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Competition or facilitation: Examination of interactions between endangered Sida hermaphrodita and invasive Phragmites australis

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by

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Abstract

Virginia Mallow (*Sida hermaphrodita*) is a perennial herb of the Malvaceae family that is native to riparian habitats in northeastern North America. Throughout most of its geographical distribution however, it is considered threatened and only two populations are known from Canada. The biology and ecology of *S. hermaphrodita* are still poorly understood and although few studies have been performed to determine the factors that contribute to the species rarity, it is considered threatened potentially due to the loss of habitat caused by exotic European Common reed (*Phragmites australis* subsp. *australis*) invasion. Allelopathic and phytotoxic conditioning of soils to inhibit native species are mechanisms that have been proposed to explain the invasion success of *P. australis*. To quantify the interaction between the two species and assess the capacity for *P. australis* to inhibit *S. hermaphrodita* performance through belowground soil modifications, a series of field vegetation surveys were conducted at the Taquanyah Conservation Area during the growing seasons of 2016, 2017, and 2018. Field performance findings suggested that proximity to *P. australis* had no significant effect on *S. hermaphrodita* seedling mortality or seedling root arbuscular mycorrhizal colonization. A supplementary greenhouse study was also conducted to examine plant performance and mycorrhizal colonization of both species in soils that correspond to different soil-vegetation levels ranging between pure stands of *S. hermaphrodita* to pure stands of *P. australis* in order to determine the potential for *P. australis* to allelopathically modify soils making them inhospitable for native species. The results provided no evidence to support previous soil conditioning reports since performance and arbuscular mycorrhizal colonization of both species were inversely promoted in their competitor's soil. Soil nutrient analysis coupled with the plant performance findings suggested that *P. australis* may not be as strictly competitive as previously believed since evidence of a belowground facilitative interaction between *S. hermaphrodita* and *P. australis* has been observed. Based on the results concluding that belowground conditions did not exclude native species, we believe aboveground competition for light is not only the main factor contributing to *S. hermaphrodita's* limited distribution where it occurs with *P. australis,* but also key to the invasion success of *P. australis*.

Future research and management treatments focussed on disrupting *P. australis'* competitive exclusion of light would be beneficial to the recovery of endangered species like *S. hermaphrodita*.

I would like to dedicate this thesis in loving memory of my grandfather, who was the first to teach me the value of appreciating the plants around me and will remain one of my biggest inspirations.

John Valentine Mulholland D.F.C.

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1.1 Biology, Ecology and Conservation Status of *Sida hermaphrodita*

1.1.1 Name and taxonomic history

Sida hermaphrodita, also commonly known as Virginia mallow, is classified as a member of the Malvaceae family. It was first described as a species of *Napaea* by Linnaeus in 1753. *Napaea hermaphrodita* was originally described together with *Napaea dioica*, but they were distinguished from one another due to *N. hermaphrodita's* hermaphroditic flowers (Linnaeus, 1753). *Napaea hermaphrodita*, however, was later transferred to the genus *Sida* by Henry Hurd Rusby in 1894 (Rusby, 1894). The genus *Sida* is considered one of the five largest genera of the Malvaceae family along with *Abutilon*, *Hibiscus*, *Nototriche* and *Pavonia*. Unfortunately, due to poorly defined distinguishing morphological characteristics, new species were uncritically placed within these genera posing significant problems for future classifications (Fryxell, 1997). Following their separation for example, *Sida hermaphrodita* and *Napaea dioica* have been misidentified in both herbarium specimen and botanical literature. The genus *Sida* includes a diverse assemblage of species and therefore has been further subdivided into subsections. Paul Fryxell placed *Sida hermaphrodita* alone within the *Pseudo-napaea* section due to its morphological distinctiveness (Fryxell 1978; Fryxell 1985; Fryxell and Fuertes, 1992; Fryxell 1997). Two more recent molecular phylogenetic studies corroborated this judgement of the distant relation between *S. hermaphrodita* and the core genus *Sida* and suggested that *S. hermaphrodita* along with the biogeographically separate *Sida hookeriana* Miguel, *Sidasodes colombiana* Fryx. & Fuertes, and *Sidasodes jamesonii* (E.G. Baker) Fryx. & Fuertes may form a sister clade to the "*Plagianthus* alliance" (Aguilar et al., 2003; Tate et al., 2005). Most recently however, due to *S. hermaphrodita*'s morphological and biogeographical uniqueness from all other members of the *Sida* core clade and other closely related genera including *Sidasodes*, *Lawrencia*, *Plagianthus*, *Hoheria*, *Asterotrichion* and *Gynatrix*, Weakley et al. (2017), proposed a transfer to classify *S. hermaphrodita* within a monotypic, isolated, North American

temperate genus called *Ripariosida*. Due to the recent nature of this proposed name change, however, to avoid confusion, the species will be referred to as *Sida hermaphrodita* throughout this document.

1.1.2 Plant description

Sida hermaphrodita is a tall perennial species native to North America that can reach heights of 1-4 meters (COSEWIC, 2010). The seedling roots develop initially a vertical tap root; however, the lateral branches develop plagiotropically (horizontally spreading) (Stevens et al., unpublished). The root system within the mature stands of *S. hermaphrodita* consists of a horizontal network occupying the top 40 cm of the soil (Stevens et al., unpublished). Mucilage cells are abundant in the cortex parenchyma. Due to the large amount of secondary phloem produced by the vascular cambium, the roots can grow up to 2 cm in diameter during the first growing season. The ring-porous wood and seasonal activity of the vascular cambium make the annual rings very clear, facilitating the determination of root age (Stevens et al., unpublished). The adventitious bud primordia which are responsible for vegetative reproduction, develop on the entire length of the root but are found to be more abundant in the vicinity of the hypocotyl (Stevens et al., unpublished).

The erect stems of *S. hermaphrodita* have a relatively common eudicot structure with extensive pith at the center. The medullary rays interrupt the cylindrical structure of the intra and inter-fascicular vascular cambium, and the inner layers of the cortex contain mucilage cells (Stevens et al., unpublished). The stems and leaves also have stellate trichomes with 4-5 branches giving the plant surface a shiny appearance and velvety texture. These trichomes, similarly to the stomata, are present on both the adaxial and abaxial leaf surfaces (Franzaring et al., 2014). The stipules are linear-lanceolate and approximately 3-4 mm in length. The stipules are free from the petioles and can be up to 0.9 mm shorter than the petioles (eFlora, 2016). The leaves grow alternately on the stem and slightly resemble maple leaves due to their palmate venation and deep lobes (the only species in the genus with palmately lobed leaves) (Fryxell, 1997; NatureServe, 2016a). The leaves range from 10-20 cm in length and have approximately 3-7 lobes that are irregularly serrate (Fryxell, 1997; NatureServe, 2016a; eFlora, 2016).

The axillary flowers of *S. hermaphrodita* are present on terminal corymbose panicles that are further sub-divided into umbelliform clusters of 2-10 flowers. The calyx is 5-lobed, 4-5 mm long, and velutinous due to the presence of hairs. The 5 petals are white, obovate and 6-10 mm long. The flowers are hermaphroditic with a monadelphous androecium and a gynoecium consisting of 6-10 carpels with capitate stigmas (Fryxell, 1997; NatureServe, 2016a; eFlora, 2016). These carpels produce a schizocarpic fruit that separates into 6-10 mericarps, each containing one seed (NatureServe, 2016a; eFlora, 2016). The seeds of *S. hermaphrodita* are red-brown with a hard, impermeable seed coat consisting of one palisade and one subpalisade cell layer. This seed coat has been observed in other *Sida* species and Malvaceae and is hypothesized to play a role in water impermeability and physical seed dormancy (Savchenko and Dimitrashko 1973; Egley and Paul 1982; Kurucz and Fári, 2013; Baskin and Baskin 2014).

1.1.3 Geographical distribution in North America

Sida hermaphrodita is native to the northeastern United States and southeastern ON. Its distribution is centered around the Great Lakes drainage basin, where is has been able to migrate throughout the Mississippi and Atlantic watersheds by way of downstream waterways. The species has been documented to occur in riverine habitats and floodplains east of the Mississippi river in the United States. Past reports state that its most extensive populations are located along the Kanawha and Ohio rivers in West Virginia (Spooner et al., 1985; NatureServe, 2016a), however, the native range of the species also includes Pennsylvania, Maryland, District of Columbia, Virginia, Indiana, Ohio, Michigan and Kentucky (Thomas, 1979; Spooner et al., 1985; Gleason and Cronquist, 1991; Bickerton, 2011; Voigt et al., 2012; NatureServe, 2016a; USDA, 2017).

Sida hermaphrodita is also native to southern Ontario, Canada which is the northernmost part of its distribution in North America. In Canada, *S. hermaphrodita* has been documented only in two locations situated approximately 35 km apart. The smaller of the two populations is located on a privately-owned quarry and an adjacent hydro corridor in the Niagara region. The larger population is

located at the Taquanyah Conservation Area in Haldimand County, which is managed by the Grand River Conservation Authority (Bickerton, 2011; Environment Canada, 2015; NatureServe: 2016a).

1.1.4 Geographical distribution outside North America

Outside of North America, *Sida hermaphrodita* was introduced as a cultivated plant to areas of the former Soviet Union in the 1930s, and to Poland in the 1950s (Borkowska and Styk, 2006). Initially, *S. hermaphrodita* was used to produce textile fibers, as fodder and also as a melliferous plant due to its long flowering period and large nectar production (Oleszek et al., 2013; Packa et al., 2014; Jablonowski et al., 2017). Since it was introduced over 50 years ago, research on the species has increased in several European countries. In Austria, Hungary and Lithuania, the species is only cultivated on a few hectares, however, in Germany, *S. hermaphrodita* is grown on approximately 100-150 ha comprised of experimental field sites and seed production plantations (Nahm and Morhart, 2018). The largest plantation, where most of the research activities have been performed to date, is located in Poland where *S. hermaphrodita* occupies more than 750 ha of land (Igliński et al., 2011; Franzaring et al., 2015). Due to its high cellulose content *S. hermaphrodita* has been identified as advantageous to the pulp and paper industry (Czarnecki and Dukarska, 2010; Smolińskiet al., 2011; Packa et al., 2014). Additionally, this species has demonstrated an ability to accumulate toxic chemicals and heavy metals from polluted soils, indicating its suitability for use in phytoremediation of degraded habitats (Antonkiewicz and Jasiewicz, 2002; Kocoń and Matyka, 2012; Werle et al., 2016). Furthermore, ongoing research is being conducted on *S. hermaphrodita* as a producer of bioenergy due to its wood-like, higher yield biomass in comparison to currently used energy plants such as corn (Borkowska et al., 2009; Oleszek et al., 2013; Barbosa et al., 2014; Szyszlak-Bargłowicz et al., 2015; Jablonowski et al., 2017). Notwithstanding its utility with respect to several industrial applications, *S. hermaphrodita* is classified as an invasive species in the Czech Republic (DAISIE, 2015; Matthews et al., 2015; Catalogue of Life, 2017).

1.1.5 Conservation status in North America

1.1.5.1 Canada

Considering its limited distribution in Canada, the species is listed under the federal Species at Risk Act (SARA) and, the provincial Endangered Species Act (ESA) as "endangered", meaning that *S. hermaphrodita* is facing imminent extirpation or extinction. Following the assessment by the Committee on the Status of Species at Risk in Ontario, in 2009, a recovery strategy was formulated for Virginia Mallow in the province (Bickerton, 2011). Resulting from this, an Ontario provincial government response statement was released acknowledging the information outlined in the strategy and declaring actions the government will take to help protect and recover Virginia Mallow. Some of the actions include educating other agencies and developing new protection regulations (Ontario Ministry of Natural Resources, 2011). Recently a federal recovery strategy was released for Virginia Mallow in order to improve conservation and recovery in Canada (Environment Canada, 2015).

1.1.5.2 U.S.A.

As previously mentioned, *S. hermaphrodita*'s distribution has been documented to extend West through Indiana, East to Maryland, South to Tennessee and its northernmost distribution is found in the Carolinian zone of Southwestern Ontario (Thomas 1979; Spooner et al. 1985; Gleason and Cronquist 1991). According to NatureServe (2016a), *S. hermaphrodita* is rare throughout most of its native range and it is currently assessed as Vulnerable in Ohio, Imperiled in Kentucky, West Virginia, and Pennsylvania, Critically Imperiled in Indiana, Maryland, and Virginia, and Possibly Extinct in Tennessee and Washington D.C.

1.1.5.3 Factors contributing to its rarity

Several assessments of the species have been made to narrow down possible threats that may have contributed to the species' rarity. To some degree, the species has been limited by its preference for riparian and floodplain habitats within the Carolinian zone of Canada and the U.S.A. Although the species has been described as thriving in riparian habitats with a degree of moderate human disturbance, human

activities such as development, methods of flood control and site maintenance have reduced the natural habitat throughout the plant's range (Bickerton 2011; Environment Canada, 2015; NatureServe, 2016a).

In previous reports, it was suggested that different biological factors could be responsible for the limited distribution of the species including specific soil requirements and low seed germination rates (Spooner et al., 1985; Kujawski et al. 1997; Bickerton, 2011). Recent studies however, have revealed that *S. hermaphrodita* has efficient vegetative and sexual propagation strategies (Packa et al., 2014; Stevens et al., unpublished) in addition to a capacity to tolerate low moisture as well as poor and moderately contaminated soils (Antonkiewicz and Jasiewicz, 2002; Kocoń and Matyka, 2012; Bickerton, 2011; Cetner et al., 2014).

It has been suggested by the Ministry of Natural Resources (2011), that the largest threat to *Sida hermaphrodita* populations in Canada is due to the loss of habitat as a result of invasive species. The increasing abundance of the Common Reed (*Phragmites australis* (Cav.) Trin. Ex Steud.) has become a concern due to its rapid and aggressive spread throughout North America. At the larger Canadian population of *S. hermaphrodita* located at the Taquanyah Conservation Area, *P. australis* has increased its abundance surrounding *S. hermaphrodita* stands, suggesting that it may be competing with *S. hermaphrodita* for light, space and nutrients (Bickerton, 2011; Environment Canada, 2015). This thesis will further explore the interaction taking place between *S. hermaphrodita* and *P. australis* at Taquanyah Conservation Area.

1.1.6 Habitat and vegetation

Virginia mallow is generally found in floodplains, bottomlands and riparian areas that are subjected to periodic flooding. Although the species prefers open sunny areas, it also survives in partial shade (Bickerton, 2011; Environment Canada, 2015). As previously mentioned, although human development continues to limit *S. hermaphrodita's* native habitat, this species is capable of surviving in disturbed habitats including railroad banks, roadside ditches and infrastructure corridors (Bickerton, 2011; Environment Canada, 2015; NatureServe, 2016a).

Sida hermaphrodita has been found to be capable of growing in a wide range of conditions. It can tolerate soil pH values between 5.4 and 7.5 and can grow in a variety of soil textures including silt loam, sandy clay loam, and clay loam (Bickerton, 2011). This species has the capacity to grow in poor soil conditions with low organic matter in addition to sewage sludge containing elements such as copper, iron and nickel (Spooner et al., 1985; Borkowska and Wardzinska, 2003; Bickerton, 2011). Additionally, it has been previously reported that both natural and cultivated populations require a significant amount of water for growth (Spooner et al., 1985), however, a recent study has determined that *S. hermaphrodita* can handle reduced moisture conditions when planted in poor soils (Cetner et al., 2014).

The dominant vegetation present at both of the two populations of *S. hermaphrodita* in Ontario has also been documented. The location of the first population in the Niagara region, is a previously disturbed site where the vegetation grows on shallow soils over limestone. The dominant species present here are open meadow species including, Fuller's Teasel (*Dipsacus fullonum* L.), Queen Anne's Lace (*Daucus carota* L.), Gray Dogwood (*Cornus racemosa* Lam.), Staghorn Sumac (*Rhus typhina* L.) and goldenrods (*Solidago* spp.) (Bickerton, 2011). At the second of the two populations located in Haldimand County, most of the vegetation occurs within a Forb Mineral Meadow Marsh (MAM 2-10) and the dominant species include Broadleaf Cattail (*Typha latifolia* L.), Common Reed (*Phragmites australis*), Purple Loosestrife (*Lythrum salicaria* L.), Spotted Touch-me-not (*Impatiens capensis* Meerb.), Fuller's Teasel (*Dipsacus fullonum*) Canadian Goldenrod (*Solidago canadensis* L.), Reed Canary Grass (*Phalaris arundinacea* L.), Black Walnut (*Juglans nigra* L.), Redosier Dogwood (*Cornus sericea* L.) and Staghorn Sumac (*Rhus typhina*) (Bickerton, 2011; Stevens et al., 2017).

1.1.7 Growth and development

As a perennial plant, *S. hermaphrodita* continues to grow every year and cultivated plants have been documented to live up to 20 years (Borkowska et al., 2009). *S. hermaphrodita* produces plagiotropic roots that persist underground through the winter to form buds that will develop into new shoots in the next year. Each spring, in late April or early May, new seedlings also emerge in the population from

Taquanyah (Stevens et al., 2017a). Plants growing from seeds only produce one stem in their first year of growth, however, stem density in consecutive years of growth has been observed to exceed more than twenty stems per plant (Borkowska et al., 2009). Due to the nature of the species' root system, populations of *S. hermaphrodita* are clonal, which makes it very difficult to distinguish mature individuals without using molecular markers (Spooner et al., 1985; Bickerton, 2011; Stevens et al., unpublished manuscript).

Shoots continue to grow throughout the summer reaching heights of up to 4 meters (Borkowska and Molas, 2012), however, it has been found that their growth begins to slow down in the autumn months during which time the leaves are shed (Franzaring et al., 2014). Several studies have determined that depending on the environmental ground and climate conditions, *S. hermaphrodita* has a higher dry matter yield ranging between 9-20 tonnes per hectare, when compared to various other plant species (Borkowska and Wardzinska, 2003; Borkowska et al., 2009; Slepetys et al., 2012; Borkowska and Molas, 2013). *Sida hermaphrodita* has also been observed to produce taller plants and increased stem densities during its 3rd and 4th year of production (Borkowska et al., 2009). Due to the allocation of biomass to the stems of *S. hermaphrodita*, this species accumulates a lower ash content and a higher fibre content when compared to other bioenergy plants (Franzaring et al., 2015). Flowering can occur during the first year and in North America, it begins in July and continues until the first frost occurs (Spooner et al., 1985; Bickerton, 2011). The production of fruits generally takes place during September and October and each plant is capable of producing several thousand seeds (Bickerton, 2011; NatureServe, 2016a). The seeds are released throughout the winter and are suspected to be dispersed by water during the following spring (Spooner et al., 1985; Krzaczek et al., 2006; Bickerton, 2011; NatureServe, 2016a).

1.1.8 Mycorrhiza

Another potential factor that could affect the growth, development and success of *S. hermaphrodita* is through an interaction with arbuscular mycorrhizal fungi (AMF). AMF are obligate biotrophs of the Glomeromycota group that are capable of forming symbiotic relationships with plants

(Wang and Qiu, 2006). This relationship is one of the most common symbiotic relationships on the planet, used by approximately 80% of vascular plant species (Remy et al., 1994; Schübler et al., 2001; Jeffries et al., 2003). In order to do so, the fungus hyphae penetrate the plant roots and differentiate into vesicles and arbuscules. The vesicles have been assumed to be involved in fungal nutrient storage whereas the arbuscules are the site of nutrient exchange between the plant and the fungus (Bonfante-Fasolo, 1984; Strack et al., 2003; Pumplin and Harrison, 2009) (Figure 1.1). Outside of the plant root, the fungal hyphae branch within the soil to essentially extend the root system past the phosphorous depletion zone. By doing so, the fungus is able to facilitate plant acquisition of water, micronutrients and macronutrients with emphasis on phosphorous (Clark, Zeto, 2000; Harrison, 2005; Besserer et al., 2006; Allen and Shachar-Hill, 2009; Smith and Smith, 2011). In return, the plant provides the fungus with carbon in the form of photosynthates (Besserer et al., 2006; Chen et al., 2010; Bapaume and Reinhardt, 2012).

Several studies have shown that the presence of AMF can affect the relative abundance of plant species and plant species diversity, in turn, altering plant community structure. It has been suggested that the mechanisms by which the presence of AMF affect plant diversity include the transport of assimilates between plants through the hyphal network and also the plant's mycorrhizal dependency (Grime et al., 1987; Habte and Manjunath, 1991).

Gerdmann (1975) defined mycorrhizal dependency as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility." A wide range of mycorrhizal dependencies has been observed in plants. Some species have no mycorrhizal dependency due to the fact that they are not capable of forming this symbiotic relationship (Bayliss, 1975). Others, however, have been observed to have mycorrhizal plants that are up to 13,000% larger than a non-mycorrhizal plant (Hall, 1975; Menge et al., 1978). Plants capable of forming mycorrhizal associations can be discriminated based on their requirement for this symbiotic interaction. Janos (1980) described both facultative and obligate mycotrophism in which facultative mycotrophic plant species can

be defined as “those that can attain reproductive maturity without mycorrhizae at least in the most fertile of their natural habitats.” Alternatively, obligate mycotrophic plant species were defined as “those that cannot grow or survive without mycorrhizae over the range of soil fertility that they naturally encounter” (Janos, 1980; Janos 2007). The growth response of a plant to AMF can be affected by a number of factors including the soil type, soil phosphorus levels, plant species and mycorrhizal fungal species (Azcon and Ocampo, 1981). Most natural ecosystems consist of several AMF species and since AMF are understood to have different degrees of host specificity, each plant within a community could potentially be colonized by several AMF species. However, the change in composition of the AMF community could alter the dynamics of the plant community (Bever, 2002). In addition, since AMF species vary in their ability to take up phosphorus and in their stimulation of plant growth, it is important to understand how much a plant species relies on this relationship to further quantify mycorrhizal community structure and how particular species of AMF may be affecting the plant community structure (Menge et al., 1978; Graham and Syvertsen, 1985; Habte and Manjunath, 1991; Van der Heijden et al., 1998). To date no studies have been performed on the mycorrhizal status of *S. hermaphrodita*.

