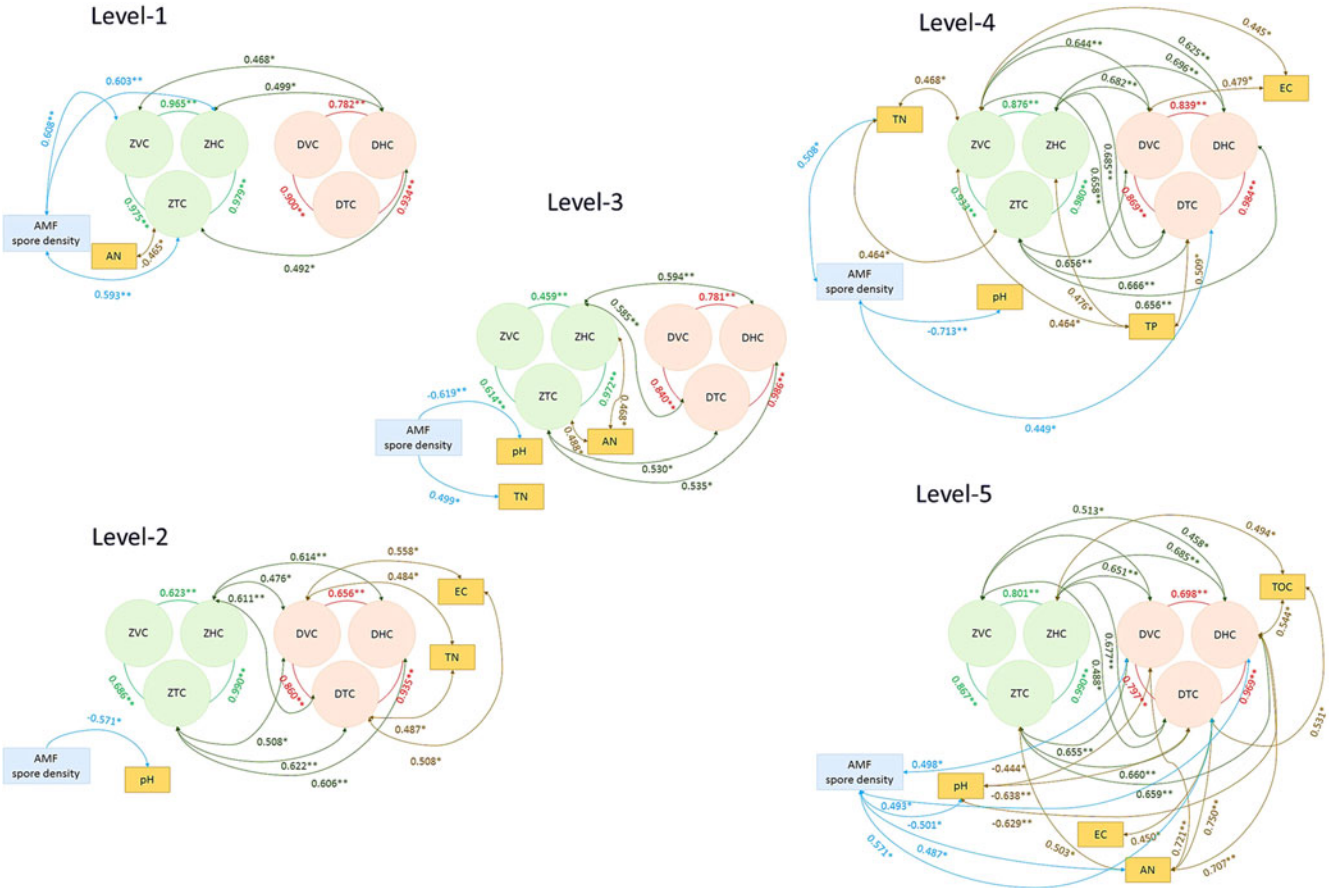

Figure 4. Correlations among the root mycorrhizal colonizations, arbuscular mycorrhizal fungal spore densities ("AMF spore density"), and soil properties in different coverage levels of *Desmodium triflorum*. ZTC, ZHC, and ZVC in light-green circles indicate the total colonization (TC), hyphal colonization (HC), and vesicular colonization (VC) of *Zoysia tenuifolia*, respectively. DTC, DHC, and DVC in light-red circles indicate the TC, HC, and VC of *D. triflorum*, respectively. Green lines and green-colored numbers indicate significant correlations between the colonization indicators of *Z. tenuifolia* and corresponding correlation coefficients, respectively. Red lines and red-colored numbers indicate significant correlations between the colonization indicators of *Z. tenuifolia* and corresponding correlation coefficients, respectively. Dark-green double arrows and dark-green numbers indicate the correlations between the colonizations of *Z. tenuifolia* and those of *D. triflorum* and corresponding correlation coefficients, respectively. Light-blue double arrows and light-blue numbers indicate the correlations between the spore densities and soil properties/root colonizations and corresponding correlation coefficients, respectively. Dark-yellow double arrows and dark-yellow numbers indicate the correlations between the soil properties and root colonizations and corresponding correlation coefficients, respectively. Correlation is significant at: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The minus sign indicates a negative correlation. Insignificant correlations are not shown.

warmer and wetter in spring. Moreover, the root mycorrhizal infection contributed to sporulation, thus inducing an increase of the spore density from spring to summer. Therefore, the root mycorrhizal colonizations were highest in autumn, while the total number of soil spores reached a peak at the same time (Figures 2 and 3). Decreasing soil temperature in winter led to a decline in the germination rate of AM fungal spores that had been produced in summer and autumn, thus inducing large amounts of spore accumulation in soils. On the other hand, the weakened plant photosynthesis in winter might limit the nutrient transport between plants and symbiotic AM fungi (van der Heijden et al. 2015), thus inducing nutrient malnutrition in fungi, leading to greater sporulation and higher AM fungal spore density in the soil (Figure 3).

Correlations of Soil Properties, Root Mycorrhizal Colonizations, and Spore Densities in Different Desmodium Triflorum Coverage Levels