1.1.9 Physiology

Very limited information is available on the life functions of *S. hermaphrodita*; however, recent work performed in Poland and Germany on the use of this species in energy production has given some insight into the species physiology. Due to the high density of stomata present on the leaf surfaces, *S. hermaphrodita* has been documented to have high photosynthetic rate and stomatal conductance (Franzaring et al., 2014). The presence of numerous trichomes on the stem and leaf surfaces may be an adaptation to reduce water loss through transpiration (Franzaring et al., 2014).

1.1.10 Reproduction

1.1.10.1 Floral biology

As previously mentioned, the flowers of *S. hermaphrodita* are hermaphroditic and anthesis at Taquanyah takes place between July and August. No specific information is available about the

pollinators of this species (Spooner et al., 1985; Bickerton, 2011; Jablonowski et al., 2017) but we observed bees (Apoidea, Hymenoptera) cross-pollinating *S. hermaphrodita* plants at Taquanyah (Figure 1.2). Based on data obtained on the Glade Mallow (*Napaea dioica*), however, it could be hypothesized that *S. hermaphrodita* is pollinated by the same insects in the Hymenoptera, Diptera, and Hemiptera orders (Iltis, 1963; Bickerton, 2011).

1.1.10.2 Potential for vegetative reproduction

Due to roots with adventitious buds, *S. hermaphrodita* is capable of vegetative reproduction. Adventitious bud primordia originate from within the roots at the periphery of the secondary vascular tissue, and their development can be stimulated by mechanical injury. These buds are capable of forming along the entire length of the root; however, they are more numerous closer to the hypocotyl (Stevens et al., Unpublished manuscript). These below ground buds act as a perennial bud bank similar to a seed bank but providing vegetative regeneration after seasonal or mechanical disturbances (Harper, 1977; Klimešová and Martínková, 2004; Klimešová and Klimeš, 2008; Stevens et al., unpublished).

1.1.10.3 Seed dispersal

From the schizocarpic fruits of *S. hermaphrodita*, several thousand seeds are released from each plant during the winter (Spooner et al., 1985; Stevens et al., unpublished). Due to the riparian and wetland habitats in which this species is found in, it is currently assumed that the seeds are dispersed by water (COSEWIC, 2010).

1.1.10.4 Viability of seeds and germination

Of the thousands of seeds released by each *S. hermaphrodita* plant, it is understood that almost all of them are viable and have the potential to germinate (COSEWIC, 2010). According to numerous studies, however, the germination capacity of recently harvested seeds is reduced to 10-15% for the first six months after harvest indicating that the seeds become dormant creating a seed bank underground (Packa et al., 2014; Baskin and Baskin, 2014). The seed dormancy in *S. hermaphrodita* has been

suggested to be both physical due to its impermeable seed coat consisting of one palisade and subpalisade cell layers (Baskin and Baskin, 2014; Packa et al., 2014; Stevens et al., unpublished), as well as physiological in which unknown chemical compounds within the seed delay germination (Packa et al., 2014). Although the longevity of the seeds is currently unknown, it has been observed that the germination capacity of the seeds was highest from seeds stored for 2.5 years (Doliński et al., 2007; Doliński, 2009). Due to the physical dormancy imposed by the seed coat, chemical, physical or biological scarification to open or damage the palisade layer is required to stimulate germination (Packa et al., 2014; Stevens et al., unpublished). In nature, this can happen through the action of microorganisms, changes in temperature, freezing and thawing fluctuations, or digestive enzymes in animals that have ingested the seeds. In laboratory or commercial growth, the seeds coat can be damaged with sulphuric acid, alcohol, high pressure, boiling and scratching (Rolston, 1978; Argel, Paton, 1999; Packa et al., 2014).

1.1.11 Population dynamics

The Taquanyah Conservation area located in Haldimand County, Ontario is a 136 ha complex of woodland, wetland and floodplain habitats and is the location of the largest population of *S. hermaphrodita* in Canada. This habitat is found within the floodplain associated with a unique cold-water stream called Mill Creek. The creek was dammed in the 1960s to be used as a reservoir to produce carp, however, the introduction of the fish in the 1980s and the subsequent rise in temperatures decreased water quality and resulted in negative impacts to the downstream ecosystems. As a result, in 2005, the dam was decommissioned and the reservoir was allowed to be recolonized by native vegetation including *S. hermaphrodita*; however, it also allowed for the growth of invasive species including *Phragmites australis*. As of 2016, there were a total of 158 separate stands of *S. hermaphrodita* located at Taquanyah Conservation Area occupying an estimated area of approximately 2,616 m^2. The average stem density within these stands was 9 stems/ m^2 and the estimated number of stems present at Taquanyah was 29,245 (Chapter 2). When comparing these numbers to the measurements taken in 2014, the estimated area occupied has increased from 2,109 m^2 whereas the estimated stem count and density are slightly lower

than the estimated 29,833 stems and 14 stems/ m^2 measured in 2014 (COSEWIC, 2010; Bickerton, 2011; Environment Canada, 2015; Stevens et al., 2017a).

1.1.12 Responses to herbicides and other chemicals

So far, there have been no studies completed on the effects of herbicides on *S. hermaphrodita*, however, some recent work has documented the impact of other chemicals such as fertilizers and heavy metals on cultivated plants. For example, it was determined that the level of nitrogen and carbon dioxide fertilization did not affect biomass yield, however, carbon dioxide fertilization slightly improved shoot regrowth after *S. hermaphrodita* was harvested (Borkowska et al., 2009; Franzaring et al., 2015). In contrast, phosphorus fertilization has been found to significantly increase the yield of *S. hermaphrodita* (Borkowska et al., 2009), which could be potentially significant for the work related to the mycorrhizal colonization of the species.

Additionally, recent studies have examined the phytoextraction capacity of *S. hermaphrodita* in contaminated soils which has highlighted some information on the response of this species to different elements. It has been observed that *S. hermaphrodita* is tolerant to contamination of soil with different metals and it is capable of absorbing different levels of Cadmium, Copper, Zinc, Manganese, Nickel, Chromium and Lead (Wierzbowska et al., 2016; Kocoń and Matyka, 2012).

1.1.13 Responses to herbivory and diseases

Very limited information is available on the response of *S. hermaphrodita* to diseases and herbivory. The only pathogen that has been observed to affect *S. hermaphrodita* is the fungus, *Sclerotinia sclerotiorum* (Lib.) de Bary (Remlein-Starosta et al., 2016). Due to its ability to infect over 450 different host plants (Boland and Hall, 1994), *S. sclerotiorum* is considered a serious pathogen to crop species, capable of destroying between 5-40% of plantation shoots (Remlein-Starosta, 2008; Starzcha et al., 2004; Mrówczyński et al., 2009; Remlein-Starosta et al., 2016). White rot symptoms that are characteristic of *S. sclerotiorum* infection were first described on *S. hermaphrodita* in Poland in 1990 (Łacicowa and Kicana,

1991). The infection of the host plant is typically initiated by the appearance of dark, water-soaked lesions on the leaves or stems which will expand, developing into necrotic tissues. Patches of fluffy white mycelium will appear over the necrotic tissues and the sclerotia will spread throughout the plant leading to chlorosis and wilting (Bolton et al., 2006). In *S. hermaphrodita*, the first shoots with wilting symptoms appeared at the end of May in Poland when the plants had reached approximately 50 cm (Remlein-Starosta and Nijak, 2007). Previous work done by Jajor et al. (2010), reported that the size of the infestation in crop plantations was related to higher temperature and humidity conditions. It has also been reported that disease incidence was significantly greater in *S. hermaphrodita* shoots that had been subjected to mechanical injuries (Remlein-Starosta, 2008). Additionally, a recent study has identified yeast-like fungal strains that are capable of inhibiting the growth of *S. sclerotiorum*, suggesting their use as a potential biocontrol on *S. hermaphrodita* plantations (Remlein-Starosta et al., 2016).

Currently, no work has been performed on herbivory factors that could affect the plant growth, however, a couple studies have described information about the plant's physiology that could suggest possible defence mechanisms that *S. hermaphrodita* may employ against herbivory. One study observed the presence of calcium oxalate crystals in the form of druses within the parenchymal tissues of *S. hermaphrodita*'s stems (Leszczuk et al., 2014). The production of calcium oxalate crystals in plants can be used to regulate calcium and also to add structural support. The presence of calcium oxalate crystals in the stems, although unlikely due to their shape, could also suggest that they are used as a defence against herbivory since some crystals are sharp fragments that herbivores find hard to eat (Leszczuk et al., 2014). Additionally, as previously mentioned, another study performed by Franzaring et al. (2014), has described the presence of numerous trichomes on the leaf and stem surfaces of *S. hermaphrodita*. These structures could also be used as a form of herbivory defence since they reduce palatability (Franzaring et al., 2014). Although these structures were hypothesized to protect against herbivory, a recent transplant study performed in 2016 at Taquanyah Conservation Area reported predation on young *Sida hermaphrodita* plants likely by Eastern Cottontail rabbits (*Sylvilagus floridanus*) (Stevens et al., 2017b).

1.2 Biology and Interactions of *Phragmites australis*

1.2.1 Name and taxonomic history

Phragmites australis also known as Common reed, is a perennial species that belongs to the Arundineae tribe in Poaceae consisting of four other species including *P. karka*, *P. mauritianus* and *P. japonicus* (Clevering and Lissner, 1999). Originally, Linnaeus had described *Phragmites australis* in 1753 as *Arundo phragmites*. Subsequently, the genus *Phragmites* was determined to be distinct from *Arundo* and common reed was transferred to the former. This species has numerous common synonyms including *P. communis*, *P. vulgaris* and *P. berlandieri*, which are more recent than *P. australis* (Saltonstall and Huber, 2007; Hocking et al., 1983; Mal and Narine, 2004).

1.2.2 Plant description

Phragmites australis is a tall perennial emergent aquatic and wetland plant that can reach heights between 4-6 m (Mal and Narine, 2004; Lambert et al., 2010; Cross and Fleming, 1989). At Taquanyah, most commonly the stems reach between 3-4 m (Personal observation). The diameter of the stem ranges between 4 and 15 mm and contains long hollow internodes approximately 10-25 cm in length. (Mal and Narine, 2004; Clayton et al., 2006; Sturtevant et al., 2018). This species has four stem types including below-ground horizontal rhizomes, above-ground vertical rhizomes, erect aerial shoots and legehalme (Haslam, 1969). The wider horizontal rhizomes (1.5-2 cm diameter) are found up to 1 meter underground and are responsible for vegetative propagation and extending the clone size (Mal and Narine, 2004; Brisson et al., 2010). The thinner vertical rhizomes (1-1.5 cm diameter) are responsible for bearing the erect aerial shoots that photosynthesize and develop the inflorescences (Haslam, 1968; Mal and Narine, 2004). Although they are not produced in all populations, *P. australis* can also develop legehalme (long runners or stolons) from the vertical rhizomes or fallen aerial shoots that bear small leaf blades (Haslam, 1969; Mal and Narine, 2004; Brisson et al., 2010). The roots of *P. australis* are fleshy and develop from the rhizomes and submerged parts of the plant (Haslam, 1968; Mal and Narine, 2004; NatureServe, 2016b). Depending on the habitat, two different root types can be produced by *P. australis*, including

short, narrow and very branched “water” roots that create dense fibrous mats in water-substrate interfaces only extending downwards approximately 10-30 cm. Or thicker and less branched “mud” roots are produced that extend downwards between 1-5 m into the substrate (Pallis. 1916; Haslam, 1972; Mal and Narine, 2004).

The smooth, glabrous and narrow linear-lanceolate leaves are alternate and can reach 20-70 cm in length and 1-5 cm in width. The leaf bases form smooth overlapping sheaths around the stems and the ligule is membranous with hairs (Mal and Narine, 2004; NatureServe, 2016b).

The inflorescence of *P. australis* is a feathery, terminal panicle, between 15 and 50 cm long. The inflorescence can vary in colour, with ascending branches bearing many spikelets. The rachillas of the spikelets are hairy, contributing the woolly appearance of the inflorescence. The numerous spikelets are approximately 1.0-1.7 cm, have two glumes at their base and include 2-8 florets each; the lower 1-2 flowers are staminate, the distal 1-2 flowers are rudimentary, and the remaining flowers are bisexual. The glumes are unequal in size, lanceolate and persistent. Lemmas are 3-veined, glabrous, awnless. Male flowers have 1-3 stamens. The upper hermaphroditic flowers have three stamens and an ellipsoid ovary with a short style and two feathery stigmas. The resulting fruit is a caryopsis less than 2 mm long (Mal and Narine, 2004; NatureServe, 2016b;).

1.2.2.1 Interspecific variation

Phragmites australis demonstrates a high degree of phenotypic plasticity depending on the origin of plants and environmental conditions (Hocking et al., 1983; Mal and Narine, 2004). It is currently understood that North American *P. australis* populations are represented by three distinct infraspecific lineages: one native one; subsp. *americanus*, one of indeterminate origin; subsp. *berlandieri*, and one invasive, introduced from the Old World, subsp. *australis.* The high degree of morphological variation makes it difficult to distinguish native versus invasive lineages in North America without genetic testing (Mal and Narine, 2004). However, subtle morphological differences have been identified to differentiate native lineages from invasive lineages. The North American native lineage, subsp. *americanus*, typically

has smooth and shiny aerial shoots in addition to yellow-green leaves that easily detach from the shoots after senescence (Swearingen and Saltonstall, 2012; Sturtevant et al., 2016). The exposed shoots display a reddish colour at the nodes and internodes where they are exposed to UV. Black spots can also appear on the shoots due to the presence of an unidentified native fungus (Swearingen and Saltonstall, 2012; Sturtevant et al., 2016). The ligules of the native plants have been observed to be approximately 1-1.7 mm long. In comparison to the invasive lineage, the inflorescences of the native plants are sparse and appear brownish in colour. Additionally, the upper and lower glumes of the spikelets range in length from 5.5-11 mm and 3-6.5 mm respectively (Saltonstall et al., 2004; Swearingen and Saltonstall, 2012; Sturtevant et al., 2016).

In contrast to most native lineages, the invasive lineage, subsp. *australis*, has typically dull and slightly rigid aerial shoots. Red colouration of the invasive shoots is rare but can appear on the lower nodes. In addition, a black sooty-like mildew can be present on the shoots (Swearingen and Saltonstall, 2012; Sturtevant et al., 2016). The leaves are blue-green in colour and tightly adhere to the shoots throughout the growing season. The ligules of the invasive plants have been observed to range between 0.4-0.9 mm in length. The inflorescences of the invasive lineage are very "bushy" in appearance and are usually purple or golden in colour. Furthermore, the upper and lower glume lengths range between 4.5-7.5 mm and 2.5-5 mm respectively (Saltonstall et al., 2004; Swearingen and Saltonstall, 2012; Sturtevant et al., 2016).

The only lineage that cannot be differentiated from the invasive lineage using these characteristics is subsp. *berlandieri*, which shares the same morphological characteristics except for the dull and rigid aerial shoots (Saltonstall et al., 2004; Swearingen and Saltonstall, 2012).

1.2.3 North American geographical distribution and habitat

Phragmites australis is one of the most widely distributed plants as it can be found on every continent except Antarctica (Roland and Smith, 1969; Mal and Marine, 2003; Gucker, 2008). The species is most commonly found in freshwater, brackish and alkaline wetlands in the temperate zones of the

globe; however, it has also been identified in some tropical wetlands. In North America it has become increasingly widespread, typically growing in tidal and non-tidal wetlands, marshes, swamps, and fens. Additionally, it can also be found in disturbed sites such as roadside ditches, construction sites, near agricultural fields or along developed shorelines (Chambers et al., 1999; Mathieu-Giroux and de Blois, 2007; Jodion et al., 2008; Lambert et al., 2010; Swearingen and Saltonstall, 2012). In North America, *P. australis* subsp. *americanus* is widespread throughout Canada and most of the U.S.A., while the 'Gulf coast lineage', *P. australis* subsp. *berlandieri*, is restricted to the southern American states (Saltonstall, 2002; Saltonstall et al., 2010). Paleontological evidence together with recent genetic analyses have clarified the evolutionary and biogeographical history of the native *P. australis* and the effects of the invasion of subsp. *australis* in North America (Saltonstall, 2003).

Fossil records of *Phragmites australis* and its rhizomes that date back ca. 4,000 years, have been found in southwestern US and north Atlantic tidal marshes (Kaplan, 1963; Neiring et al., 1977; Clark, 1986; Kane and Gross, 1986; Orson et al., 1987). A *P. australis* Pleistocene fossil was also identified from between 11,000 to 40,000 years ago within Shasta ground sloth dung collected from caves in the Grand Canyon (Hansen, 1978), while the oldest fossil record suggests that *P. australis* was present in North America during the Cretaceous period (Lamotte, 1952). It is based on these records, that the species is considered a native plant to North America, however, due to the dramatic change in its abundance over the past 200 years, studies are being performed in order to assess genotypic differences in the *P. australis* populations present in North America (Clevering and Lissner, 1999; Mal and Narine, 2004; Swearingen and Saltonstall, 2012; NatureServe, 2016b).

1.2.3.1 Native

Phragmites australis subsp. *americanus* has the highest genetic diversity among all lineages (Saltonstall et al., 2010). Thirteen endemic haplotypes have been identified in North America by 5 uniquely shared mutations that distinguish them from all other haplotypes worldwide (Saltonstall, 2002; Saltonstall et al., 2010). These haplotypes broadly cluster corresponding to 3 geographic regions

including the Atlantic Coast, the Midwest, and the West. Haplotypes E, F, Z, AA, AB, and AC have been found along the Atlantic Coast ranging from Cape Cod in the South to Georgia in the north. Haplotypes E, G, and S have been documented in the Midwestern area which includes the Great Lakes region and southern Canada. Lastly, haplotypes A, B, C, D, and K have been identified in the Pacific Northwest and Southwestern U.S. (Saltonstall, 2002; Saltonstall 2003a; Saltonstall et al., 2010). Of these haplotypes, haplotype E had the greatest distribution ranging from East to West coasts and North into all Canadian provinces and the Northwest Territories (Mal and Narine, 2004; Lambert et al., 2010). Due to its large distribution and its close relation to other haplotypes, it is hypothesized that haplotype E is the ancestor of all other native *P. australis* subsp. *americanus* lineages (Saltonstall, 2003).

Comparing historic to modern samples, most of the 13 native North American haplotypes showed little change in their distributions (Saltonstall, 2002; Saltonstall et al., 2010). However, subsequent sampling of populations from the Atlantic coast region has shown a large reduction in genetic diversity. Most of the native haplotypes that were historically common in this region, were not identified in modern populations. Only haplotypes F and Z were found to persist at one site in Virginia and Maryland respectively (Saltonstall, 2003; Meadows and Saltonstall, 2007). All other Atlantic coast types have been suspected to be eliminated due to the rapid spread of the invasive lineage (Saltonstall, 2002).

1.2.3.2 Gulf coast

The second lineage, *P. australis* subsp. *berlandieri* has been identified to have the lowest genetic diversity (Saltonstall et al., 2010). Only a single type (haplotype I) has been recognized to dominate the Gulf coast region of the U.S. Its distribution, which has remained unchanged between historic and modern samples, extends west into the Gulf of California and south into Mexico and northern parts of South America (Saltonstall, 2003). This haplotype, which shares no mutations with the other 13 haplotypes native to North America, has been shown to be most closely related to Asian haplotypes (Saltonstall et al., 2010). Due to its relation to haplotypes present in other parts of the world, it has been suggested that this haplotype is not native to North America and instead, it is synonymous with the Australian and Asian

species *P. karka*, however, it is not known when this species may have been introduced and genetic analysis has not been performed to confirm this hypothesis (Saltonstall, 2002; Ward, 2010).

1.2.3.3 Invasive

Lastly, the invasive lineage, *P. australis* subsp. *australis* has shown differing levels of genetic diversity depending on the source of DNA. Most populations have been suggested to belong to a single type, haplotype M (Saltonstall, 2003). More recently, other closely related haplotypes have also been identified in isolated populations, including haplotype L found in eastern Washington (Saltonstall, 2003) and L1 found in Quebec (Meyerson and Cronin, 2013). These may be the result of local mutations or secondary introductions of the haplotype M (Saltonstall et al., 2010). Haplotype M is the most common haplotype worldwide and is most closely related to haplotypes found across Europe and continental Asia (Saltonstall et al., 2010; Saltonstall, 2003). Its global distribution has been suspected to be indicative of its plasticity and ability to colonize new habitats, including those in North America (Saltonstall et al., 2010).

Due to its high prevalence on the Atlantic Coast, it was hypothesized that haplotype M was first accidentally introduced there from the United Kingdom (Plut et al., 2011) in the ballast material of ships during the late 1700s or early 1800s (Saltonstall, 2002; Saltonstall, 2003). From there is has been able to spread throughout the Atlantic coast where is has displaced almost all the haplotypes native to this region. In the Midwest region, although all native haplotypes persist, haplotype M has become increasingly prevalent around the Great Lakes and St. Lawrence River. Lakeshores, rivers, and roadsides bordered by drainage ditches have been suggested to provide corridors to facilitate its dispersal further into the Midwest where the threat to native haplotypes is high (Saltonstall, 2003; Wilcox et al., 2003; Maheu-Giroux and de Blois, 2007; Lelong et al., 2007; Jodoin et al., 2008). In the remaining regions, haplotype M is rare and only found in urban areas of the West and along the Mississippi River Delta in the Gulf coast (Saltonstall, 2003). Comparably to the Midwest region, although native haplotypes are dominant, the future dispersal of the invasive haplotype M into these regions is thought to be clearly possible (Saltonstall, 2003; Hauber et al., 2011).

1.2.4 Growth, development and reproduction

Phragmites australis has an underground rhizome system that persists perennially. Considered the juvenile stem type that restores and maintains *P. australis* populations, the horizontal rhizomes produce the most growth during the late summer and early fall when their nutrient reserves are at their maximum (Haslam, 1968; Hocking et al., 1983). Extending horizontally underground at a rate of 0.5-4 m per year, the horizontal rhizomes turn upwards and terminate into vertical rhizomes in the spring after remaining dormant through the winter (Haslam, 1968; Mal and Narine, 2004). New horizontal rhizomes typically develop from vertical rhizomes before the end of summer (Mal and Narine, 2004). The vertical rhizomes grow most rapidly during the early spring and terminate into aerial shoots whenever they break the soil surface (Haslam, 1968). Lateral buds present on the vertical rhizomes also give rise to new aerial shoots. Bud formation on the rhizomes begins in the midsummer and continues gradually throughout the winter to prepare for shoot emergence in the early spring, which has been observed to occur over 1-3 months (Haslam, 1969; Hocking et al., 1983). The aerial shoot growth has been documented to be at its highest during the summer months and then begins to slow in the autumn during which time the leaves will senesce (Hocking et al., 1983; Swearingen and Saltonstall, 2012). Legehalme may also be developed directly from vertical rhizome buds or from fallen aerial shoots. Due to their non-rigid structure, the legehalme extend horizontally along the soil surface, with a growth rate documented to exceed 10 m per year (Haslam, 1968; Haslam, 1972; Brisson et al., 2010). Legehalme branches may turn upwards to develop into non-flowering aerial shoots or turn downwards to produce new horizontal rhizomes, thereby further propagating the *P. australis* stand (Haslam, 1968). In addition to the four different stem types, *P. australis* also produce fleshy roots from its rhizome system. Roots have been documented to be sparse when originating from horizontal rhizomes and are more densely branched when originating from the vertical rhizomes (Haslam, 1972). During the spring and summer months is when the roots proliferate the most (Hocking et al., 1983).

League et al. (2006), observed differences in growth between native and invasive *P. australis*. Field observations revealed that native *P. australis* subsp. *americanus* was typically characterized by a lower stem density and biomass, and exhibited less expansion (League et al., 2006), however, there may be variability within the native lineage causing denser stem growth comparable to the invasive lineage (Snyder, Personal Communication). The native plants have been observed to have a larger rhizome bud density than the invasive plants; however, the increased buds did not give rise to greater shoot production. In contrast to the longer bud stage of the native subsp. *americanus*, the buds of the exotic subsp. *australis* developed quickly, giving rise to a significantly greater density of newly emerging shoots (League et al., 2006). The early shoot emergence, together with a later flowering period allow the invasive lineage to benefit from a longer growing season (Mal and Narine, 2004; League et al., 2006). It is suggested that this may contribute to the differences observed in shoot growth in which invasive shoots were on average 28 cm taller and had up to five times the shoot biomass of the native *P. australis* (League et al., 2006). The observed shoot to root ratio was also greater, suggesting that the invasive subsp. *australis* has a more efficient root and rhizome system, through which it can allocate more resources aboveground (League et al., 2006).

Vegetative reproduction has been documented to be the main means by which *P. australis* is capable of maintaining their populations and spreading into to new areas (Mal and Narine, 2004); however, this may be accurate only for the Gulf coast lineage (Pellegrin and Hauber, 1999; Saltonstall et al., 2010). Although rhizomes have been documented to typically live between 3-7 years (Haslam, 1972; Hocking et al., 1998), *P. australis*. populations can maintain themselves for over 100 years through vegetative propagation (Rudescu et al., 1965). Rhizome fragments as short as 20 cm can serve as propagules for colonization into new areas when dispersed by water, animals or human activities (Haslam, 1969; Small and Catling, 2001). The horizontal rhizomes are responsible for the gradual expansion of populations. Although growth and expansion are slow for the first couple years, *P. australis* populations are capable of expanding clonally at a rate of 4 m per year (Clevering and van der Toorn,

2000), likely facilitated by significantly greater elongation of rhizome internode lengths in the invasive lineage (League et al., 2006).

Although range expansion has previously been solely attributed to vegetative reproduction, seed dispersal has recently been suggested to be important for the establishment of new stands of both native and invasive lineages (Brisson et al., 2008; Saltonstall et al., 2010). Plants have the capacity to flower 4 months after germination. Flowering periods have been observed to vary between lineages, with the invasive lineage, *P. australis* subsp. *australis* flowering between July through September. *Phragmites australis* subsp. *americanus* has been noted to flower between June and October, while the Gulf coast lineage, subsp. *berlandieri* flowers later, between October and November (Saltonstall et al., 2010). Like other Poaceae, the florets are wind pollinated. Each individual plant is capable of producing thousands of caryopses/seeds each year which are dispersed by wind, water and animals during the winter months (Bittmann, 1953; Haslam, 1972; Hocking et al., 1983). The germination percentage of the seeds has been documented to be extremely variable between 2 and 100% (Haslam, 1973; Kraska et al., 1992), with the invasive lineage displaying the highest germination rate. (Meyerson et al., 2009; Saltonstall et al., 2010). Seedling mortality has been reported to be relatively high due to different habitat conditions; thus, sufficient seeds must be dispersed each year to overcome this challenge (Hocking et al., 1983; Mal and Narine, 2003; Swearingen and Saltonstall, 2012). Additionally, Brisson et al. (2008), observed that in eastern Canada, as long as the seedlings withstand the critical period of the first winter, they will be likely to form mature individuals facilitating the establishment of new populations (Brisson et al., 2008).

1.2.4.1 Intraspecific hybridization

Due to the overlapping geographical ranges and flowering periods of the three North American *Phragmites australis* lineages, it was expected that hybridization and gene flow between these lineages would have occurred in natural populations (Saltonstall et al., 2010). Until recently, phenological or physiological barriers were suggested to prevent the cross-pollination between native and invasive lineages (Saltonstall 2002; Saltonstall, 2003b; Meyerson et al., 2010). However, recent studies determined

that through manual cross-pollination, viable hybrid seedlings can be produced (Meyerson et al., 2010), and have also provided the first evidence of natural hybridization among native and invasive *P. australis* populations in Ontario (Paul et al., 2010). Although constraining mechanisms like limited pollen dispersal and flowering time have been suspected to impact the interbreeding between lineages, the continuous expansion of subsp. *australis* into the native *P. australis* habitats increases the potential for identifying new hybrids. Future examination and monitoring of hybrid individuals will be necessary since the fitness of these new hybrids as well as their potential to threaten native biodiversity is unknown (Meyerson et al., 2010; Paul et al., 2010).

1.2.5 Mycorrhiza

Arbuscular mycorrhizal fungi have been observed to form symbiotic relationships with the majority of terrestrial plant biomass. However, since the soils in wetland habitats are often saturated and lack oxygen availability for aerobic soil microorganisms, the presence of AMF in wetland ecosystems was historically thought to be rare. As a result, although the effects of AMF on plants and soils in terrestrial habitats is well known, little attention has been given to these fungi in wetland habitats (Dolinar and Gaberščik, 2010; Wang et al., 2015). It is now recognized based on recent studies, that AMF are also prevalent in wetlands; however, the factors that affect AMF colonization and the relationships between plants and rhizospheric microorganism communities in wetland habitats are still poorly understood (Stevens et al., 2011; Wang et al., 2015).

Due to *P. australis*' tolerance for both terrestrial and aquatic environments, it has recently been examined for mycorrhizal colonization. AMF have been reported within the roots of *P. australis,* but it was dependent on the soil moisture content and the plant phenology (Cooke and Lefor, 1998; Oliveira et al., 2001), and in some instances, the colonization was not confirmed under flooded conditions (Wirsel, 2004). It is hypothesized that one of the reasons why *P. australis* supports mycorrhizal colonization is due to its ability to use pressurized through-flow of air to vent its below ground tissues (Brix et al., 1996). Although a mycorrhizal symbiosis has been identified, limited information is available and the objective

of the majority of these studies was to determine how colonization by AMF improved the phytoremediation ability of *P. australis* in contaminated habitats. Several studies have determined that the mycorrhizal symbiosis with *P. australis* had beneficial effects on the water quality and growth of the plants under cadmium and salinity stress conditions (Al-Garni, 2006; Huang et al., 2017). Furthermore, these studies have all taken place in areas of Europe and Asia including Germany, Slovenia, Portugal, Estonia and China (Oliveira et al., 2001; Al-Garni, 2006; Dolinar and Gaberščik, 2010; Huang et al., 2017). Further examination of the relationship between *P. australis* and AMF in North American habitats would be beneficial.

Although studies on the mycorrhizal colonization of *P. australis* have not taken place in North America, studies on the association with other fungi have been conducted in the United States. Diverse communities of fungal endophytes have been identified to colonize a variety of *P. australis* tissues including, roots, rhizomes, stems and leaves (Clay et al., 2016; Soares et al., 2016). Fungal endophytes have previously been identified to form symbiotic relationships with other plant species, mediating the host plant growth and improving their ability to adapt to new environments (Clay and Schardl, 2002; Rodrigues et al., 2009; Porras-Alfrano and Bayman, 2011). Recent work using endophytes recognized to associate with *P. australis* has supported these studies suggesting that *P. australis* endophytes may be capable of enhancing the host's growth (Clay et al., 2016) and its tolerance to extreme habitats (Soares et al., 2016). Although the capacity for *P. australis* to form symbiotic relationships with these fungi has been confirmed, the functional role of fungal endophytes is still not fully understood (Kowalski et al., 2015; Clay et al., 2016).

1.2.6 Potential invasion mechanisms

Plant invasions by exotic species are often very detrimental to ecosystems as the invading plant species not only compete with the native species for resources, but they also often replace the native biodiversity resulting in devastating ecosystem and detrimental economic effects (Mack et al, 2000; DiTomaso, 2000; Zedler and Kercher, 2004). As previously mentioned, *P. australis* is currently

considered one of the most invasive species causing considerable negative effects on native biodiversity (Marris, 2005; Rudrappa et al., 2007). Typically, it is expected that due to the lack of predators in the new habitat, introduced species are more aggressive (Inderjit et al., 2006; Rudrappa et al., 2007). However, numerous recent studies have been performed to determine the specific mechanism that enhance the invasive capacity of *P. australis*. Increasing attention has been given to allelopathy as a major pathway for the invasive process of weeds including *P. australis* (Bais et al., 2003; Callaway and Ridenour, 2004; Pisula and Meiners, 2010). Allelopathy refers to a type of chemical warfare by which one organism is capable of either directly or indirectly inhibiting the growth of another organism through the release of chemical compounds into the environment (Rice, 1984; Bais et al., 2006; Hong et al., 2008). Although a considerable number of studies have been performed, allelopathic interactions are not fully understood and several hypotheses have been proposed about the mechanism by which *P. australis* allelopathically interacts with neighboring plants.

Rudrappa et al. (2007) suggested that the invasive success of *P. australis* could be attributed to its release of exudates containing gallic acid (3,4,5-trihydroxybenzoic acid) from its root system (Root Allelopathy). Identified as the active phytotoxin, gallic acid was hypothesized to directly induce rhizotoxicity and inhibit the growth of native plant roots (Rudrappa et al., 2007). It was suggested that gallic acid phytotoxicity was due to the generation of toxic levels of reactive oxygen species which would trigger a cell death cascade by disrupting the microtubule assembly in native plant roots (Rudrappa and Bais, 2008). Additionally, it was reported that the gallic acid secreted by *P. australis* roots, could also undergo photo-degradation when exposed to ultraviolet light, resulting in mesoxalic acid (2-oxomalonic acid) which negatively affects neighboring plants similarly to its precursor (Rudrappa et al., 2009).

In a subsequent study, Bains et al. (2009) suggested that *P. australis* allelopathically affects native plants indirectly through interactions with rhizospheric microorganisms. Through examination of the pathway for free gallic acid production, it was hypothesized that instead of directly excreting gallic acid, the exotic lineage of *P. australis* contains elevated levels of polymeric gallotanin within its roots.

These gallotanins were determined to be hydrolyzed by tannase enzymes produced in high amounts by acid-degrading microbes and native *P. australis* plants to release the phytotoxic compound gallic acid (Bains et al., 2009). Due to the observed community size of acid-degrading microbes associated with exotic rhizosphere samples, it was suggested that not only is exotic *P. australis* capable of root allelopathy through indirect inhibition of native plant growth, but it is also capable of altering the microbial community structure of the rhizosphere to enhance its invasive potential (Bains et al., 2009).

Although gallic acid was reported to be responsible for the invasive success of *P. australis*, contradictory results were published disputing the role of this phytotoxin. After sampling soil, rhizomes and foliage of several *P. australis* populations, Weidenhamer et al. (2013) were unable to detect free gallic acid within the soils associated with *P. australis* and only detected trace amounts within the plants (Weidenhamer et al., 2013). Even though it was previously reported that gallic acid was highly toxic to neighboring plants (Rudrappa et al., 2007), other studies found that gallic acid was less active than other phenolic compounds and required at least a 10 mM concentration to have any inhibitory effects (Reigosa et al., 1999; Chung et al., 2002; Weidenhamer et al., 2013). Furthermore, it was discovered that due to the rapid degradation of gallic acid in non-sterile soil, the persistent high concentrations required for allelopathic interaction could not be maintained. Based on these results, it was suggested that the exudation of gallic acid could not be the primary explanation for *P. australis*' allelopathic effect (Weidenhamer et al., 2013).

Due to the contradictory results of gallic acid's effects on plant growth and previous reports stating that phytotoxic effects typically result from a mixture of phytotoxins rather than a single compound (Reigosa et al., 1999; Inderjit and Duke, 2003), other studies have been performed to examine the effects of aqueous extracts released from various *P. australis* tissues through decomposition (Allelopathic Phytotoxicity) (Uddin et al., 2012; Uddin et al., 2014a; Uddin et al., 2014b; Uddin et al., 2014c; Uddin et al., 2017). The presence of gallic acid was confirmed in various organ extracts of *P. australis* in combination with other unidentified phenolics, supporting the suggestion that a combined

effect of total phenolic compounds may better explain *P. australis*' allelopathic effect (Uddin et al., 2012; Uddin et al., 2014a). It was observed that *P. australis* extracts from different organs have inhibitory effects on seed germination and establishment due to oxidative stress caused by the production of reactive oxygen species (Uddin et al., 2014a). These inhibitory effects of residue decomposition on native seedling growth and consequently native vegetation structure, were also observed to be significantly greater under anaerobic conditions (Uddin et al., 2012; Uddin et al., 2014c). When comparing the effects of tissue extracts, *P. australis* leaf extracts were reported to have the greatest inhibitory effects followed by the extracts from rhizomes, roots, stems and inflorescences (Uddin et al., 2012; Uddin et al., 2014a). Based on these results it was hypothesized that through the degradation of the large volumes of biomass produced by *P. australis*, especially leaf degradation, allelopathic phytotoxins are directly released into the surrounding soil consequently inhibiting the growth of native species (Uddin et al., 2014a). Additionally, it was also observed that *P. australis* infected soils had lower arbuscular mycorrhizal fungal inoculum potential suggesting that *P. australis* may have the potential to allelopathically effect neighboring species indirectly by interfering with belowground mutualisms (Uddin et al., 2017).

Lastly, following what was mentioned previously, other studies have examined the mutualistic relationship between *P. australis* and endophytic microorganisms in order to determine if this symbiotic association plays a role in the invasive character of *P. australis* (Fischer and Rodriguez, 2013; Clay et al., 2016). Diverse communities of endophytes were found to colonize the root, rhizome, stem and leaf tissues of *P. australis* (Li et al., 2010; Clay et al., 2016). Soares et al. (2016a) demonstrated that endophytic bacteria associated with the shoot meristems of *P. australis* were capable of enhancing the nutrient uptake of the host by scavenging for nitrogenous compounds present in the rhizosphere (Soares et al., 2016a). Subsequently, it was also established that fungal endophytes associated with *P. australis* roots could help the host adapt to high saline soils (Soares et al., 2016b). The functional roles of specific endophytes are not fully known but one fungal endophyte, genus *Stagonospora*, identified on root, stem, leaf and seed samples of *P. australis* has been suggested to enhance host growth (Ernst et al., 2003; Clay et al., 2016).

Subsequently, White et al. (2018) found supporting evidence that seed associated endophytes not only can improve nutrient availability for the host, but also inhibit soil fungal pathogens, supress diseases, and allow *P. australis* to inhibit competitor plants through the release of compounds by the associated endophytes (Endophytic Allelopathic Exclusion). Using a strain of *Pseudomonas fluorescens*, isolated from *P. australis*, it was observed that bacterial inoculation inhibited the growth of test species which was hypothesized to be due to the production of hydrogen cyanide (HCN) by the bacteria (White et al., 2018). More research is required to examine the effects of other associated endophytes however, based on this research it is possible that the invasive success of *P. australis* may be attributed to its association with microorganisms.

Due to the different hypotheses regarding the allelopathic potential of *P. australis* it is difficult to understand the method by which *P. australis* affects different plants including endangered species like *S. hermaphrodita*. It is unknown whether the allelopathic chemicals detrimentally affect plants directly, through their interaction with rhizospheric and symbiotic microorganisms, or by negatively affecting the beneficial plant-fungus relationship with arbuscular mycorrhiza.

Current ecology theory suggests that an invasive species has the capacity to modify the below ground environment which would increase its fitness while making the surrounding soil inhospitable for competing native species (Jordan et al., 2008). *Phragmites australis* has been suggested to affect neighboring plants through allelopathic effects such as modifying various aspects of the soil's chemical and physical properties, including pH values, organic matter content, soil structure and altering microbiotic communities (Jordan et al., 2008). Research has shown that positive feedback between soils and invasive species contributes to the effect of plant invasion in which the invasive species can use the changes induced to improve its fitness by taking over new areas. (Corbin and D'Antonio 2004; Ehrenfeld 2004; Wolfe and Klironomos 2005; Eppstein and Molofsky 2007). Nonetheless, research is uncertain whether these effects always benefit invasive species more than native species (Jordan et al., 2008).

1.3 Objectives and Hypotheses

As previously indicated, the biology and ecology of *S. hermaphrodita* are poorly understood and the factors contributing to its rarity have only recently begun to be examined. Several different suggestions have been made about the potential allelopathic invasion mechanism of *P. australis*, the common mode of action has been through belowground processes. It is unknown if *S. hermaphrodita* is affected by *P. australis* but, it could be expected that any effects on plant growth would be through these belowground processes. This study shall examine the reciprocal interaction between the endangered *S. hermaphrodita* and the invasive *P. australis* in order to determine if each species impacts the performance of the other. Additionally, since to date there is limited information on the relationships that form between these two plants and AMF, this study shall also examine the mycorrhizal colonization of these plants grown in soils obtained from Taquanyah Conservation Area. The specific objectives of my M.Sc. thesis were to (1) determine how seedling performance and AMF root colonization of *S. hermaphrodita* in the field relates to the presence/absence of *P. australis* and (2) determine how chemical compounds and microorganisms present within the soils associated with *S. hermaphrodita* and *P. australis* affect the performance and mycorrhizal colonization of both plants. The former will be done by assessing the performance of *S. hermaphrodita* seedlings as well as the AMF colonization of *S. hermaphrodita* seedling roots at locations where *P. australis* plants are present or absent at Taquanyah Conservation Area. The latter will be accomplished by examining the performance of both species in soils obtained in different vegetation levels ranging from high density *S. hermaphrodita* to high density *P. australis*. For the first objective, based on the literature reports according to which *P. australis* is capable of negatively impacting the growth of neighboring species via direct or indirect allelopathic soil modification interactions (Rudrappa et al., 2007; Bains et al., 2009; Weidenhamer et al., 2013; Uddin et al., 2014a; White et al., 2018), I hypothesized that seedling mortality of *S. hermaphrodita* will increase in close proximity to *P. australis*. Additionally, I also hypothesized that due to the suggestion that *P. australis* may have an impact on arbuscular mycorrhizal fungal colonization potential of native plants (Uddin et al., 2017), any AMF colonization observed in *S. hermaphrodita* seedlings would decrease in close proximity

to *P. australis* stands. For the second objective I hypothesized that the performance of *S. hermaphrodita* will increase in soils obtained from farther distances from *P. australis*, while based on the understanding of the highly invasive characteristics of *P. australis* (Mal and Narine, 2003; Marris, 2005), the performance of *P. australis* will remain consistent across all sites. Similarly, I also hypothesized that the arbuscular mycorrhizal colonization of *S. hermaphrodita* will increase in farther distances from *P. australis*, while *P. australis* will not be colonized since *P. australis* colonization has previously been limited (Cooke and Lefor, 1998; Oliveira et al., 2001; Wirsel, 2004).

1.4 Figures

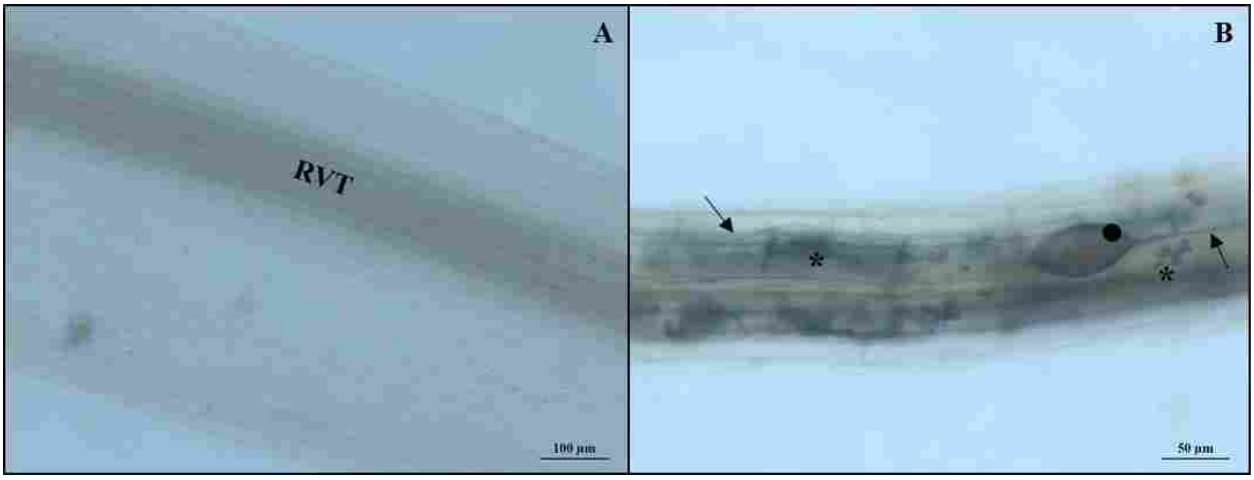

Figure 1.1: Root samples of *P. australis* that were cleared in a 10% KOH solution, and stained with a 5% ink in vinegar solution for the observation of AMF colonization. Depicted are segments of a non-colonized root (A) with the Root Vascular Tissue (RVT) visibly running through the centre of the root, and a segment of root colonized with blue stained AMF structures (B). AMF structures include intra-radicular hyphae (arrow) within the cortex of the root running parallel to the root vascular tissue which differentiate into vesicles (circle) used for nutrient storage, and arbuscules (asterisk) which are the site of nutrient exchange between the plant and the fungus.