According to Pearson correlation analyses, the relationships among the soil properties, spore density, and root colonization of the two plants exhibited different trends within the five *D. triflorum* coverage levels. Pairwise significant positive correlations were observed among the TC, HC, and VC of *Z. tenuifolia* and among those of *D. triflorum* in all of the five *D. triflorum* coverage levels (Figure 4; Supplementary Table S2), which showed a mutual influence of the hyphae and the vesicle after mycorrhizal infection. However, the correlations between the mycorrhizal colonizations of *D. triflorum* and those of *Z. tenuifolia* were considerably different within the five *D. triflorum* coverage levels. Positive correlations were observed between the root colonizations (TC, HC, and VC) of *Z. tenuifolia* and the HC of *D. triflorum* in the soil of level 1, which had the lowest spreading status of *D. triflorum* (Figure 4). Both the HC and TC of *Z. tenuifolia* showed significant positive correlations with the root colonizations (TC, HC, and VC) of *D. triflorum* in the level 2 soil. Subsequently, both the HC and TC of *Z. tenuifolia* showed significant positive correlations with the HC and TC but not the VC of *D. triflorum* in the level 3 soil. Finally, pairwise significant positive correlations were observed among the six root colonization indicators (TC, HC, and VC of *Z. tenuifolia*, and TC, HC, and VC of *D. triflorum*) in the level 4 and level 5 soils (Figure 4), thus showing an interactive relationship between the mycorrhizal colonizations of the two plants. The

positive correlations between the root colonizations of native species (*Z. tenuifolia*) and those of the invasive species (*D. triflorum*) (Figure 4) implied that a mutualistic relationship based on the mycorrhizal infections of the two species was established immediately after the spread of *D. triflorum* and the formation coexistence within the lawn. Moreover, the significantly positive correlations between the HC/VC of *Z. tenuifolia* and the HC of *D. triflorum* in the level 1 soil presumably imply that *D. triflorum* was colonized by the extraradical mycelia of *Z. tenuifolia* because of the close root-to-root contact of the two plants (Enkhtuya et al. 2005; Sykorova et al. 2003); this pathway of mycorrhizal infection still needs more evidence (micrographic observations). Furthermore, the root colonizations of the two plants were more significantly correlated with each other, especially in the higher coverage level soils (Figure 4), which reflected the ability of AM fungal mycelia to establish a potentially large network interconnecting with different plants in the coexistent community (Giovannetti et al. 2004; Opik and Moora 2012). Therefore, the exact role of extraradical mycelia in the coexistence system needs to be further investigated to understand the mycorrhizal function during the invasion process of *D. triflorum*.