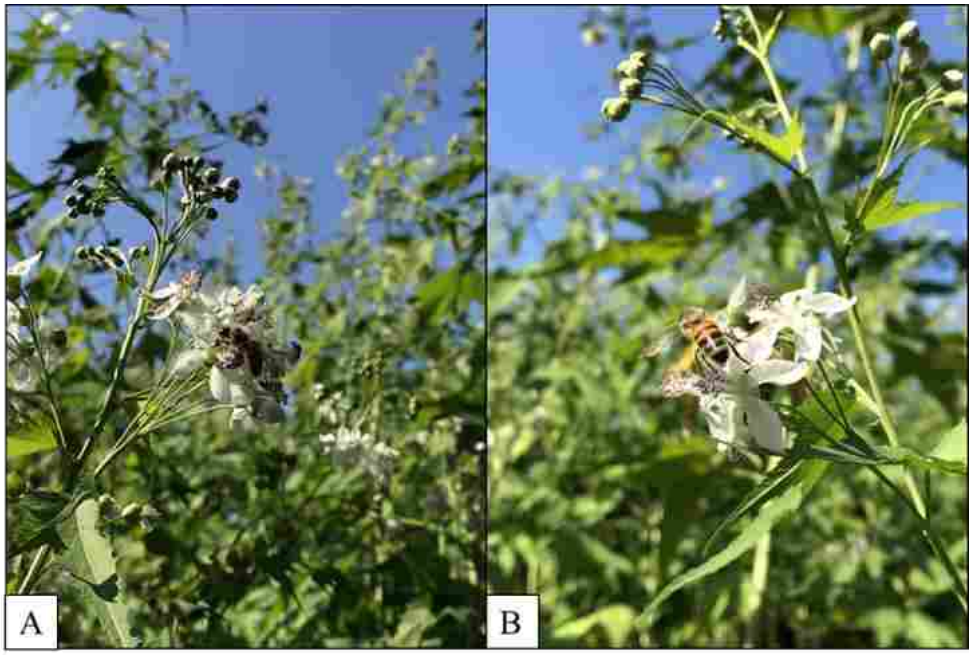

Figure 1.2: Honeybees observed pollinating the hermaphroditic flowers of *S. hermaphrodita* stands at Taquanyah Conservation Area in July 2019 (A & B).

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Chapter 2: Seedling performance of endangered *Sida hermaphrodita* does not support a putative detrimental soil conditioning by *Phragmites australis*

In preparation for *Conservation Biology*

Following the defense, this manuscript will be expanded to also incorporate aboveground competition results obtained by the Rooney lab.

2.1 Abstract

Virginia Mallow (*Sida hermaphrodita*) is a perennial herb native to riparian habitats in northeastern North America. Throughout most of its geographical distribution, however, it is considered threatened potentially due to the loss of habitat caused by exotic European common reed (*Phragmites australis*) invasion. The biology and ecology of *S. hermaphrodita* are still poorly understood, and factors contributing to the species rarity are unknown. Allelopathic and phytotoxic conditioning of soil to inhibit native species are mechanisms that have been proposed to explain the invasion success of *P. australis*. Field vegetation surveys were conducted to quantify *S. hermaphrodita* seedling performance and arbuscular mycorrhizal colonization in areas ranging in regard to their proximity to *P. australis* stands to determine the potential of *P. australis* to allelopathically alter soils and inhibit native seedling growth. Results did not support previous allelopathic soil conditioning reports since field results suggested that the proximity to *P. australis* has no significant effect on *S. hermaphrodita* seedling mortality or seedling root AMF colonization. Interestingly, seedling emergence was highest in areas of intermediate proximity to *P. australis* stands. The *S. hermaphrodita* field performance findings coupled with the AMF results suggested that nutrient stress may be promoting the coexistence of both species and that *S. hermaphrodita* stands currently have the capacity to increase at Taquanyah Conservation Area. Our results also suggested that instead of belowground soil conditioning, the key to *P. australis* invasion may be through competition for light and that the effective control and management of this species (and thus conservation of *S. hermaphrodita* populations and other native plants) may be achieved through the disruption of its aboveground competition potential.

2.2 Introduction

Biological invasions by alien plant species are considered one of the greatest threats to natural communities, ecosystems and global biodiversity (Mack et al., 2000; Pimentel et al., 2000; Sala et al., 2000; Alvarez and Cushman, 2002). The introduction of alien invasive species into new habitats is often the result of human actions, in which the alien species are either intentionally or inadvertently moved

outside of their native range where they can grow exponentially and become aggressive invaders (Vitousek et al., 1997; Mitchell and Power 2003; Sanon et al., 2009). The aggression by which alien species can negatively impact the new ecosystems and the native species that occupy them has been attributed to numerous factors including escape from natural predators (Mitchell and Powers 2003), higher productivity (Ruiz and Carlton 2003; Uddin and Robinson 2018), adaptability to environmental disturbance (Mitchell and Gopal, 1991), higher competitive ability (Thébaud and Simberloff, 2001), allelopathic potential (Bais et al., 2003; Callaway and Ridenour 2004; Pisula and Meiners 2010; Uddin et al., 2012) and the modification of soil microbiota including the disruption of symbioses with Arbuscular Mycorrhizal Fungi (AMF) (Roberts and Anderson, 2001; Levine et al., 2003; Mummey and Rillig, 2006; Stinson et al., 2006; Sanon et al., 2009; White et al., 2018). Through the use of such mechanisms, invasive alien species displace native species, leading to their endangerment and potential extinction (Vitousek et al., 1997; Wilcove et al., 1998; Simberloff, 2003). Alien species now dominate many marine, freshwater and terrestrial habitats throughout the world, and their rate of increase is frequently exponential (Ruiz and Carlton 2003). Consequentially, interest in understanding the mechanisms attributed to alien invasions and the impacts the invaders have on native plant communities, has grown in hopes of determining the best control measures suitable to conserving native species and their natural ecosystems (Rejmánek and Richardson 1996; Byers et al., 2002; Levine et al., 2003).

Sida hermaphrodita (L.) Rusby (Virginia Mallow; Malvaceae) is a herbaceous perennial species native to floodplains and riparian habitats of Northeastern North America (Spooner et al., 1985; COSEWIC 2010). It is a large clonal species that can reach 1-4 m in height (COSEWIC 2010; Borkowska and Molas 2012), and reproduces both sexually through the release of seeds from its hermaphroditic flowers, and vegetatively through the budding of its robust plagiotrophic root system (Thomas 1980; Spooner et al., 1985; Bickerton 2011; Stevens et al., unpublished). Although information on the species' physiology and ecology is limited, and its capacity to form AMF relationships has not been determined, the species is understood to tolerate a variety of soil conditions as well as a degree of moderate human

disturbance (Bickerton 2011; Kocoń and Matyka, 2012; Oleszek et al., 2013; Cetner et al., 2014). Despite *S. hermaphrodita's* vigorous growth, good reproductive potential (Stevens et al., unpublished) and tolerance to various environmental conditions, the species is currently endangered throughout its native geographical distribution and has been designated Canadian national conservation and Global conservation statuses of Critically Imperiled (N1) and Vulnerable (G3) respectively (Spooner et al., 1985; Klimešová and Klimeš, 2008; COSEWIC 2010; Environment Canada, 2015; NatureServe 2019). Recently, the largest threat to the conservation of *S. hermaphrodita* was suggested to be the loss of habitat as a result of the increasing abundance of the invasive *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed; Poaceae) (Bickerton 2011).

Invasive *P. australis* is a tall perennial aquatic and wetland grass that can reach heights between 4-6 m (Mal and Narine, 2003; Lambert et al., 2010; Cross and Fleming, 1989). It makes use of optimal reproductive strategies whereby the combination of sexual reproduction through the release of seeds and vegetative reproduction through the growth of its extensive rhizome system, contribute to its dispersal and range expanse into new territories (Clevering and Lissner, 1999; Mal and Narine 2003; Lambert et al., 2010; Belzile et al., 2010; McCormick et al., 2010; Kirk et al., 2011). Considered to be native to Eurasia, the invasive haplotype M can be currently found on every continent except Antarctica (Roland and Smith, 1969; Gucker, 2008; Mal and Marine, 2003). Since its introduction into Eastern North America likely from the United Kingdom (Plut et al., 2011) in the 19th century, invasive *P. australis* has created large, rapidly expanding monospecific stands that have displaced entire communities of flora and fauna (Burk, 1877; Saltonstall 2003; Mal and Narine 2003; Saltonstall et al., 2010).

Globally considered one of the most aggressive invasive species (Fell et al., 1998; Marris 2005; Uddin et al., 2012), considerable efforts have been made to determine specific mechanisms to explain the competitive success of *P. australis*. Biological characteristics, such as high rates of sexual and vegetative propagation and the development of dense canopies, are thought to provide competitive advantages for its expansion into new ecosystems (Meyerson et al. 2000; Mozdzer and Zieman 2010). However, numerous

studies have also hypothesized that *P. australis* can enhance its invasion by directly or indirectly inhibiting the growth of neighbouring plants by allelopathically and phytotoxically conditioning soil properties (Rudrappa et al., 2007; Uddin et al., 2012; Uddin et al., 2014; Weidenhamer et al., 2013; Crocker et al., 2017), modifying soil microbial communities (Jordan et al., 2008; Song et al., 2015; Shearin et al., 2018), and disrupting belowground symbiotic relationships with AMF (Uddin et al., 2017). Unfortunately, the literature surrounding *P. australis'* allelopathy and phytotoxicity is not robust enough to conclude that *P. australis'* invasion relies on these mechanisms (Uddin et al., 2017), thus more work is needed to examine *P. australis* underground plant interactions to fully understand its threat to native species like *S. hermaphrodita*.

The objective of this study was to observe underground interactions between *P. australis* and the endangered *S. hermaphrodita* in field settings to gain insight into how *P. australis* may pose a significant threat to *S. hermaphrodita*. Additionally, since it is currently unknown whether *S. hermaphrodita* can form symbioses with AMF, this study will also examine the AMF colonization of seedling roots. More specifically, this study seeks to address the putative detrimental soil conditioning effects of *P. australis* populations and determine how proximity to *P. australis* may affect *S. hermaphrodita* seedling survival and AMF root colonization levels. This objective was achieved by assessing the emergence and mortality of *S. hermaphrodita* seedlings over three consecutive growing seasons and the AMF colonization of *S. hermaphrodita* seedling roots at locations where *P. australis* plants were present and absent. A confirmation of the belowground competition mechanism may suggest that *P. australis* has similar interactions with other plants and would provide insight into potential targets for control of its threat to other native species.

2.3 Materials and Methods

2.3.1 Site description

Taquanyah Conservation Area (TCA) is a 136 ha complex of woodland, grassland and wetland habitats within a floodplain associated with a cold water stream located in Haldimand County, Ontario

(42°57’17.0”N, 79°54’46.0”W). The conservation area is one of the only two locations where *S. hermaphrodita* remains in Canada (Bickerton, 2011). Based on morphological identification, the invasive, non-native *Phragmites australis* haplotype (Saltonstall et al., 2005) has become one of the most dominant species in this area, gradually displacing the native species (Bickerton, 2011).

2.3.2 Field seedling vegetation survey

A long-term monitoring program of *S. hermaphrodita* was initiated at TCA in 2014. Population boundaries were determined by walking each individual *S. hermaphrodita* stand with an SX Blue II GPS and the areas of each stand were subsequently mapped using ArcMap. Due to close ramet development, stands were considered separate if there was greater than one-meter distance between two adjacent groups of plants. To determine the stem density within the stands, all stems were counted when stands occupied less than 1 m^2. In larger stands, a 1 m^2 grid was superimposed using ArcMap and 1 m x 1 m quadrats were randomly placed throughout the stand. Stems were counted in these quadrats until the total area sampled was equivalent to 5% of the total stand area. For classification of all other vegetation observed within the quadrats used for density assessment, see Table S2.3. This population monitoring at TCA has been repeated every two years (2014, 2016 and 2018).

To understand plant community dynamics at the boundaries of the *S. hermaphrodita* stands, 28 permanent 1 m x 1 m quadrats were marked using 15.2 cm galvanized framing spikes (Paulin) in 2014 at various locations surrounding large existing *S. hermaphrodita* stands (with an area greater than ca. 5 m^2) at TCA. Since *Phragmites australis* has been suggested to represent a significant threat to the *S. hermaphrodita* population, emphasis was placed on establishing quadrats in areas were the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The first proximity level included areas where *S. hermaphrodita* and *P. australis* stands overlapped. The second proximity level included areas where the species were close to one another (less than 5 m). The third proximity level included areas where the species were further away from one another (greater than 10 m) (Figure 2.1). Monitoring of these quadrats has taken place each year since 2014.

Vegetation surveys of the quadrats have been completed each year. Quadrats were divided into four 0.5 m x 0.5 m sub-quadrats using two 30.5 cm bungee cords (Home Essentials) and all the plant species were identified and noted for the vegetation composition (Figure 2.2). For classification of all vegetation observed within each quadrat used for vegetation surveys, see Table S2.1. Additionally, the seedling emergence of *S. hermaphrodita* was quantified. Seedlings can be distinguished from the new vegetative shoots produced from belowground roots by the presence of morphologically distinct cotyledons (Figure 2.3), which permits the seedlings to be tracked throughout the growing season (Stevens et al., 2017a). During the growing seasons of 2016, 2017 and 2018, two successive trips to TCA were made approximately 4 weeks apart from each other to repeat vegetation surveys. New *S. hermaphrodita* seedlings found within the 28 quadrats during each survey were counted and tagged with 10.2 cm zip-ties (Commercial Electric) to determine the emergence of seedlings between vegetation surveys. The number of tagged surviving seedlings observed following the initial survey, were used to determine seedling mortality (Figure 2.4).

2.3.3 Seedling collection

In 2016 a preliminary AMF assessment of *S. hermaphrodita* seedling roots was completed in which approximately 5 seedlings were collected from the peripheral soil of the 28 quadrats previously described to confirm whether *S. hermaphrodita* can form AMF relationships (n = 140). Subsequently, in 2018, during the two consecutive trips to TCA for field vegetation surveys, approximately 10 seedlings were collected from the peripheral soil of each quadrat (n = 560). To prevent damage to the seedling roots, soil samples surrounding seedlings were excavated (Figure 2.5) with Hori-Hori digging blades (Sun Joe) and sealed in 26.8 cm x 27.3 cm freezer bags (Ziploc) for transport from TCA. Seedlings were carefully removed from soil samples in-lab, rinsed of any soil and debris and placed in 10 mL Falcon tubes with 50% Ethanol (EtOH) to store until roots could be stained for AMF colonization.

2.3.4 AMF assessment

Seedlings were removed from the 50% EtOH and thoroughly rinsed with deionized water. Seedling roots were separated from shoots and the clearing and staining followed a modified ink-vinegar staining technique protocol (Vierheilig et al., 1998; Vierheilig et al., 2005). For clearing, the roots were submerged in 10% Potassium hydroxide (KOH) and were heated at a temperature of 95°C in a vacuum oven (Thermo Scientific Lindberg Blue M) under 25 inches Hg pressure for approximately 20-25 minutes. The roots were then rinsed twice with a 10% vinegar ($C_2H_4O_2$) solution and then submerged in 5% Sheaffer ink-vinegar solution to stain the AMF structures within the roots. The roots were heated for approximately 5-10 minutes under the same conditions as previously mentioned for the clearing step and subsequently checked under a Zeiss SteREO Discovery V8 dissecting microscope (Carl Zeiss Inc., Germany) for sufficient staining. Lastly, the roots were rinsed with 5% vinegar to de-stain and then stored in 50% glycerol ($C_3H_8O_3$).

All roots were mounted in 50% glycerol on frosted microscopic slides (Fisherbrand™). Prepared slides were viewed under a Zeiss Axioscope 5 microscope (Carl Zeiss Inc., Germany) under 200x magnification (objective 20x, ocular 10x) and images were taken with Zeiss Zen Axiocam imaging software (blue edition).

Mycorrhizal colonization was assessed using the gridline intersect technique (McGonigle et al., 1990). Using this technique, intra-radicular hyphae, arbuscules, and vesicles were counted to obtain an estimate of the proportion of roots in a plant that contained mycorrhizal structures.

2.3.5 Statistical analyses

Preliminary seedling root AM colonization in 2016 was analyzed using a one-way analysis of variance (ANOVA) in JMP (Statistical Analysis Software version 14) to determine if there was a relationship between the *P. australis* proximity level and the measurements of seedling root colonization. Seedling performance and 2018 seedling mycorrhizal colonization were analyzed using a two-way

ANOVA to determine if there was an interaction between the survey year or survey month and the *P. australis* proximity level and their effect on *S. hermaphrodita* seedling performance or seedling colonization respectively. To meet the assumptions of normality and homogeneity of variance, seedling emergence, seedling mortality, 2016 vesicular colonization and 2018 hyphal colonization were square-root transformed. Since we were interested only in specific comparisons and not in all possible pair-wise comparisons, multiple comparisons were conducted using the LS means function in JMP with a non-corrected student's T specification.

2.4 Results

2.4.1 Seedling performance

The interaction between the *P. australis* proximity level and the survey year did not have a significant effect on *S. hermaphrodita* seedling emergence (Table 2.1). The emergence of *S. hermaphrodita* seedlings significantly increased ($p < 0.001$) throughout the three years of sampling (Table 2.1). Average seedling emergence in 2018 (mean: 19.717 seedlings/m^2 ± 0.385 seedlings/m^2) was approximately 4.5 times greater than the average seedling emergence observed in 2016 (mean: 4.408 seedlings/m^2 ± 0.385 seedlings/m^2) (Figure 2.6). The change in *P. australis* proximity level also had a significant effect ($p < 0.05$) on *S. hermaphrodita* seedling emergence (Table 2.1). Closer proximities to *P. australis* led to theoretically anomalous effects in which the average *S. hermaphrodita* seedling emergence in intermediate proximity to *P. australis* was significantly higher (17.181 seedlings/m^2 ± 0.391 seedlings/m^2) than the average seedling emergence in close (9.223 seedlings/m^2 ± 0.452 seedlings/m^2) and far proximity (8.542 seedlings/m^2 ± 0.296 seedlings/m^2) to *P. australis* stands (Figure 2.6).

Additionally, the interaction between the *P. australis* proximity level and the survey year did not have a significant effect on *S. hermaphrodita* seedling mortality (Table 2.1).The mortality of *S. hermaphrodita* seedlings differed significantly ($p < 0.0001$) among the three years of sampling (Table 2.1). Average seedling mortality in 2018 (96.236% ± 2.251%) was more than double the average seedling mortality in 2016 (38.270% ± 2.164%) (Figure 2.7). The change in *P. australis* proximity level, however,

did not have a significant effect on *S. hermaphrodita* seedling mortality (Table 2.1) since average seedling mortality remained consistent across all proximity levels (close: 64.238% ± 2.599%; intermediate: 65.905% ± 2.272%; far: 62.012% ± 1.682%) (Figure 2.7).

2.4.2 Seedling AMF colonization

Preliminary assessment of arbuscular mycorrhizal fungal colonization in 2016 indicated that AM fungi were able to colonize *S. hermaphrodita* seedlings and that colonized roots had both arbuscules and vesicles (Figure 2.10). Initial assessment suggested that the change in *P. australis* proximity level did not significantly affect *S. hermaphrodita* seedling root colonization since no significant differences were observed between *P. australis* proximity levels and the various measures of AM colonization (Table 2.2). Mean hyphal colonization of *S. hermaphrodita* seedling roots remained consistent across all proximity levels (close: 67.578% ± 5.574%; intermediate: 68.522% ± 4.827%; far: 65.261% ± 3.787%). Similar results were also obtained for mean arbuscular colonization (close: 42.101% ± 5.701%; intermediate: 33.717% ± 4.937%; far: 31.189% ± 3.873%) and mean vesicular colonization (close: 3.178% ± 5.049%, intermediate: 3.612% ± 4.373%, far: 0.543% ± 3.430%) (Figure 2.8).

After evaluating the capacity for *S. hermaphrodita* to form AMF symbioses, additional assessments of AMF colonization in 2018 suggested that the interaction between *P. australis* proximity level and the survey month did not have a significant effect on *S. hermaphrodita* seedling AM colonization (Table 2.3). Our additional results confirmed that change in *P. australis* proximity level did not significantly affect *S. hermaphrodita* root colonization (Table 2.3). All metrics of seedling AM colonization were not significantly affected by *P. australis* proximity since mean hyphal colonization levels (close: 79.710% ± 6.810%; intermediate: 83.911% ± 5.898%; far: 87.633% ± 4.640%), mean arbuscular colonization levels (close: 44.743% ± 5.071%; intermediate: 36.902% ± 4.392%; far: 47.030% ± 3.455%), and mean vesicular colonization levels (close: 21.530% ± 5.394%; intermediate: 25.414% ± 4.671%; far: 31.657% ± 3.675%) remained consistent across the proximity levels (Figure 2.9). Additionally, mean measurements of AM colonization between the consecutive sampling periods

remained consistent for hyphal colonization (July: 83.458% ± 4.739%; August: 84.168% ± 4.816%), arbuscular colonization (July: 40.387% ± 3.528%; August: 45.397% ± 3.586%), and vesicular colonization (July: 27.901% ± 3.753%; August: 24.500% ± 3.814%), indicating that *S. hermaphrodita* seedling AM colonization did not differ significantly among the survey months (Table 2.3 & Figure 2.9).