The correlations between the soil AM fungal spore densities and the root mycorrhizal colonizations also differed within the five *D. triflorum* coverage levels (Figure 4; Supplementary Table S2). The soil AMF spore density was positively correlated with the TC, HC, and VC of *Z. tenuifolia* in the level 1 soil, thus showing a possible dominance of the AMF-infected *Z. tenuifolia* roots for sporulation. However, the correlation between the spore density and the root colonization was not significant in the level 2 and level 3 soils. The AMF spore density was significantly correlated with the TC of *D. triflorum* in the level 4 soil and with all three colonizations of *D. triflorum* in the level 5 soil (Figure 4), possibly explaining the various relationships between the AM fungal spore density and the root mycorrhizal colonization at different coverage levels of the *D. triflorum* plants. Root AMF infection could benefit sporulation (Soteras et al. 2012), the dominant contributor of soil spores changed with the development of *D. triflorum* in the lawn. At the early stage (level 1) of the *D. triflorum* spreading process, the hyphae and vesicles in the root of *Z. tenuifolia* contributed to the formation of AM fungal spores. With the development of *D. triflorum* in the lawn, the hyphal and vesicular structures in the roots of *D. triflorum* gradually contributed to sporulation; therefore, soil AM fungal spores might be a result of sporulation from both species in level 2 and level 3 soils. When *D. triflorum* dominated in the lawn (level 4, level 5), the AM fungal spores were predominantly produced by the mycorrhizal structures of *D. triflorum*, thus inducing significant correlations between the spore densities and the root colonizations of *D. triflorum* (Figure 4). In the native–invasive relationships, the host preference of AMF might lead not only to different AMF communities in the roots of the co-occurring plants but also to a change in the AMF community composition in the soil of native species (Hawkes et al. 2006; Klironomos 2003; Vandenkoornhuyse et al. 2003; Zhang et al. 2010). The AM fungal spore composition, therefore, might also be affected by the altered soil, as well as the root mycorrhizal fungal groups, because of exotic species invasion (Zhang et al. 2010). Therefore, we argue that the spread of *D. triflorum* gradually changed the AM fungal community composition of the native soil to facilitate the invasion and its own host–fungus specificity. To verify this speculation, the AMF species in the roots of the two plants and the AM fungal spore groups in soils that support the coexistence system need to be identified in follow-up analyses.

The correlations between the soil properties and the spore densities, as well as the soil properties and root colonizations of the native (*Z. tenuifolia*) and invasive (*D. triflorum*) plants indicated a difference among the five coverage levels of *D. triflorum* (Figure 4; Supplementary Table S2). Except for the level 1 soil, all of the *D. triflorum* coverage level soils showed a significant negative correlation between pH and spore density (Figure 4), which indicated that the more acidic soil supported a higher number of spores in the soil. The soil AN was significantly correlated with the TC of *Z. tenuifolia* in the level 1 soil, the HC and VC of *Z. tenuifolia* in the level 3 soil, and not only the TC of *Z. tenuifolia* but also all the colonization indicators of *D. triflorum* in the level 4 soil (Figure 4). Both the EC and TN showed significant correlations with the TC and VC of *D. triflorum* in the level 2 soil (Figure 4). The relationship among the soil properties, root mycorrhizal colonizations, and spore densities was most significant in the level 4 soil, where growth of *D. triflorum* plants was stimulated more than *Z. tenuifolia* plants: the TP and EC showed significant correlations with the root colonizations of both *Z. tenuifolia* and *D. triflorum*, while the TN was significantly correlated with the colonizations of only *Z. tenuifolia* (Figure 4). At the highest coverage level of *D. triflorum* (level 5), the soil TOC and AN showed significant correlations with the root colonizations of both plants, while the pH was significantly correlated with all of the colonization indicators of only *D. triflorum*, thus showing different relationships among the soil properties and mycorrhizal colonizations during the development of *D. triflorum* in the *Z. tenuifolia* lawn. The positive correlations of the mycorrhizal colonizations and the soil N/P (AN, TN, TP) concentrations potentially indicate the nutritional needs of mycorrhizae after formation (Della Monica et al. 2015; Deng et al. 2017; Jiang et al. 2018). However, the nutrient (N, P) concentrations of the two plants' tissues are further needed for a better understanding of the relationships between soil nutrients and host plants via the mediation of the root mycorrhizal structures. Moreover, the soil property–root colonization correlation was most significant in the level 4 soil (Figure 4), indicating a complex relationship between the soil and AMF in this stage. Except for the close linkage with the root mycorrhizal colonization, the AMF sporulation was suggested to be strongly influenced by other soil traits, such as soil depth and physiochemical properties (Cuenca and Lovera 2010; Liu et al. 2013). However, unlike the previous finding of a positive soil pH–spore relationship (Verbruggen et al. 2013), in our study, except in the level 1 soil, the spore density was negatively correlated with the pH (Figure 4). This inconsistency might be due to the differences between the studied plant species and the native soil properties. Thus, the lower soil pH would benefit AMF sporulation in the soil.