2.5 Discussion

Our assessment of *S. hermaphrodita* seedling performance and root arbuscular mycorrhizal colonization did not support the negative soil conditioning effect hypothesized for the exotic *P. australis*. The plant-soil feedback theory suggests that invasive plant species can alter soils to engineer a positive plant-soil feedback and create a competitive advantage over native plant species (Bever et al., 1997; Reinhart et al., 2003; Callaway et al., 2004; Reinhart and Callaway 2006; Van Grunsven et al., 2007; Van Der Putten et al., 2007; Crocker et al., 2017). Previous research has shown that *P. australis* is capable of conditioning soil to inhibit the germination and growth of native species through the active allelopathic release of inhibitory phenolic compounds (Rudrappa et al., 2007; Rudrappa et al., 2009; Bains et al., 2009), the indirect release of phytotoxic tissue extracts resulting from litter decomposition (Uddin et al., 2012; Uddin et al., 2014), and through the allelopathic disruption of AMF symbioses (Uddin et al., 2017).

Globally, the number of documented occurrences of *S. hermaphrodita* are low and declining and the impacts of the increasingly abundant *P. australis* surrounding natural populations has been suspected to be responsible (Environment Canada, 2015; NatureServe, 2016). Interestingly, results of yearly field surveys indicated that *S. hermaphrodita* seedling emergence at TCA has significantly increased each year (Figure 2.6). Population density assessments have also indicated that *S. hermaphrodita* stands at TCA have grown since the area occupied by *S. hermaphrodita* and the estimated number of vegetative stems at TCA have increased between the survey periods in 2014 (total area occupied: 2,109 m^2; total stem count: 29,833 stems), 2016 (total area occupied: 2,616 m^2; total stem count: 29,245 stems) and 2018 (total area occupied: 3,827 m^2; total stem count: 65,911 stems) (Table S2.2 & Figure S2.1-Figure S2.3). The

observed increase in seedling emergence each year may be a result of an increase in seeds contributed to the seedbank each year.

2.5.1 Seedling performance

Soil conditioning by *P. australis* was hypothesized to inhibit *S. hermaphrodita* seedling emergence in close proximity. Seedling emergence results did vary significantly across the proximity levels; however, results did not support the initial hypothesis since *S. hermaphrodita* seedling emergence was generally lowest in quadrats farther from *P. australis* (Figure 2.6). The higher seedling emergence observed in areas of intermediate and close proximity may indicate that *S. hermaphrodita* seedlings are not negatively impacted by *P. australis*. Life stage has been considered a factor that could impact the outcome of interactions between plant species (Goldsmith, 1978; Keddy and Shipley, 1989; Callaway and Walker, 1997). Nurse plant syndrome has been used to explain associations in which adults of one species can ameliorate harsh environmental conditions by stabilizing soils and increasing soil organic matter, nutrients and moisture, thereby facilitating the establishment of another species' seedlings (Niering et al., 1963; Steenberg and Lowe, 1969; Arriaga et al., 1993; Walker, 1994; Callaway et al., 1996). *Phragmites australis* has been documented facilitating seedling emergence and establishment of other species by trapping seeds and reducing wind and erosion (Liu et al., 2012). *Sida hermaphrodita*, like other species in the Malvaceae family, have seeds protected by an impermeable seed coat which enforces physical dormancy and controls germination (Rolston, 1978; Kelly et al., 1992; Baskin et al., 2000; Packa et al., 2014). Previous studies have identified that *S. hermaphrodita* germination is usually low without seed coat damage to disrupt the physical dormancy, and because optimal environmental conditions are rarely encountered (Kurucz and Fári, 2013; Packa et al., 2014; Stevens et al., Unpublished). Numerous dormancy breaking techniques have been recognized and Packa et al. (2014) found that only slight damage to the seed coat is necessary to enable imbibition and increase seed germination capacity (Packa et a., 2014). Although further examination is needed for confirmation, it is possible that established *P. australis* stands may create microclimates of altered humidity levels, soil hydrology, as well as air and

soil temperatures that could influence cycles of freezing and thawing, resulting in the disruption of *S. hermaphrodita* seed physical dormancy (Báldi, 1999; Isselstein et al,. 2002; Flores and Jurado, 2003; Cavieres et al., 2007; Drezner and Garrity, 2008). Furthermore, our results from additional examinations of interactions between both species, provided further support for altered soil hydrology, since levels of soil moisture were highest within *P. australis* stands (See Chapter 3). Through the potential alteration of soil hydrology, *S. hermaphrodita* seed germination may be impacted by improving the imbibition capacity of seeds where water availability is increased beneath *P. australis* stands. Due to the observed increase in seedling emergence in close and intermediate *P. australis* proximity levels, it is possible that established *P. australis* stands at TCA may create conditions necessary for *S. hermaphrodita* seedling germination and emergence through the promotion of imbibition and mitigation of environmental conditions in areas closer to *P. australis* stands.

In many cases of nurse plant facilitation, patterns of mortality have been observed in which the beneficiary seedlings become significant competitors as they mature and eventually supress the growth of the nurse species (McAuliffe, 1984; McAuliffe, 1986; Valiente-Banuet et al., 1991; Flores-Martinez et al., 1994; Callaway and Walker, 1997). However, the results from our vegetation surveys indicate that *P. australis* nurse plant mortality did not occur. Instead, *S. hermaphrodita* seedling mortality increased significantly each year (Figure 2.7). We hypothesized that if *P. australis* was capable of conditioning soils to create a competitive advantage, then *S. hermaphrodita* seedling mortality would be highest in close proximity to *P. australis*, however, *S. hermaphrodita* seedling mortality remained consistently high across all proximity levels (Figure 2.7). Since *S. hermaphrodita* seedling mortality did not differ among the three *P. australis* proximity levels, it may be possible that although *P. australis* may create conditions that support *S. hermaphrodita* germination and emergence, environmental conditions at TCA including limited light availability, combined with the competitive effects generated by other species (see below), do not support further development of the seedlings.

2.5.2 Seedling AMF colonization

Assessments of arbuscular mycorrhizal colonization of *S. hermaphrodita* seedlings provided the first empirical evidence that *S. hermaphrodita* is capable of forming relationships with arbuscular mycorrhizal fungi (Figure 2.10). Following the confirmation of AMF colonization, we expected that soil conditioning by *P. australis* would result in significant negative impacts on *S. hermaphrodita* seedlings by interfering with AMF symbioses. However, our results did not support previous AMF interference findings (Uddin and Robinson, 2017; Uddin et al., 2017) since all measurements of AMF colonization were consistently high during each assessment period and across the *P. australis* proximity levels (Figures 2.8 & 2.9). Owing to reports that in general, plant symbioses with AMF are formed to improve plant acquisition of water and nutrients, and AMF abundance commonly increases where plants are nutrient limited (Bolan, 1991; Koide, 1991; Read, 1991; Treseder, 2004), the consistently high levels of AMF colonization observed in *S. hermaphrodita* seedlings suggests that limited nutrient availability at TCA may be contributing to the low *S. hermaphrodita* seedling survival. However, our additional results indicate instead that *S. hermaphrodita* performance does not coincide with common AMF relationships and that soil nutrient availability may be facilitated between *S. hermaphrodita* and *P. australis* due to unknown abiotic stressors contributing to plant performance (See Chapter 3).

2.5.3 Synthesis of detrimental interaction

Light availability may be the major factor responsible for the observed *S. hermaphrodita* performance. Although this study was designed to examine the potential effect that *P. australis* proximity has on *S. hermaphrodita* seedlings, all the quadrats used for vegetation surveys contained a variety of other plant species. Not including *P. australis*, some of the most frequently encountered species during the field surveys included *Phalaris arundinacea* L., *Solidago canadensis* L., *Dipsacus fullonum* L., and *Geum urbanum* L., (Table S2.1), all of which are powerful competitors and have the capacity to impede access to light for *S. hermaphrodita* seedlings (Figure S2.4). *Phalaris arundinacea* (Reed Canary Grass; Poaceae) is another aggressive perennial rhizomatous grass with culms ranging in height between 60-150

cm (Hitchcock, 1950), and has been well documented to establish monocultures rapidly and pre-empt the establishment of native species by intercepting light (Apfelbaum and Sams, 1987; Galatowitsch et al., 1999; Budelsky and Galatowitsch, 2000; Green and Galatowitsch, 2001; Perry and Galatowitsch, 2004). *Solidago canadensis* (Canada Goldenrod; Asteraceae) is a widespread rhizomatous perennial herb with stem height ranging from 25-200 cm (Werner et al., 1980), that clonally develops a dense aboveground canopy (Hartnett and Bazzaz, 1985). Due to its morphological and physiological plasticity, it can adapt to varying light intensity (Sun et al., 2008), by continuously producing leaves and branching its inflorescence to completely shade the undergrowth and increase its light interception (Abrahamson and Gadgil, 1973; Potvin and Werner, 1983; Dong et al., 2006). *Dipsacus fullonum* (Fuller's Teasel; Dipsacaceae) is a large biennial herb that produces a robust flowering stem between 50-250 cm in height as well as a low rosette up to 60 cm in diameter of thick leaves capable of shading neighbouring species (Werner, 1975a; Werner, 1975b; Rector et al., 2006). *Geum urbanum* (Avens; Rosaceae) is a smaller rhizomatous perennial herb with branched stems reaching 70 cm tall and is considered a fast colonizing species that favours partially shaded areas (Waldren et al., 1988; Taylor, 1997; Baeten et al., 2009). This species also displays high physiological plasticity, allowing it to adapt to different levels of light availability and continuously grow both cauline and rosette leaves in high and low light conditions (Pons, 1977; Endels et al., 2004; Baeten et al., 2010), contributing to the diverse botanical canopy above younger plants. *Phragmites australis* specifically, is characterized by tall dense canopies of standing live and dead shoots (litter) that shade the understory (Swearingen and Saltonstall, 2010; Holdredge and Bertness, 2011). The high *S. hermaphrodita* seedling mortality observed throughout TCA may coincide with previous reports that light may be the key limiting resource affecting native plant growth, especially in areas invaded by *P. australis* that monopolizes light (MacDougall and Turkington, 2005; Minchinton et al., 2006; Holdredge and Bertness, 2011). Additional unpublished results from the Rooney lab also support this hypothesis since they suggest *S. hermaphrodita's* photosynthetic rates are impacted within *P. australis* stands.

Given that *S. hermaphrodita* seedlings or AMF colonization were not excluded in close proximity to *P. australis,* our results did not provide evidence to support *P. australis* soil conditioning mechanisms. Instead, our results suggest that competitive exclusion of light may be the main biotic mechanism of *P. australis* through which it displaces *S. hermaphrodita*. Additionally, although further assessment of other environmental conditions and interactions with other species at TCA need to be examined, the high AMF colonization and additional soil fertility results (See Chapter 3) suggest that the limiting soil nutrients may differ between *S. hermaphrodita* and *P. australis*, which may explain the observed interaction between the two species. It has been demonstrated that soil nutrient enrichment promotes the spread of *P. australis* (Minchinton and Bertness, 2003), however, previous studies have suggested that under sub-optimal conditions (e.g., water table level, nutrient level, strong competition), *P. australis* may not become vigorous enough to displace other strong competitors and instead may co-exist with them (Haslam, 1971; Güsewell and Klötzli, 1998; Uddin and Robinson, 2017; Uddin and Robinson, 2018). The growing *S. hermaphrodita* stands at TCA indicate that light competition or an unknown abiotic stressor may be preventing *P. australis* from threatening *S. hermaphrodita's* vegetative spread. *Sida hermaphrodita* seedling germination and emergence may be favoured near *P. australis*, however, *P. australis'* ability to competitively exclude light, likely impedes *S. hermaphrodita* seedling survival. Our results suggest that *S. hermaphrodita* has the capacity to increase its abundance at TCA, however, conservation efforts focussed on cutting and removing or burning aboveground *P. australis* shoots and litter as well as other invasive plants to disrupt the canopy cover, would help restore native community structure and promote the vegetative and seedling expansion of *S. hermaphrodita* into areas of its native distribution previously taken over by *P. australis* and other invasive plants.

2.6 Tables and Figures

Table 2.1: Output of two-way ANOVA used to assess differences between the three survey years, the three *P. australis* proximity levels (Proximity) in addition to the interaction between survey year and location and their effect on the measures of *S. hermaphrodita* seedling performance. Proximity levels included locations where *S. hermaphrodita* and *P. australis* stands overlapped (n = 6), locations where *P. australis* stands were less that 5 m away (n = 8), and locations where *P. australis* stands were greater than 10 m away from *S. hermaphrodita* stands (n = 14). Square-root transformations were applied to the seedling performance response variables tested including seedling mortality and seedling emergence. Seedling performance was examined at each of the 28 locations during consecutive vegetation surveys completed during the growing seasons of 2016, 2017 and 2018. Significance level of $p < 0.05$ was used for all analyses and significant effects are indicated with an asterisk (*)

	Year			**Proximity**			**Year x Proximity**		
	Df	**F**	**P**	**Df**	**F**	**P**	**Df**	**F**	**P**
Seedling Emergence	2/83	9.439	0.0002*	2/83	3.316	0.042*	4/83	0.885	0.477
Seedling Mortality	2/70	67.395	<0.0001*	2/70	0.388	0.680	4/70	0.644	0.633

Table 2.2: Output of one-way ANOVA used to assess any preliminary relationships between measures of *S. hermaphrodita* seedling root AM colonization and the three *P. australis* proximity levels (Proximity). Proximity levels included locations where *S. hermaphrodita* and *P. australis* stands overlapped (n = 6), locations where *P. australis* stands were less that 5 m away (n = 8), and locations where *P. australis* stands were greater than 10 m away from *S. hermaphrodita* stands (n = 14). Response variables tested were hyphal colonization (HC), arbuscular colonization (AC), and vesicular colonization (VC). Square-root transformation was only applied to the measurement of vesicular colonization to meet ANOVA assumptions. Five seedlings were collected from each of the 28 locations during the growing season in 2016 and assessed for measures of root colonization using a modified magnified intersections method (McGonigle et al., 1990). Significant effects were not observed.

	Proximity		
	Df	**F**	**P**
HC (%)	2/26	0.156	0.857
AC (%)	2/26	1.266	0.300
VC (%)	2/26	2.747	0.084

Table 2.3: Output of two-way ANOVA used to assess differences between two survey months , the three *P. australis* proximity levels (Proximity), in addition to the interaction between survey month and location and their effect on the measures of *S. hermaphrodita* seedling AM root colonization. Proximity levels included locations where *S. hermaphrodita* and *P. australis* stands overlapped (n = 6), locations where *P. australis* stands were less that 5 m away (n = 8), and locations where *P. australis* stands were greater than 10 m away from *S. hermaphrodita* stands (n = 14). Response variables tested were hyphal colonization (HC), arbuscular colonization (AC), and vesicular colonization (VC). Square-root transformation was only applied to the measurement of hyphal colonization to meet ANOVA assumptions. Ten seedlings were collected from each of the 28 locations during consecutive vegetation surveys completed in July and August 2018 and assessed for measures of root colonization using a modified magnified intersections method (McGonigle et al., 1990). Significant effects were not observed.

	Month			**Proximity**			**Month x Proximity**		
	Df	**F**	**P**	**Df**	**F**	**P**	**Df**	**F**	**P**
HC (%)	1/53	0.031	0.861	2/53	1.342	0.271	2/53	0.199	0.820
AC (%)	1/53	0.992	0.324	2/53	1.681	0.197	2/53	0.028	0.972
VC (%)	1/53	0.404	0.528	2/53	1.351	0.269	2/53	0.071	0.932

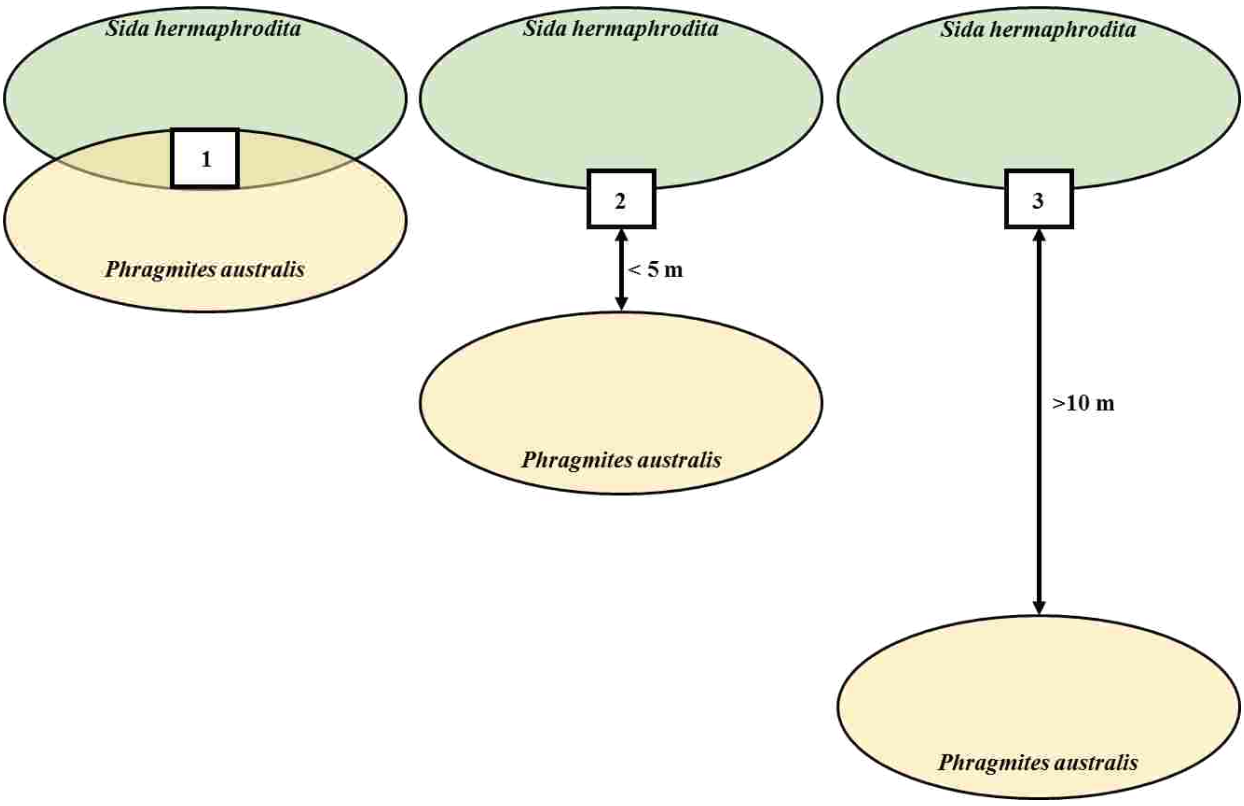

Figure 2.1: Experimental layout for each of the 28 1 m x 1 m quadrats placed at TCA in 2014, where field vegetation surveys were conducted each year. 28 quadrats were placed in locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The proximity levels were characterized as locations where both *S. hermaphrodita* and *P. australis* stands overlapped (n = 6) (1), intermediate locations where the boundaries of both species were in close vicinity to one another (< 5 m; n = 8) (2) and locations where the species boundaries were farther from one another (> 10 m; n = 14) (3). Vegetation surveys have been completed each year in which all species present within the quadrats were identified and noted for vegetation composition and *S. hermaphrodita* seedling growth was monitored throughout the growing season.

Figure 2.2: Photographs taken at TCA of quadrats where field vegetation surveys were completed. 28 1 m x 1 m quadrats were placed in locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The proximity levels were characterized as locations where both *S. hermaphrodita* and *P. australis* stands overlapped (1), intermediate locations where the boundaries of both species were in close vicinity to one another (< 5 m) (2) and locations where the species boundaries were farther from one another (> 10 m) (3). Vegetation surveys were completed each year in which all species present within the quadrats were identified and noted for vegetation composition and *S. hermaphrodita* seedling growth was monitored throughout the growing season.

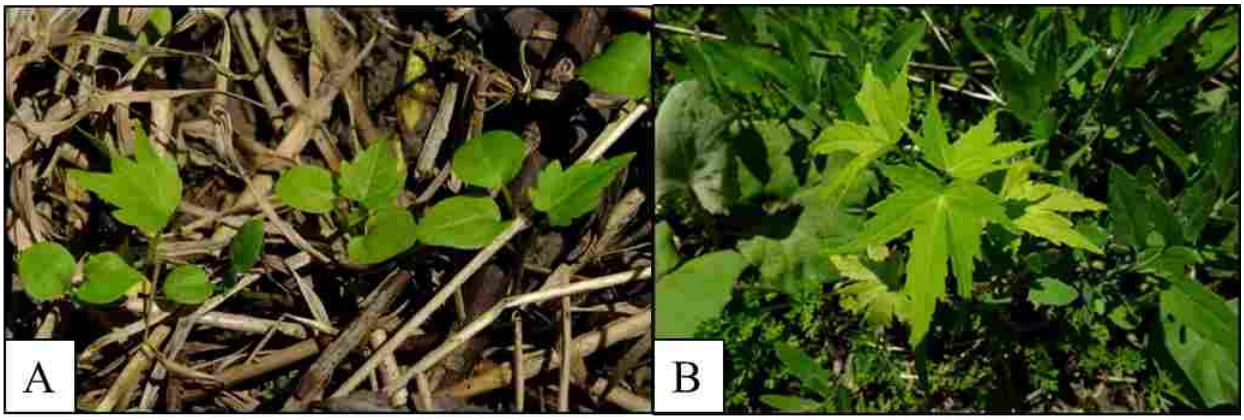

Figure 2.3: Seedlings and small plants of *S. hermaphrodita* located at the periphery of established stands at TCA. Seedlings with 1-2 true leaves. Seedlings are identified by the presence of ovate cotyledons subtending the toothed true leaves (A). Young plants establishing from perennating organs were distinguishable from seedlings due to their more extensive development and absence of cotyledons (B).

Figure 2.4: *S. hermaphrodita* seedlings were tagged with ties during each vegetation survey to monitor their performance throughout the growing season and to distinguish them from newly emerging seedlings. Seedlings that did not continue to grow and develop true leaves throughout the growing season subsequently lost their cotyledons (A) or wilted (B & C). Tagged seedlings with these appearances were used to determine seedling mortality in relation to proximity to *P. australis*.