Dynamics of the Soil AMF Community within Different Desmodium Triflorum Coverage Levels

Except for the level 3 soil, the four other *D. triflorum* coverage level soils showed that Ascomycota was the dominant fungal phylum, with a relative abundance of more than 50% of the entire fungal community (Supplementary Table S3). Basidiomycota was the second most-abundant phylum in soil, with a relative abundance of 5.6% to 35.4%, whereas Glomeromycota, the fungal phylum to which AMF belongs, only occupied a relative abundance of 0.4% to 1.6% in the whole fungal community (Supplementary Table S3). Moreover, AMF OTUs were 18, 16, 28, 17, and 27 in the level 1, level 2, level 3, level 4 and level 5 soils, respectively (Figure 5B). Furthermore, a total of 41 AMF OTUs

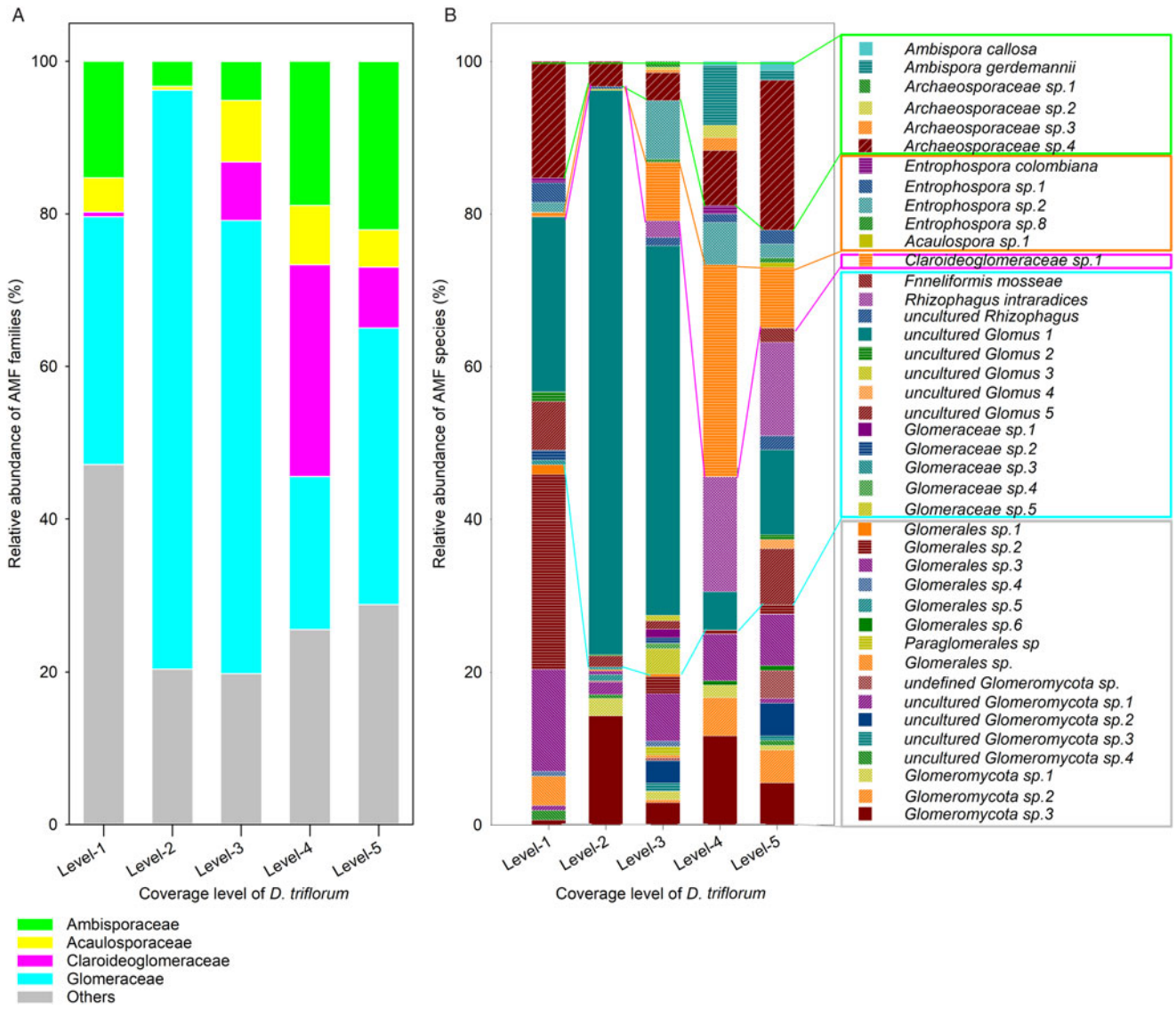

Figure 5. The relative abundance and community composition at the family (A) and species levels (B) of arbuscular mycorrhizal fungi (AMF) in soils of different *Desmodium triflorum* coverage levels.

were observed in the lawn soil, including 6 OTUs in Archaeosporales, 5 OTUs in Diversisporales, 20 OTUs in Glomerales, 1 OTU in Paraglomerales, and 9 other OTUs from unknown orders (Figure 5B). For the AMF community in the five different *D. triflorum* coverage level soils, Glomerellaceae was dominant, with the highest relative abundance in the level 2 soil (75.9%) and the lowest relative abundance in the level 4 soil (20.0%) (Figure 5A). Opposite results were observed in Claroideoglomeraceae: the highest and the lowest relative abundances were 27.8% in the level 4 soil and 0% in the level 2 soil, respectively. The abundance of Ambisporaceae was the lowest (3.3%) in the level 2 soil but the highest (22.1%) in the level 5 soil. Acaulosporaceae occupied the lowest relative abundance (0.5% to 8.1%) in the five families defined, showing different contributions of AMF species in different families (Figure 5A).