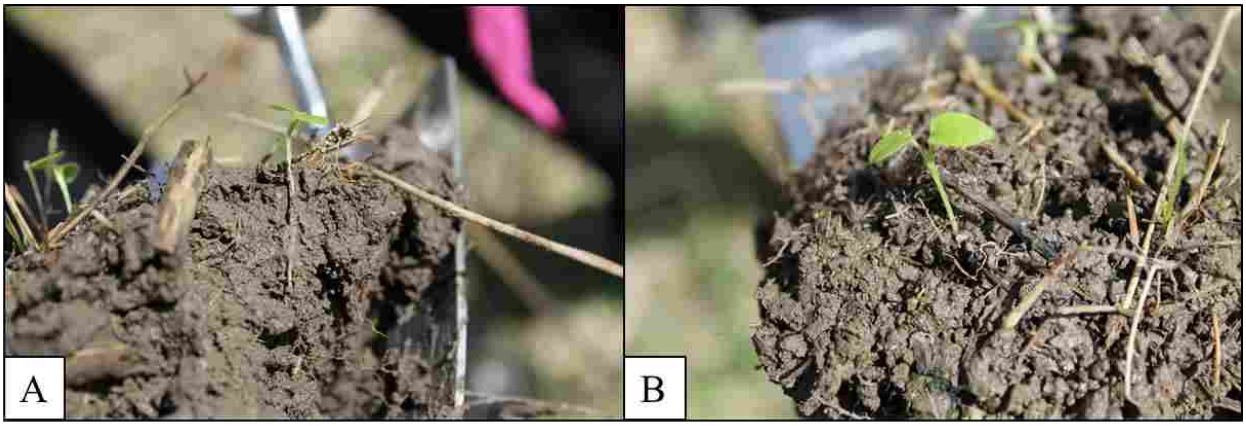

Figure 2.5: In 2016 and 2018, *S. hermaphrodita* seedlings were collected from the peripheral soil of each of the 28 1 m x 1 m quadrats used for vegetation surveys. To prevent damage to seedling roots, soil samples surrounding the seedlings were excavated (A and B). The excavated seedlings were carefully separated from soil samples in lab and were later cleared and stained to assess the proportion of AMF colonization within seedling roots.

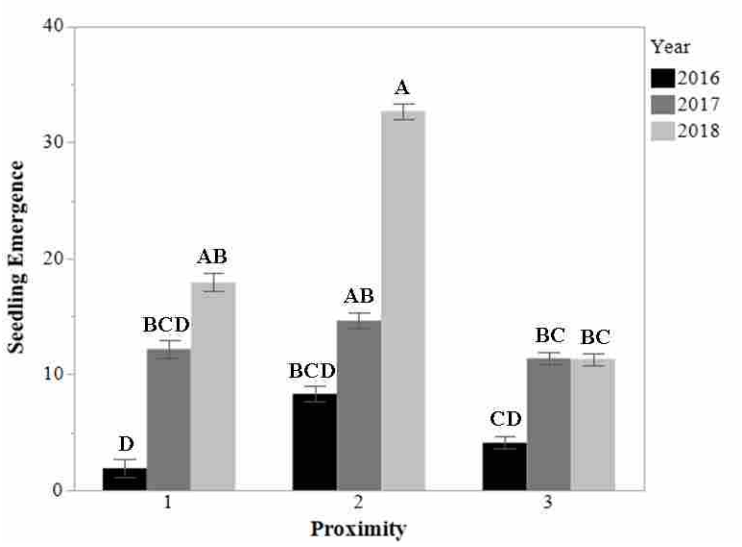

Figure 2.6: Seedling emergence of *S. hermaphrodita* seedlings surveyed in quadrats placed in locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The proximity levels included locations where both *S. hermaphrodita* and *P. australis* stands overlapped (n = 6) (1), intermediate locations where the boundaries of both species were in close vicinity to one another (< 5 m; n = 8) (2) and locations where the species boundaries were farther from one another (> 10 m; n = 14) (3). The interaction between survey year and *P. australis* proximity level and their effect on *S. hermaphrodita* seedling emergence as a representative of seedling performance was assessed using a two-way analysis of variance (ANOVA). Square-root transformation was applied to meet ANOVA assumptions and differences in seedling emergence among years and across proximity levels were determined using Student's T multiple comparisons. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

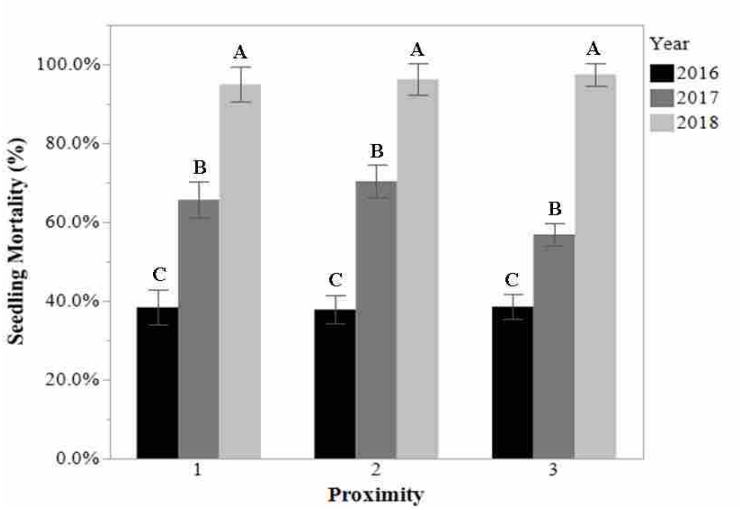

Figure 2.7: Seedling mortality of *S. hermaphrodita* seedlings surveyed in quadrats placed in locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The proximity levels included locations where both *S. hermaphrodita* and *P. australis* stands overlapped (n = 6) (1), intermediate locations where the boundaries of both species were in close vicinity to one another (< 5 m; n = 8) (2) and locations where the species boundaries were farther from one another (> 10 m; n = 14) (3). The interaction between survey year and *P. australis* proximity level and their effect on *S. hermaphrodita* seedling mortality as a representative of seedling performance was assessed using a two-way analysis of variance (ANOVA). Square-root transformation was applied to meet ANOVA assumptions and differences in seedling mortality among years and across proximity levels were determined using Student's T multiple comparisons. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

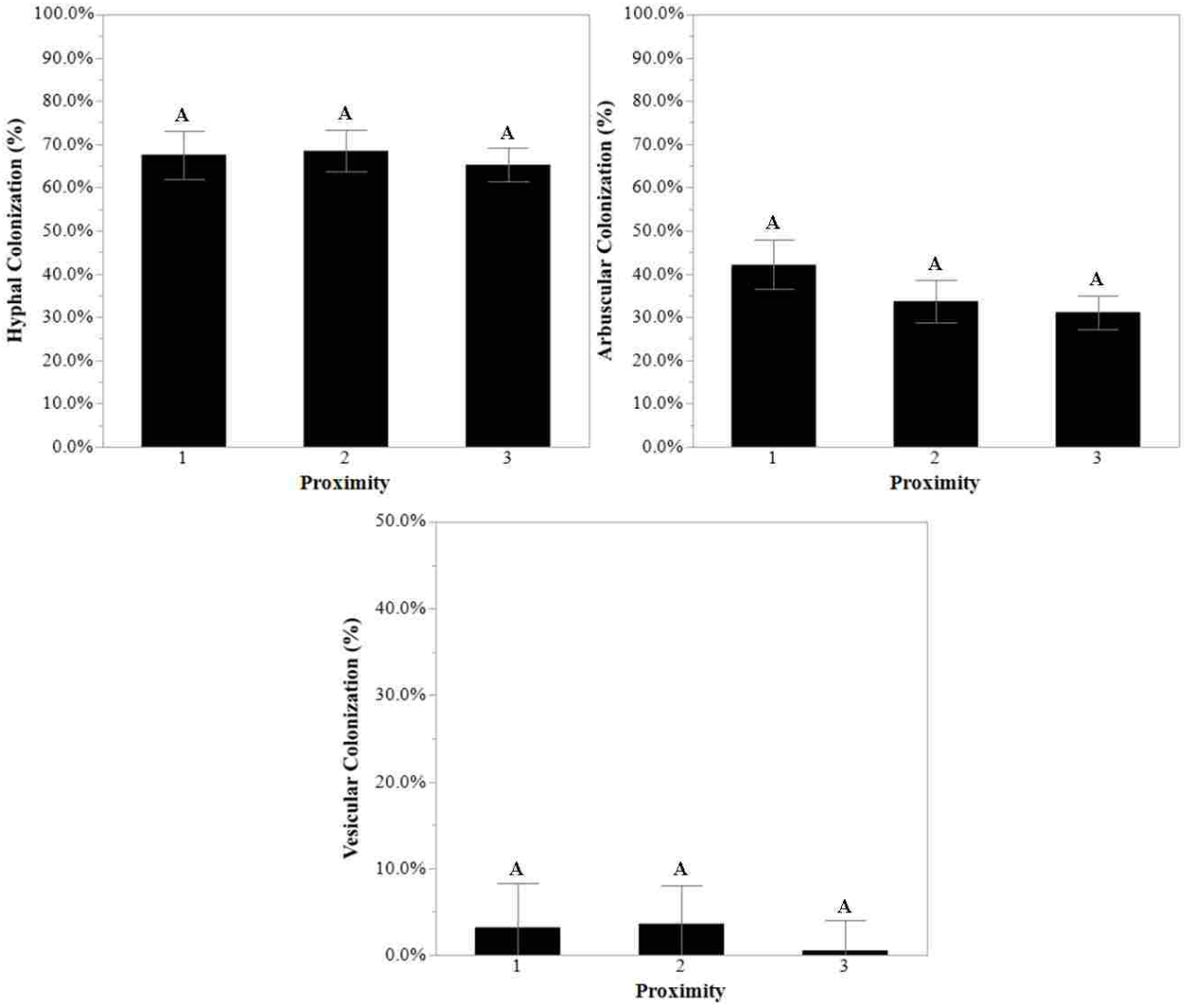

Figure 2.8: Preliminary seedling root AMF colonization of *S. hermaphrodita* seedlings collected in 2016 from locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The proximity levels included locations where both *S. hermaphrodita* and *P. australis* stands overlapped (n = 6) (1), intermediate locations where the boundaries of both species were in close vicinity to one another (< 5 m; n = 8) (2) and locations where the species boundaries were farther from one another (> 10 m; n = 14) (3). The relationship between the *P. australis* proximity level and the measurements of AMF colonization including hyphal colonization (left), arbuscular colonization (right) and vesicular colonization (bottom) were assessed using a one-way analysis of variance (ANOVA). Square-root transformation was only applied to the measurement of vesicular colonization to meet ANOVA

assumptions and differences between proximity levels were determined using a Student's T comparison. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

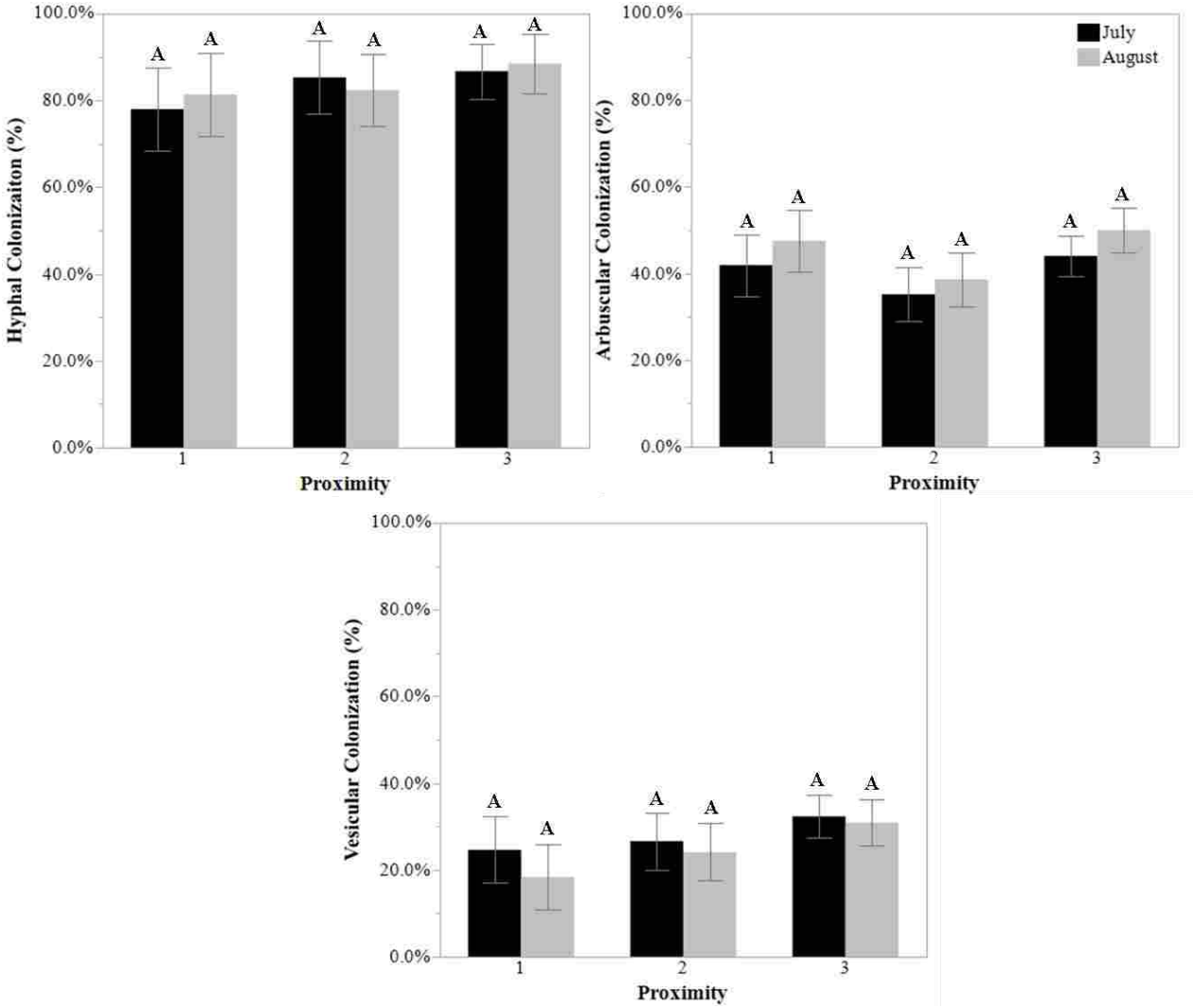

Figure 2.9: Seedling root AMF colonization of *S. hermaphrodita* seedlings collected in 2018 from locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The proximity levels included locations where both *S. hermaphrodita* and *P. australis* stands overlapped (n = 6) (1), intermediate locations where the boundaries of both species were in close vicinity to one another (< 5 m; n = 8) (2) and locations where the species boundaries were farther from one another (> 10 m; n = 14) (3). The interaction between the survey month and the *P. australis* proximity level and their effect on hyphal colonization (left), arbuscular colonization (right), and vesicular colonization (bottom) as representatives of seedling root AMF colonization were assessed using a two-way analysis of variance (ANOVA). Square-root transformation was only applied to the measurement of hyphal colonization to meet ANOVA

assumptions and differences in seedling colonization among months and across proximity levels were determined using Student's T multiple comparisons. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

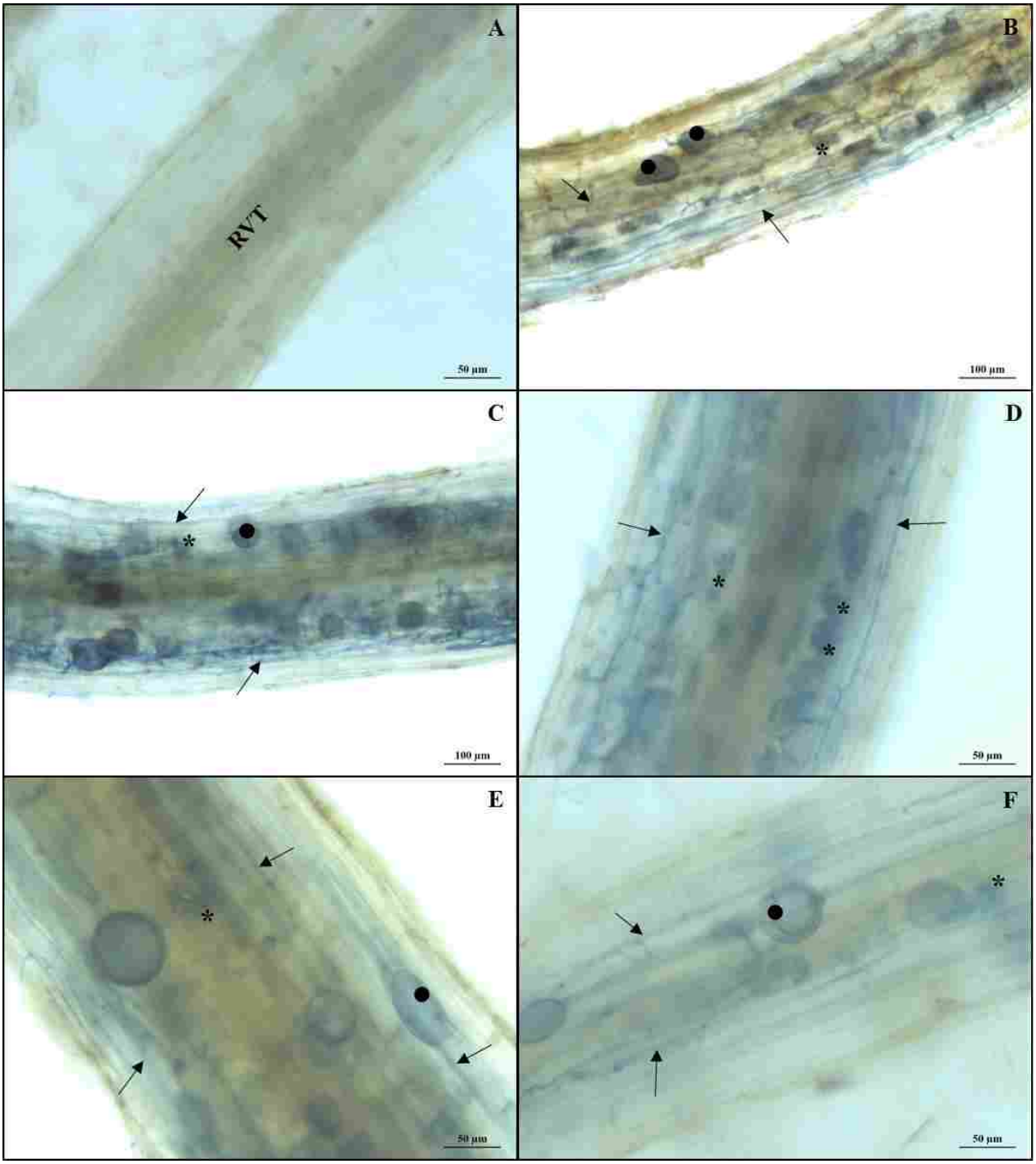

Figure 2.10: Cleared and stained roots of *S. hermaphrodita* seedlings collected from locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. Root samples were cleared in a 10% KOH solution, stained with a 5% ink in vinegar solution and AMF colonization was quantified using a modified version of the magnified intersections method (McGonigle et al., 1990)

A: Un-colonized area of a *S. hermaphrodita* root with the Root Vascular Tissue (RVT) running through the centre of the root. B-F: Areas of *S. hermaphrodita* seedling roots colonized with blue stained AMF structures. B-C: Heavily colonized seedling root sections collected in during July and August 2018 respectively. Both sections exhibit intra-radicular hyphae (arrow) within the cortex of the root and numerous arbuscules (asterisk) and vesicles (circle). D: Seedling root section collected in July 2018 exhibiting intra-radicular hyphae (arrow) within the cortical tissue, running parallel to the root vascular tissue and numerous arbuscules (asterisk). E-F: Seedling root sections collected in July and August 2018 respectively, exhibiting intra-radicular hyphae (arrow), arbuscules (asterisk) and numerous vesicles (circle).

2.7 Supplemental Information

Table S2.1: Classification of all species identified within the quadrats set up at TCA where vegetation surveys were conducted in locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. Plants were identified using the species key by Dickinson et al. (2004). Status as introduced (I) or native (N), and physiognomy (Phys) was determined from the USDA PLANTS database. Each species frequency of occurrence within the 28 1 m x 1 m quadrats assessed twice (56) throughout the growing seasons of 2016, 2017, and 2018 is presented. Minimum (Min) and maximum (Max) density (/m^2) of each species is also presented. Unidentified species are indicated by UN.