Our molecular analysis results showed markedly different lawn soil AMF community compositions among the five *D. triflorum* coverage levels (Figure 5), presumably due to the different host–fungal specificity of the invasive *D. triflorum* in comparison to that of *Z. tenuifolia* (Zhang et al. 2010). Except in the level 4 soil, Glomerellaceae was the most abundant in the lawn soil, which implied that both *Z. tenuifolia* and *D. triflorum* are easily colonized by the AMF species of this family. The relative abundances of both Ambisporaceae and Acaulosporaceae were the lowest, but that of Glomerellaceae was the highest, while a lack of Claroideoglomeraceae and the lowest number of AMF OTUs were observed in the level 2 soil in comparison to soils of other levels (Figure 5). This reflects that the level 2 soil might have the simplest AMF community composition. In contrast, the relative abundance of Claroideoglomeraceae was the highest, while that of Glomerellaceae was the lowest in the level 4 soil, indicating a most responsive AMF community in the stage when *D. triflorum* began to become dominant in the lawn. Moreover, the soil AM fungal spore density differed significantly in response to the increased *D. triflorum* coverage levels (Figure 3). However, this change trend is not in line with previous results that more highly invaded grass plots held more abundant AMF spores than those in the less-invaded plots (Madawala 2014). Geography, microclimate, and soil status might partly contribute to this difference, because