Family	Genus, Species	Common Name	I/N	Phys	Frequency, Min/Max		
					2016	2017	2018
Aceraceae	*Acer rubrum* L.	Red Maple	N	Tree	0/56, 0/0	0/56, 0/0	1/56, 1/1
Anacardiaceae	*Rhus typhina* L.	Staghorn Sumac	N	Shrub/Tree	7/56, 1/1	1/56, 1/1	0/56, 0/0
Apiaceae	*Daucus carota* L.	Queen Anne's Lace	I	Forb/Herb	10/56, 1/27	12/56, 1/105	12/56, 1/82
Asclepiadaceae	*Ascelpias syriaca* L.	Common Milkweed	N	Forb/Herb	6/56, 1/5	4/56, 1/2	3/56, 1/3
Asteraceae	*Ambrosia artemisiifolia* L.	Annual Ragweed	N	Forb/Herb	0/56, 0/0	0/56, 0/0	3/56, 1/2
	Arctium minus Bernh.	Lesser Burdock	I	Forb/Herb	0/56, 0/0	6/56, 1/12	4/56, 2/124
	Bidens frondosa L.	Devil's Beggartick	I	Forb/Herb	0/56, 0/0	0/56, 0/0	3/56, 2/7
	Cirsium arvense (L.) Scop.	Creeping Thistle	I	Forb/Herb	6/56, 1/9	6/56, 1/6	8/56, 1/5
	Cirsium vulgare (Savi) Ten.	Bull Thistle	I	Forb/Herb	0/56, 0/0	9/56, 1/10	11/56, 1/14
	Erigeron annuus (L.) Pers.	Eastern Daisy Fleabane	N	Forb/Herb	2/56, 1/2	3/56, 1/4	1/56, 3/3
	Lactuca serriola L.	Prickly Lettuce	I	Forb/Herb	2/56, 5/8	0/56, 0/0	1/56, 1/1
	Solidago canadensis L.	Canada Goldenrod	N	Forb/Herb	53/56, 1/135	53/56, 1/162	55/56, 1/131
	Sonchus arvensis L.	Field Sow Thistle	I	Forb/Herb	7/56, 1/24	17/56, 1/33	11/56, 1/28
	Symphyotrichum laeve (L.) Á. Löve & D. Löve	Smooth Blue Aster	N	Forb/Herb	0/56, 0/0	2/56, 29/40	2/56, 1/2

	Symphyotrichum lanceolatum (Willd.) G.L. Nesom	White Panicle Aster	N	Forb/Herb	3/56, 5/105	10/56, 1/20	4/56, 2/12
	Symphyotrichum novae-angliae (L.) G.L. Nesom	New England Aster	N	Forb/Herb	3/56, 1/54	13/56, 1/12	8/56, 1/8
	Taraxacum officinale F.H. Wigg.	Common Dandelion	I/N	Forb/Herb	15/56, 1/8	4/56, 1/3	5/56, 1/4
	Tussilago farfara L.	Coltsfoot	I	Forb/Herb	4/56, 1/48	4/56, 2/5	1/56, 4/4
Balsaminaceae	*Impatiens capensis* Meerb.	Spotted Touch-Me-Not	N	Forb/Herb	6/56, 1/7	2/56, 1/1	0/56, 0/0
Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Garlic Mustard	I	Forb/Herb	4/56, 1/15	6/56, 1/93	4/56, 8/44
Chenopodiaceae	*Chenopodium album* L.	Lamb's Quarters	I/N	Forb/Herb	2/56, 2/13	3/56, 2/5	0/56, 0/0
Cornaceae	*Cornus sericea* L.	Redosier Dogwood	N	Shrub	3/56, 1/1	7/56, 1/8	6/56, 1/14
Cyperaceae	Carex sp.	UN	UN	Graminoid	0/56, 0/0	0/56, 0/0	2/56, 7/13
Dipsacaceae	*Dipsacus fullonum* L.	Fuller's Teasel	I	Forb/Herb	18/56, 1/165	18/56, 2/83	22/56, 1/162
Fabaceae	*Medicago lupulina* L.	Black Medick	I	Forb/Herb	7/56, 1/106	0/56, 0/0	0/56, 0/0
	Melilotus albus Medik.	White Sweet-Clover	I	Forb/Herb	0/56, 0/0	1/56, 1/1	0/56, 0/0
	Melilotus officinalis (L.) Lam.	Yellow Sweet-Clover	I	Forb/Herb	5/56, 2/48	3/56, 2/84	6/56, 60/107
	Trifolium repens L.	White Clover	I	Forb/Herb	0/56, 0/0	6/56, 1/14	5/56, 2/15
Juglandaceae	*Juglans nigra* L.	Black Walnut	N	Tree	0/56, 0/0	5/56, 1/1	4/56, 1/2
Lamiaceae	*Prunella vulgaris* L.	Common Selfheal	I/N	Forb/Herb	0/56, 0/0	5/56, 1/9	4/56, 3/25
Lythraceae	*Lythrum salicaria* L.	Purple Loosestrife	I	Forb/Herb	6/56, 2/95	10/56, 1/28	7/56, 2/104
Malvaceae	*Sida hermaphrodita* (L.) Rusby.	Virginia Mallow	N	Forb/Herb	55/56, 1/121	52/56, 3/163	56/56, 1/110
Onagraceae	*Circaea canadensis* (L.) Hill	Enchanter's Nightshade	N	Forb/Herb	0/56, 0/0	0/56, 0/0	2/56, 4/17
Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	25/56, 1/197	31/56, 1/229	33/56, 2/246
	Phragmites australis	Common Reed	I	Graminoid	13/56, 1/26	11/56, 1/28	12/56, 1/24

	(Cav.) Trin. Ex Steud.						
	Poaceae sp.	UN	UN	Graminoid	12/56, 1/200	14/56, 10/200	12/56, 50/200
Rosaceae	*Fragaria vesca* L.	Woodland Strawberry	N	Forb/Herb	0/56, 0/0	1/56, 1/1	0/56, 0/0
	Geum urbanum L.	Avens	I	Forb/Herb	16/56, 1/22	22/56, 2/45	18/56, 1/23
	Rubus strigosus Michx.	Wild Red Raspberry	N	Shrub	0/56, 0/0	1/56, 1/1	3/56, 1/3
Salicaceae	Salix sp.	UN	UN	Tree	0/56, 0/0	1/56, 2/2	1/56, 1/1
Solanaceae	*Solanum dulcamara* L.	Climbing Nightshade	I	Vine	2/56, 1/1	6/56, 1/4	5/56, 1/2
Urticaceae	*Urtica dioica* L.	Stinging Nettle	I/N	Forb/Herb	4/56, 1/2	2/56, 2/2	2/56, 17/21

Table S2.2: Classification of *S. hermaphrodita* stands present at TCA in 2014, 2016, and 2018. All stands were mapped using SX Blue II GPS and each stands area was determined using ArcMap. Stands were separated into 10 classes distinguished by surface area (m^2). The number of separate stands (NS), average area occupied by the stand of that size class (ASA), and the average estimated stem density of vegetative *S. hermaphrodita* stems (AESD) within stands of that size class are presented. The total number of separate stands, total surface area occupied by *S. hermaphrodita* stands and the estimated total number of *S. hermaphrodita* stems present at TCA during the year of monitoring are also presented at the bottom of the table.

Size Class	**2014**			**2016**			**2018**		
	NS	**ASA**	**AESD**	**NS**	**ASA**	**AESD**	**NS**	**ASA**	**AESD**
<1 m^2	96	0.175m^2	1.064	95	0.169m^2	1.028	107	0.118m^2	0.879
>1 m^2 <5 m^2	34	2.188m^2	37.649	19	2.825m^2	63.676	7	2.352m^2	43.185
>5 m^2 <10 m^2	11	6.721m^2	92.036	13	7.231m^2	85.289	4	8.326m^2	133.593
>10 m^2 <20 m^2	15	14.211m^2	240.766	5	15.842m^2	93.342	5	14.911m^2	229.322
>20 m^2 <50 m^2	16	29.837m^2	392.171	12	34.825m^2	417.567	9	31.886m^2	511.193
>50 m^2 <100 m^2	4	75.817m^2	1094.017	5	67.101m^2	641.317	5	71.661m^2	1216.347
>100 m^2 <150 m^2	1	124.376m^2	1190.459	5	124.122m^2	1196.342	3	121.931m^2	1902.820
>150 m^2 <200 m^2	0	0	0	2	174.369m^2	1570.947	1	187.319m^2	2664.092
>200 m^2 <250 m^2	2	227.477m^2	3155.825	0	0	0	2	227.013m^2	4055.760
>250 m^2	1	386.531m^2	5745.582	2	325.323m^2	4511.105	5	407.175m^2	7324.820
Total	180	2109.15m^2	29833.44	158	2616.12m^2	29245.10	148	3827.61m^2	65911.16

Table S2.3: Classification of all species identified within the quadrats used for *S. hermaphrodita* population density assessment in 2018. 182 separate 1 m x 1 m quadrats were used to assess 5% of the total *S. hermaphrodita* stand area to estimate the number of *S. hermaphrodita* stems present at TCA during that growing season. All plants found associated with *S. hermaphrodita* within the quadrats used for density assessment were identified using the species key by Dickinson et al. (2004). Status as introduced (I) or native (N), and physiognomy (Phys) was determined from the USDA PLANTS database. Each species frequency of occurrence within the 182 quadrats is presented. Minimum (Min) and maximum (Max) percent vegetation cover (PVC) of each species is also presented. Unidentified species are indicated by UN.

Family	Genus, Species	Common Name	I/N	Phys	Frequency	Min PVC	Max PVC
Asteraceae	*Ambrosia artemisiifolia* L.	Annual Ragweed	N	Forb/Herb	3/182	5%	5%
	Arctium minus Bernh.	Lesser Burdock	I	Forb/Herb	13/182	5%	90%
	Cirsium arvense (L.) Scop.	Creeping Thistle	I	Forb/Herb	15/182	5%	10%
	Solidago canadensis L.	Canada Goldenrod	N	Forb/Herb	90/182	5%	60%
	Sonchus arvensis L.	Field Sow Thistle	I	Forb/Herb	5/182	5%	10%
	Symphyotrichum novae-angliae (L.) G.L. Nesom	New England Aster	N	Forb/Herb	9/182	5%	5%
	Taraxacum officinale F.H. Wigg.	Common Dandelion	I/N	Forb/Herb	1/182	5%	5%
	Tussilago farfara L.	Coltsfoot	I	Forb/Herb	6/182	5%	90%
Balsaminaceae	*Impatiens capensis* Meerb.	Spotted Touch-Me-Not	N	Forb/Herb	15/182	5%	80%
Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Garlic Mustard	I	Forb/Herb	31/182	5%	30%
Cornaceae	*Cornus sericea* L.	Redosier Dogwood	N	Shrub	4/182	5%	20%
Cyperaceae	Carex sp.	UN	UN	Graminoid	5/182	5%	20%
Dipsacaceae	*Dipsacus fullonum* L.	Fuller's Teasel	I	Forb/Herb	9/182	5%	40%

Fabaceae	*Melilotus officinalis* (L.) Lam.	Yellow Sweet-Clover	I	Forb/Herb	1/182	60%	60%
Juglandaceae	*Juglans nigra* L.	Black Walnut	N	Tree	4/182	5%	5%
Lythraceae	*Lythrum salicaria* L.	Purple Loosestrife	I	Forb/Herb	1/182	10%	10%
Malvaceae	*Sida hermaphrodita* (L.) Rusby.	Virginia Mallow	N	Forb/Herb	182/182	5%	40%
Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	64/182	5%	90%
	Phragmites australis (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	34/182	5%	90%
	Poaceae sp.	UN	UN	Graminoid	5/182	5%	90%
Rosaceae	*Geum urbanum* L.	Avens	I	Forb/Herb	5/182	5%	10%
	Rubus strigosus Michx.	Wild Red Raspberry	N	Shrub	9/182	5%	60%
Salicaceae	Salix sp.	UN	UN	Tree	3/182	5%	20%
Solanaceae	*Solanum dulcamara* L.	Climbing Nightshade	I	Vine	1/182	5%	5%
Typhaceae	*Typha latifolia* L.	Broadleaf Cattail	N	Forb/Herb	3/182	5%	30%
Urticaceae	*Urtica dioica* L.	Stinging Nettle	I/N	Forb/Herb	14/182	5%	30%
Vitaceae	*Vitis riparia* Michx.	Riverbank Grape	N	Vine	1/182	10%	10%

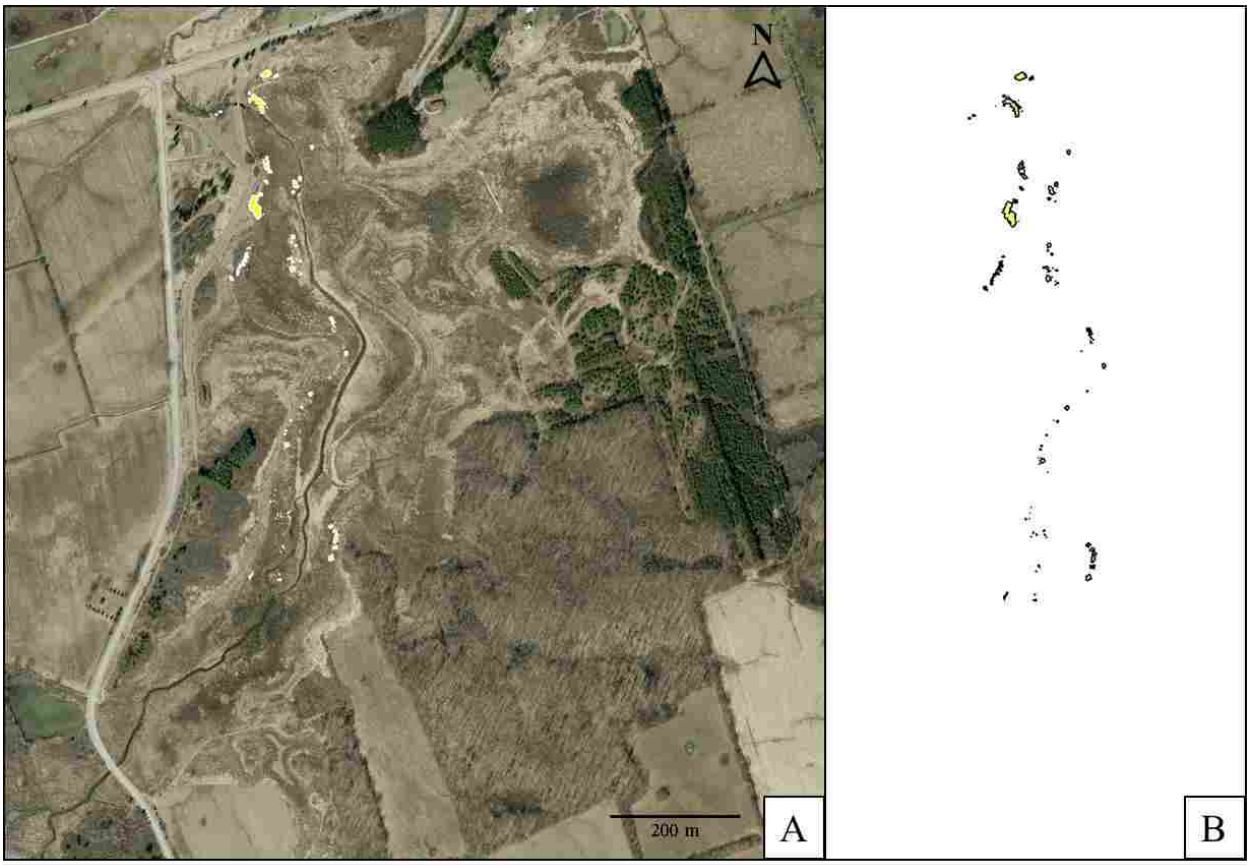

Figure S2.1: Overview of the 180 separate *S. hermaphrodita* stands mapped at TCA using SX Blue II GPS in 2014. The stands are highlighted in yellow and depicted with (A) and without (B) orthoimagery included to depict more clearly where each stand is located throughout the conservation area.

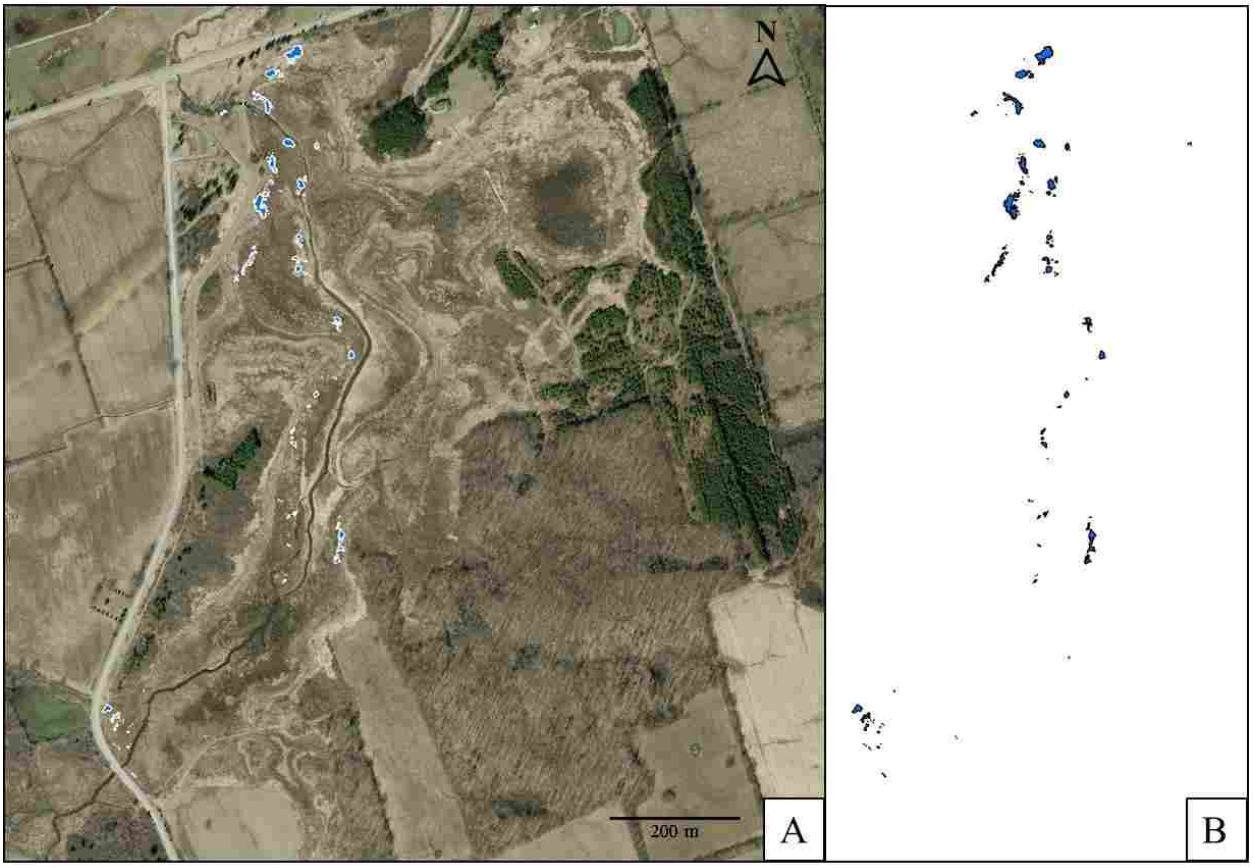

Figure S2.2: Overview of the 158 separate *S. hermaphrodita* stands mapped at TCA using SX Blue II GPS in 2016. The stands are highlighted in blue and depicted with (A) and without (B) orthoimagery included to depict more clearly where each stand is located throughout the conservation area.

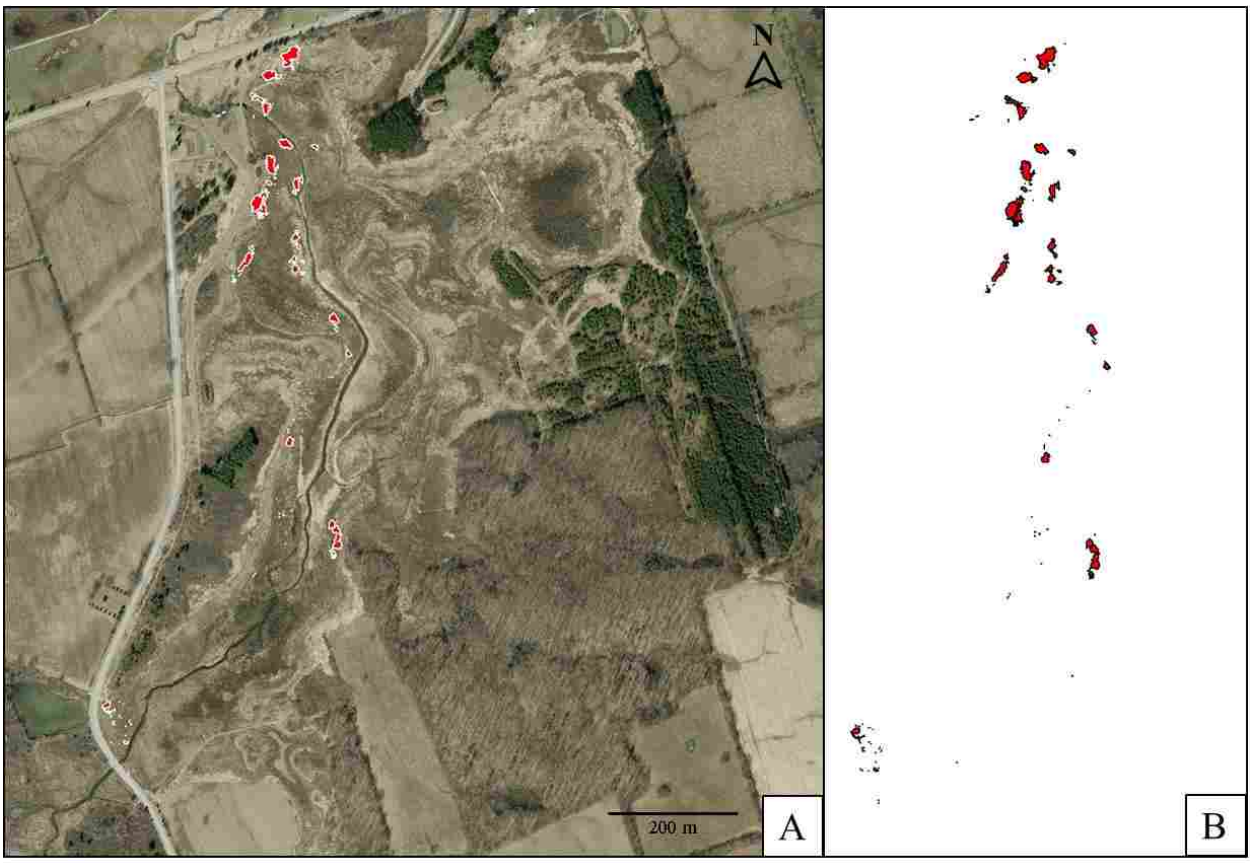

Figure S2.3: Overview of the 149 separate *S. hermaphrodita* stands mapped at TCA using SX Blue II GPS in 2018. The stands are highlighted in red and depicted with (A) and without (B) orthoimagery included to depict more clearly where each stand is located throughout the conservation area.