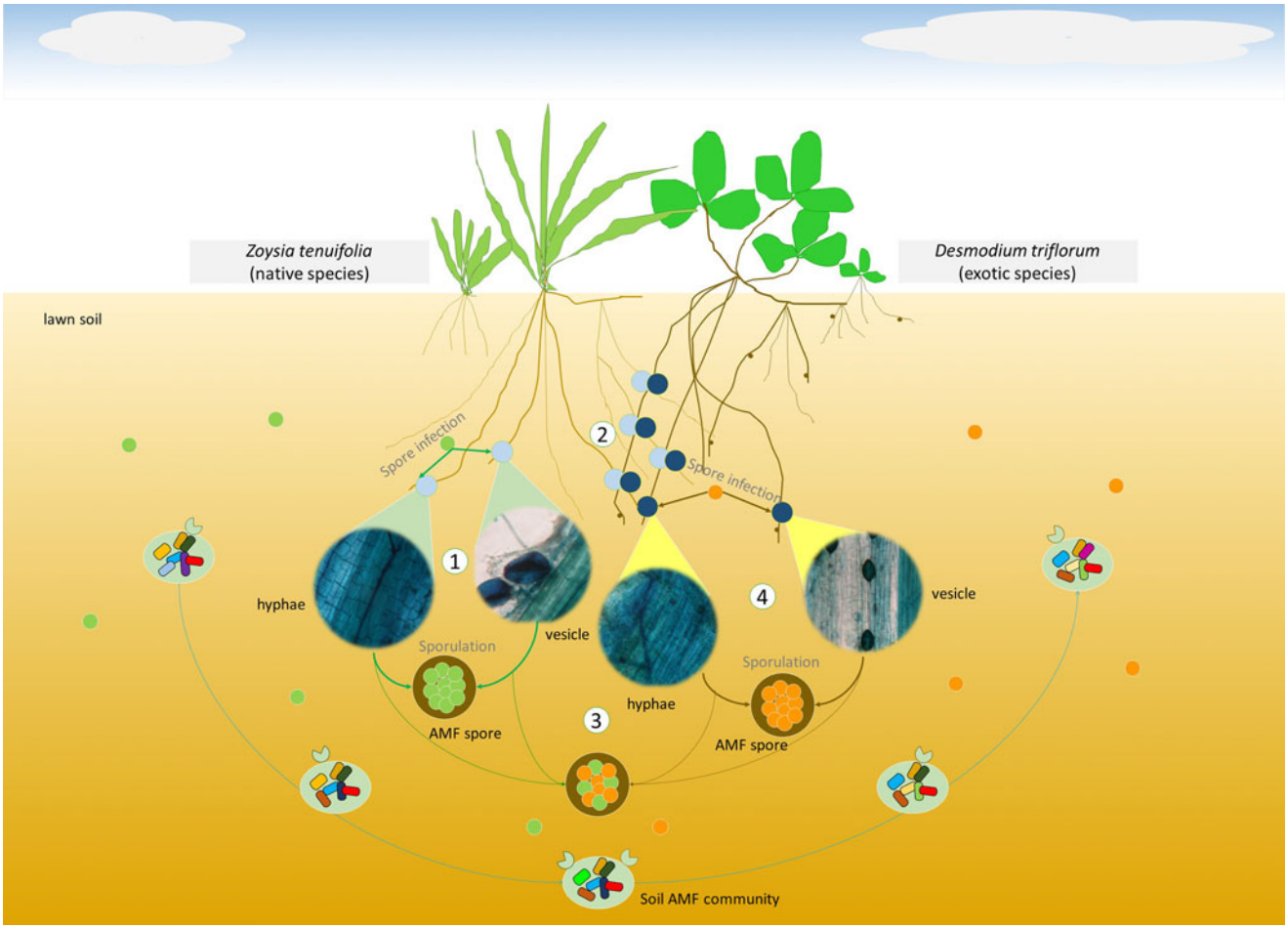

Figure 6. Conceptual framework demonstrating possible mechanisms of soil arbuscular mycorrhizal fungi (AMF) during the spreading process of *Desmodium triflorum* in the *Zoysia tenuifolia* lawn. Numbers 1, 2, 3, and 4 indicate different spreading stages of the invasive plant *D. triflorum*. Corresponding mycorrhizal structures were shown as the four microscopic views. Light-green and medium-yellow circles indicate AM fungal spores predominantly produced by the root mycorrhizal structures of *Z. tenuifolia* and *D. triflorum*, respectively. Medium-green and dark-yellow lines indicate the life cycle of spores in *Z. tenuifolia* plants and in *D. triflorum* plants, respectively. The AM fungi might influence the spread of *D. triflorum* by the following steps: (1) the early stage of the lawn's development with only *Z. tenuifolia* growing but without *D. triflorum* present. This occurs at the very beginning of the lawn establishment, and the AM fungal spores that previously existed in the lawn soil first infected the fine roots of *Z. tenuifolia* and completed the life cycle on their own. (2) The early spreading stage of *D. triflorum* (level 1). The roots of the two plants come into contact with each other, inducing the external hyphae that originally grow closely on the *Z. tenuifolia* roots to infect the roots of *D. triflorum*. The difference between the mycorrhizal infections of the two host plants contributes to higher root mycorrhizal colonizations of *D. triflorum* compared with *Z. tenuifolia*. However, at this stage, *D. triflorum* is not as competitive as *Z. tenuifolia* in the lawn, although it has advantages in terms of mycorrhizal infections. Therefore, the soil AM fungal spores are still predominantly produced by the mycorrhizal structures of the AMF-infected *Z. tenuifolia* roots. (3) The intermediate spreading stage of *D. triflorum* (levels 2 and 3). *Desmodium triflorum* continues to spread in the lawn. The contact of the two plants becomes more frequent and further induces a much closer relationship between the AM infections of the two plants. The increased *D. triflorum* plants in the lawn and the advantage of *D. triflorum* in root mycorrhizal infections facilitate the contribution of the mycorrhizal structures of the *D. triflorum* roots to sporulation. Thus, in this stage, the soil AM fungal spores were produced by the mycorrhizal structures of both plants, thereby inducing insignificant correlations between the spore densities and the root colonizations of either *Z. tenuifolia* or *D. triflorum*. (4) The late spreading stage of *D. triflorum* (levels 4 and 5). *Desmodium triflorum* is dominant in the lawn. The large numbers of *D. triflorum* plants and the AM infection advantage of *D. triflorum* facilitate AMF sporulation in the soil, thereby inducing significant correlations between the spore densities and the root colonizations of *D. triflorum*. At the different spreading stages of *D. triflorum*, the soil AM fungal communities also change as a result of the changed contributions of the AMF-infected host plants to the sporulation.

AMF occupied two niches simultaneously in the soil–plant connections: the intraradical niche for carbon supply and the bulk soils for survival and development (Helgason et al. 2014).

Possible Mechanisms of AMF During the Spreading Process of Desmodium Triflorum

Based on the results of root mycorrhizal colonizations and correlation analyses, we analyzed the possible role of AMF in the spreading process of *D. triflorum* (Figure 6). (1) At the beginning of the lawn establishment with only the *Z. tenuifolia* plants growing but without *D. triflorum* spreading, the soil AM fungal spores that existed in the soil before the lawn was established, first infected the fine roots of *Z. tenuifolia* and completed the life cycle on their own. (2) At the first spreading stage (level 1) of *D. triflorum*, the roots of the two plants came into contact with each other, which induced the external hyphae that originally grew closely on the *Z. tenuifolia* roots to infect the roots of *D. triflorum*. The inherent competitive advantages of *D. triflorum* in not only mycorrhizal symbiosis but also nutrient absorption finally contributed to higher root mycorrhizal colonizations of *D. triflorum* compared with *Z. tenuifolia*. However, at this stage, the *D. triflorum* plants were still not as competitive as the *Z. tenuifolia* plants. Therefore, the soil AM fungal spores were still predominantly produced by the mycorrhizal structures of the infected *Z. tenuifolia* roots. (3) *Desmodium triflorum* continued spreading in the lawn (level 2,

level 3). The aboveground and belowground contact of the two plants became more frequent and gradually induced a closer relationship between the AM infections of the two plants (Figure 4). Moreover, the increased numbers of the *D. triflorum* plants in the lawn and the advantages of *D. triflorum* in mycorrhizal infections facilitated the contribution of the mycorrhizal structures of the *D. triflorum* roots to sporulation. Therefore, the soil AM fungal spores were produced by the mycorrhizal structures of both plants, thereby inducing insignificant correlations between the spore densities and the root colonizations of either *Z. tenuifolia* or *D. triflorum* (Figure 4). (4) At the late spreading stage (level 4, level 5), the AM infection advantage of *D. triflorum* as well as the large numbers of the *D. triflorum* plants finally resulted in the mycorrhizal structures of *D. triflorum* predominantly contributing to sporulation in the soil, thereby inducing significant correlations between the spore densities and the root colonizations of *D. triflorum* (Figures 4 and 6). Notably, at the different spreading stages of *D. triflorum* in the lawn, the soil AM fungal communities varied as a result of the changed contributions of the AMF-infected host plants to sporulation (Figures 5 and 6).

Previous studies have presented a series of potential AMF functions that help exotic plants succeed in invasion, such as changing the root AM dependency, growth response, and fungal community difference between native and invasive species (Bunn et al. 2014; Endresz et al. 2013; Klironomos 2002; Lekberg et al. 2013; Zhang et al. 2010). In the current study, the significantly higher root colonizations of *D. triflorum* compared with those of *Z. tenuifolia* might explain the successful invasion of *D. triflorum* plants to some extent. On the other hand, the investigated soil spores and AM fungal communities in the present study are only representatives of the current spreading status in the lawn, while the life-history strategies of AMF (López-García et al. 2014) during the whole period still remain unclear. Moreover, the resident AMF species in the roots of the two different plant species still remain a mystery but are essential, especially to the initial root colonization in *D. triflorum* plants because of the priority effects and host specificity of AMF in infecting plant roots (Herrera et al. 2013; Opik et al. 2009; Santos-Gonzalez et al. 2007; Weber et al. 2015; Werner and Kiers 2015). Therefore, the future work of this study will focus mainly on the identification of AMF species that colonized the *Z. tenuifolia* and *D. triflorum* plant roots and the identification of AMF spore species in the soils where the two plants were growing. It is worth mentioning that we explored the invasion mechanism of *D. triflorum* from only the mycorrhizal infection aspects. Whether AMF induced differences by influencing the plant growth or root morphology constructions of the two plants (Enkhtuya et al. 2005; Fan et al. 2011; Wu et al. 2010; Wu QS et al., 2011) and the competitive relationships of the two plants (Danieli-Silva et al. 2010; Li et al. 2009) needs future exploration. Moreover, as a leguminous plant, *D. triflorum* might have more advantages in nutrient usage/absorption, biomass accumulation, root secretion production, and other physiological processes than the native species, *Z. tenuifolia*. The relationship of non-leguminous (*Z. tenuifolia*) and leguminous (*D. triflorum*) species in AMF interactions (Klabi et al. 2014) should be taken into consideration; therefore, a future laboratory incubation experiment simulating different *D. triflorum*/*Z. tenuifolia* stem densities in the lawn needs to be conducted to achieve a better understanding of the possible advantages of *D. triflorum* in plant growth, nutrient use, root morphology construction, and biomass responses under the infection of AMF.

In the present study, we compared the root mycorrhizal colonizations, soil AM fungal spore densities, and soil AMF communities under different invasion pressures of *D. triflorum* in a *Z. tenuifolia* lawn. Our results suggest that the development of *D. triflorum* in the lawn was likely to have been initiated by the root hyphal connection from *Z. tenuifolia* roots, while the preference of root mycorrhizal structures in contributing to soil sporulation tended to change from *Z. tenuifolia* to *D. triflorum*. Meanwhile, the different soil AMF community compositions and relative abundances of AMF OTUs among *D. triflorum* coverage levels might also reflect changes in the mycorrhizal colonizations, soil properties, and interspecific relationships of native *Z. tenuifolia* and invasive *D. triflorum* during this spreading period. Taken together, the successful spread of *D. triflorum* in the lawn is due not only to the advantage of root mycorrhizal infection, but also to the dependency of soil properties, spore properties, and root colonization of *D. triflorum* in comparison to *Z. tenuifolia*. Future evidence, such as the detailed root AMF/soil AMF spore species that initiated and supported the spreading process, as well as data on plant growth, nutrient utilization, root morphology construction, and interspecific competitive advantages of *D. triflorum* are needed to fully unveil the mechanisms of *D. triflorum* in successfully outcompeting *Z. tenuifolia* in the lawn.

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Supplementary Materials. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2019.50

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