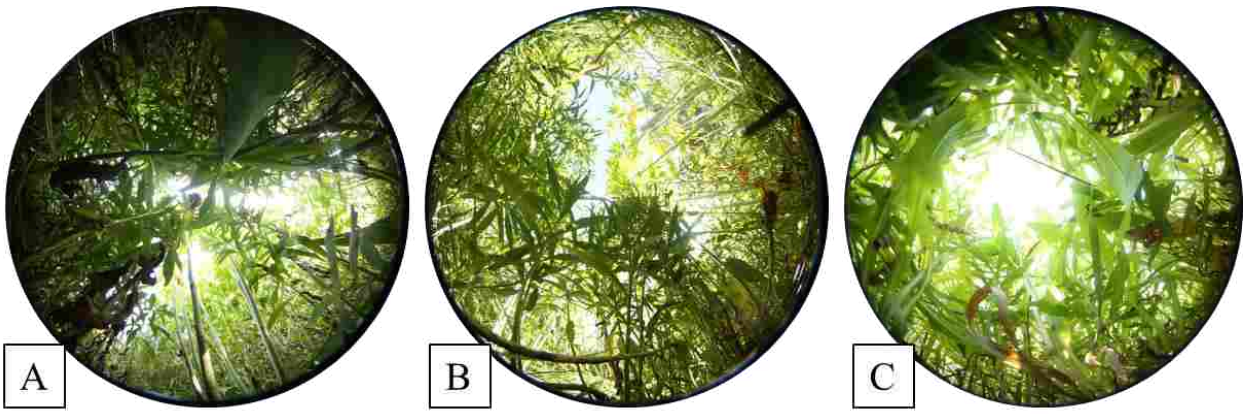

Figure S2.4: Photographs taken at TCA within quadrats where vegetation surveys were conducted. 28 1 m x 1 m quadrats were placed in locations where the proximity of *S. hermaphrodita* stands to *P. australis* stands differed. The proximity levels included locations where both *S. hermaphrodita* and *P. australis* stands overlapped (1), intermediate locations where the boundaries of both species were in close vicinity to one another (< 5 m) (2), and locations where the species boundaries were farther from one another (> 10 m) (3). These figures are 180° hemispherical images of plant cover within each quadrat to depict the light availability for understory plants like *S. hermaphrodita* seedlings.

2.8 References

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Chapter 3: Reciprocal belowground facilitation not allelopathic inhibition occurs between invasive *Phragmites australis* and endangered *Sida hermaphrodita*

In preparation for *Plant and Soil*

Title: Reciprocal belowground facilitation not allelopathic inhibition occurs between invasive *Phragmites australis* and endangered *Sida hermaphrodita*

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3.1 Abstract

Virginia Mallow (*Sida hermaphrodita*) is a perennial herb native to riparian habitats in northeastern North America. Throughout most of its geographical distribution, however, it is considered threatened potentially due to the loss of habitat caused by exotic European Common reed (*Phragmites australis*) invasion. The biology and ecology of *S. hermaphrodita* are still poorly understood, and few studies have been performed to determine factors that contribute to the species' rarity. Allelopathic and phytotoxic alterations of soil environments have been mechanisms proposed to explain the invasion success of *P. australis.* A greenhouse study was conducted to quantify seedling growth and arbuscular mycorrhizal fungal colonization of both species in soils obtained from different vegetation levels ranging from pure stands of *S. hermaphrodita* to pure stands of *P. australis* to determine the potential for *P. australis* to allelopathically alter soils making them inhospitable to native species. Results obtained did not support previous allelopathic exclusion reports and indicated that species performance and AMF colonization was best when plants were grown within the competitor's soil. The soil nutrient analysis coupled with plant performance findings, suggest a potential belowground facilitative interaction between species however, in natural settings, light stress resulting from *P. australis* nutrient enriched growth may cause a competitive shift, explaining its success in interactions with native species like *S. hermaphrodita*.

3.2 Introduction

Invasive alien plant species are recognized as one of the greatest threats to natural ecosystems and global biological diversity (Sanon et al., 2009; Pimentel et al., 2000; Meiners, 2007). It is now well-established that alien plant invasions cause habitat destruction and are capable of displacing native species (Vitousek et al., 1997; Simberloff, 2003). Alien invasive species are often introduced to new areas as a result of human actions, either deliberately or unintentionally (Vitousek et al., 1997). After the initial introduction, populations of alien plants can grow exponentially and become aggressive invaders (Sanon et al., 2009; Mitchell and Power 2003). Their aggression has been attributed to numerous factors including higher competitive ability than native species (Thébaud and Simberloff, 2001), lack of natural

predators (Mitchell and Power 2003), direct or indirect chemical inhibition of neighbouring species (allelopathy) (Callaway and Ridenour 2004; Pisula and Meiners 2010; Bais et al., 2003; Uddin et al., 2012), and the modification of belowground soil microbiota including the disruption or alteration of symbiotic relationships with Arbuscular Mycorrhizal Fungi (AMF) (Sanon et al., 2009; Mummey and Rillig, 2006; Stinson et al., 2006). Utilizing such mechanisms, exotic invasive species can cause the endangerment and even the extinction of native species having significant consequences on global conservation and recovery efforts (Vitousek et al., 1997; Wilcove et al., 1998; Simberloff, 2003).

Sida hermaphrodita (L.) Rusby (Virginia Mallow; Malvaceae) is native to riparian habitats of Northeastern North America (Spooner et al., 1985; COSEWIC, 2010). This herbaceous perennial can reach 1-4 m in height (Spooner et al., 1985; Oleszek et al., 2013; Borkowska, and Molas, 2012) and it develops a strong plagiotropic root system with buds which are responsible for its vegetative propagation and clonal growth (Stevens et al., unpublished). The species can tolerate a variety of soil conditions as well as a degree of moderate human disturbance (Bickerton 2011; Kocoń and Matyka, 2012; Oleszek et al., 2013; Cetner et al., 2014).

Information on the species physiology and ecology is limited; also to date, symbiotic relationships between *S. hermaphrodita* and AMF have not been clarified. Although *S. hermaphrodita* grows vigorously, it has good reproductive potential, both vegetative and sexual (Stevens et al., unpublished), and is tolerant of different environmental conditions, it is currently endangered throughout its native distribution area in the U.S.A. and Canada (Spooner et al., 1985; Klimešová and Klimeš, 2008; COSEWIC 2010; NatureServe 2019). Recently, the largest threat to the conservation of *S. hermaphrodita* in North America was suggested to be the loss of habitat as a result of the increasing abundance of the invasive *Phragmites australis* (Cav.) Trin. ex Steud. (Common Reed; Poaceae) (Bickerton 2011).

Invasive *P. australis* is a perennial wetland grass that can reach up to 4-6 meters in height and forms extensive rhizome systems which allow the species to expand vegetatively into new territories (Cross and Fleming 1989; Clevering and Lissner, 1999; Mal and Narine 2003; Lambert et al., 2010).

Considered to be native to Eurasia, the invasive haplotype M has currently a nearly cosmopolitan distribution, being found on every continent except Antarctica (Roland and Smith, 1969; Gucker, 2008; Mal and Marine, 2003). Since its introduction to North America in the 19th century, it has spread rapidly displacing entire communities of native flora and fauna by creating large monospecific stands (Burk, 1877; Saltonstall 2003; Mal and Narine 2003; Saltonstall et al., 2010).

Considered one of the most aggressive plant invaders worldwide (Marris, 2005; Rudrappa et al., 2007), substantial efforts have been made to explain the invasiveness of *P. australis* and determine specific mechanisms which allow this species to take over native plant communities. Biological characteristics, such as high rates of sexual and vegetative propagation and dense canopy development, are thought to facilitate its expansion into new ecosystems and provide competitive advantage (Meyerson et al. 2000; Mozdzer and Zieman 2010). However, many studies have also hypothesized that *P. australis* can enhance its invasion by directly or indirectly inhibiting the growth of neighbouring plant species through the allelopathic and phytotoxic alteration of soil properties (Rudrappa et al., 2007; Uddin et al., 2012; Uddin et al., 2014a; Weidenhamer et al., 2013), the modification of microbial communities (Jordan et al., 2008; Song et al., 2015) and the interference of belowground mutualisms including symbioses with AMF (Uddin et al., 2017). Considering that *S. hermaphrodita* is similar to *P. australis* in many respects (e.g., high vigour and reproductive potential), the interaction of the two species offers an interesting case in which to study invasion.

The main objective of this study was to determine the reciprocal below ground interaction between the endangered *S. hermaphrodita* and the invasive *P. australis* in order to determine if and how each species impacts the performance of the other. Additionally, since to date there is very limited information on the possible relationships between these two plants and AMF, this study also examined their mycorrhizal colonization. More specifically we addressed the question of how chemical compounds and microorganisms present within the soils associated with *S. hermaphrodita* and *P. australis* affect their performance and mycorrhizal colonization. This objective was achieved by examining the growth of both

species in soils that corresponded to different soil-vegetation levels ranging between pure stands of *S. hermaphrodita* to pure stands of *P. australis*. A confirmation of the hypothesized *P. australis* belowground mechanism of action would be important beyond the case *S. hermaphrodita* as it likely can be expanded to other endangered wetland plants.

3.3 Materials and Methods

3.3.1 Site description

Taquanyah Conservation Area (TCA) is a 136 ha habitat comprised of woodland, grassland and wetland communities located in Haldimand County, Ontario (42°57'17.0" N, 79°54'46.0" W), and is one of the only two locations where *S. hermaphrodita* remains in Canada. The habitat of *S. hermaphrodita* is found within the floodplain associated with a cold-water stream (Bickerton 2011) and the local dominant plant species include *Typha latifolia* L., *Impatiens capensis* Meerb., *Solidago canadensis* L., *and Phalaris arundinacea* L. Based on morphological identification, the invasive, alien *Phragmites australis* haplotype (Saltonstall et al., 2005) has also become a dominant species in this area, by gradually displacing the native species (Bickerton, 2011).

3.3.2 Soil collection and nutrient analyses

In late August 2017, five transects running northeast of the stream were set up. Each transect included five 1 m x 1 m quadrats placed in locations of different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands (Figure 3.1). The first soil core location was placed within a high-density stand of *S. hermaphrodita* (ca. 25 stems/m^2) (Figure 3.2a), and the second was selected along the border of the same stand to obtain soil from a moderately dense *S. hermaphrodita* area (ca. 10 stems/m^2). The third quadrat was positioned in vegetation between the *S. hermaphrodita* stand and an adjacent *P. australis* stand (Figure 3.2b), whereas the last two quadrats were arranged on the border and within the *P. australis* stand similarly to the moderate and high-density locations of the *S. hermaphrodita* quadrats (ca. 25 and 60 *P. australis* stems/m^2, respectively) (Figure 3.2c). The locations of quadrats one and five were selected to obtain soil from within pure *S. hermaphrodita* or *P. australis* vegetation. For

classification of all vegetation observed within each quadrat, see Table S3.1-Table S3.5. Two soil cores were obtained from the corners of each quadrat to ensure that 40 soil cores were collected from each transect, for a total of 200 soil cores. The soil cores were uniformly extracted using split-core sampler with an auger tip (AMS, American Falls, ID). Removable clear polyvinyl chloride (PVC) sleeves, 7.5 cm height by 7.5 cm in diameter enclosed within the sampler to contain intact, undisturbed soil cores that were sealed with vinyl end caps (Uline) and subsequently frozen until the greenhouse portion of the study.

In addition to the soil cores taken for the plant interaction study, one soil core was obtained from the center of each quadrat to measure soil moisture, pH, available Phosphorus (P), sodium bicarbonate, Potassium (K), Magnesium (Mg), Zinc (Zn), and Manganese (Mn) content. Additional soil analyses were also conducted for total Phosphorus, total Nitrogen, Ammonium (NH_4+) and Nitrate (NO_3-) content. Fertility analyses were conducted at the University of Guelph Soil and Nutrient Laboratory.

3.3.3 Soil core experimental design

The intact soil core sleeves were individually wrapped in black polyethylene film (Uline) to prevent soil exposure to UV radiation while in the greenhouse. The individual cores were then set on 8.9 cm diameter steel blue seed germination blotter (Anchorpaper) and placed in 9.4 cm diameter polystyrene dome lids (Polar Pak) to serve as a basin to keep the samples intact, separated from other soil samples, and to control water levels. 10 cores each were enclosed in propagation trays (54.6 cm x 28.4 cm x 6.4 cm) (Mondi™) under mini greenhouse domes (54.6 cm x 27.9 x 19.1 cm) (Mondi™). Deionized water was added to each core basin every other day to ensure that every sample had available water levels as needed. For the first week, soil cores were allowed to acclimate to experimental conditions to ensure that all cores were brought to the same moisture level. Any plant species that emerged during this time were removed from the soil sample. Following acclimation, the cores were maintained under greenhouse conditions (16/8-hour day/night cycle and average day/night temperature of 26/19°C) for 12 weeks.

3.3.4 Seed germination, seedling planting and harvest

Seeds of *S. hermaphrodita* and *P. australis* (collected during the summer of 2014 from TCA and stored dry at 4°C) were surface sterilized with 5-7% Sodium Hypochlorite (NaClO) (Fisher Scientific) and 70% ethanol (EtOH) (Fisher Scientific) using a modified method described by Schulz et al. (1993). Seeds were submerged into EtOH for 1 minute and then transferred to NaClO for 5 minutes, back into EtOH for 1 minute, and finally rinsed thoroughly with deionized water. To ensure uniform germination of *S. hermaphrodita*, the seed coats were scarified with a minutien pin (Spooner et al., 1985). The *P. australis* seeds were not scarified. Following surface sterilization and scarification, the seeds were germinated on 8.9 cm diameter steel blue seed germination blotter (Anchorpaper), kept moist with deionized water in 100 mm x 15 mm sterile polystyrene petri dishes (Fisher Scientific) that were sealed with Parafilm M™ wrapping film. The dishes were maintained under the previously specified greenhouse conditions. Radicle emergence indicated germination, at which time three seeds of each species were sown into each soil core. Since two cores were taken from each individual location at TCA, one core from each site was sown with *P. australis* seeds and the other was sown with *S. hermaphrodita* seeds (Figure 3.3).

Following twelve weeks of growth, the plants were carefully removed from the soil cores and rinsed to remove any soil and debris. Roots and shoots were separated immediately after harvest to obtain root and shoot fresh weights of each plant using a Mettler Toledo NewClassic MS analytical balance. The shoots were then individually scanned with an Epson Expression 10000 EL scanner and surface area measurements were determined using WinRhizo Arabidopsis 2012d software (Regent Instruments, Quebec, Canada). Roots were also scanned using the same WinRhizo software to obtain root length, surface area, and average diameter measurements. Following scanning, root segments were separated from the original sample, placed in 10 mL Falcon tubes with 50% EtOH to estimate the proportion of roots that are colonized by AMF.

3.3.5 AMF assessment

Roots were removed from the 50% EtOH and thoroughly rinsed with deionized water. The clearing and staining of the roots followed a modified ink-vinegar staining technique protocol (Vierheilig et al., 1998; Vierheilig et al., 2005). For clearing, the roots were submerged in 10% Potassium hydroxide (KOH) and were heated at a temperature of 95°C in a vacuum oven (Thermo Scientific Lindberg Blue M) under 25 inches Hg pressure for approximately 50-60 minutes. The roots were then rinsed twice with a 10% vinegar ($C_2H_4O_2$) solution and then submerged in 5% Sheaffer ink-vinegar solution to stain the AMF structures within the roots. The roots were heated for approximately 10-15 minutes under the same conditions as previously mentioned for the clearing step and subsequently checked under a Zeiss SteREO Discovery V8 dissecting microscope (Carl Zeiss Inc., Germany) for sufficient staining. Lastly, the roots were rinsed with 5% vinegar to de-stain and then stored in 50% glycerol ($C_3H_8O_3$).

Roots were cut into approximately 3 cm long segments, 7 of which were randomly selected and mounted in 50% glycerol on frosted microscopic slides (Fisherbrand™). Prepared slides were viewed under a Zeiss Axioscope 5 microscope (Carl Zeiss Inc., Germany) under 200x magnification (objective 20x, ocular 10x) and images were taken with Zeiss Zen Axiocam imaging software (blue edition).

Mycorrhizal colonization was assessed using the gridline intersect technique (McGonigle et al., 1990). Using this technique, intra-radicular hyphae, vesicles, and arbuscules were counted to obtain an estimate of the proportion of roots in a plant that contained mycorrhizal structures.

3.3.6 Statistical analyses

All plant growth responses and mycorrhizal colonization measurements were analysed using a two-way analysis of variance (ANOVA) in JMP (Statistical Analysis Software version 14). All analyses were performed to determine if there was an interaction between the plant species and the soil-vegetation level (location) and their effect on the measure of plant performance or AMF colonization. To address any variability that may be associated with the placement of transects, data was analysed as a randomized

complete block design with transect as the blocking factor. Two levels of species, five levels of location and five replicate blocks (transects) were included in the design. To meet assumptions of normality and homogeneity of variance, plant performance response variables, including measurements of shoot biomass, total biomass, shoot project area, root project area, root average diameter, and all measures of root AMF colonization were square-root transformed. The remaining plant performance response variables, including measurements of root biomass, root/shoot ratio, and root length were log transformed. Since we were interested only in specific comparisons and not in all possible pair-wise comparisons, multiple comparisons were conducted using the LS means function in JMP with a non-corrected student's T specification. The soil chemistry and plant performance and mycorrhizal analyses were used for principal component analyses (PCA) to identify patterns of soil nutrient, plant performance and fungal properties for each species. Multiple linear regressions were used to clarify the relationships between plant performance and colonization in addition to selected environmental variables.

3.3 Results

3.3.1 *Sida hermaphrodita* and *Phragmites australis* plant growth response

The results show marked differences between the two species (Figure 3.4). Across all measures of plant growth, the performance of *S. hermaphrodita* was significantly different ($p < 0.0001$) than the performance of *P. australis* (Table 3.1). Measurements of shoot biomass and shoot surface areas of *P. australis* plants (mean: 0.737 g ± 0.036 g; mean: 33.589 cm^2 ± 0.221 cm^2) were approximately double those of *S. hermaphrodita* plants (mean: 0.316 g ± 0.038 g; mean: 14.629 cm^2 ± 0.236 cm^2) (Figures 3.4 & 3.6). Whereas average measurements of *P. australis* root performance (root biomass, root surface area, root length) were between 6 and 8 times greater than the root performance of *S. hermaphrodita* (Figures 3.4, 3.6, 3.7). Root average diameter was the only plant performance variable in which *S. hermaphrodita* (mean: 0.401 mm ± 0.006 mm) had greater performance than *P. australis* (mean: 0.315 mm ± 0.006 mm) (Figure 3.7). Interactions were observed between the plant species and the soil-vegetation level which had significant effects on several plant performance response variables, including total biomass ($p < 0.05$),

shoot biomass ($p < 0.01$), root biomass ($p < 0.01$), shoot surface area ($p < 0.001$), and root length ($p < 0.01$) (Table 3.1). In each case, although the measurement of *P. australis* performance was significantly greater than the measurement of *S. hermaphrodita* performance, the magnitude of the difference between the two species depended on the location. Following harvest, the average total biomass of *P. australis* plants (2.137 g ± 0.059 g) was three times greater than the average total biomass of *S. hermaphrodita* plants (mean: 0.616 g ± 0.063 g) (Figure 3.5). However, the relative total biomass among both species differed across locations in which measurements of *P. australis* total biomass was significantly higher in pure *S. hermaphrodita* soils (3.136 g ± 0.149 g) than the measurements of total biomass in pure *P. australis* soils (1.525 g ± 0.126 g). The measurements of *S. hermaphrodita* total biomass had the inverse relationship, in which total biomass was lowest in pure *S. hermaphrodita* soils (0.466 g ± 0.151 g) and highest in pure *P. australis* soils (0.865 g ± 0.129 g) (Figure 3.5). Comparable interactions were also obtained for other response variables in which reductions in plant performance between pure *S. hermaphrodita* soils and pure *P. australis* soils were observed for *P. australis* plant performance parameters such as shoot biomass (39%), root biomass (61%), shoot surface area (44%) and root length (53%) (Figures 3.4, 3.6, 3.7). *S. hermaphrodita* performance had an inverse relationship in which increases in plant performance between pure *S. hermaphrodita* soils and pure *P. australis* soils were observed for the performance parameters of *S. hermaphrodita* plants, including shoot biomass (66%), root biomass (62%), shoot surface area (71%) and root length (57%) (Figures 3.4, 3.6, 3.7). Similar results were obtained for the root surface area (Figure 3.6), in which *P. australis* and *S. hermaphrodita* performance was highest in soils collected within dense stands of their competitor, however, no significant interaction was observed (Table 3.1).

Contrasting results were obtained for the remaining growth parameters including root average diameter and root-to-shoot ratio. No significant interactions were observed between the species and the soil-vegetation level on the growth parameters, however, the root average diameter of both *S. hermaphrodita* and *P. australis* were lowest in pure *S. hermaphrodita* soils (*S. hermaphrodita* mean:

0.396 mm ± 0.015 mm; *P. australis* mean: 0.293 mm ± 0.015 mm), and highest in pure *P. australis* soils (*S. hermaphrodita* mean: 0.417 mm ± 0.012 mm; *P. australis* mean: 0.346 mm ± 0.011 mm) (Figure 3.5). The root-to-shoot biomass ratio of *P. australis* was highest in pure *S. hermaphrodita* soils (2.152 ± 0.169) and lowest in pure *P. australis* soils (1.438 ± 0.142). Similarly, the root-to-shoot biomass ratio of *S. hermaphrodita* was highest in pure *S. hermaphrodita* soils (1.144 ± 0.171) but lowest in moderately dense *S. hermaphrodita* soils (0.687 ± 0.163) and pure *P. australis* soils (0.749 ± 0.146) (Figure 3.5).

Additionally, the transect blocking factor had a significant effect ($p < 0.05$) on the root biomass of the investigated plants (Table 3.1). Specifically, there was a significant difference in root biomass among the five transects with the southernmost transect producing less root biomass (57%) in comparison to the other four transects. Plant performance was lower in the southernmost transect compared to the other four transects for all other response variables except root average diameter, however, significant effects were not observed (Table 3.1).

3.3.2 AMF colonization

Arbuscular mycorrhizal fungi were able to colonize both investigated plant species and colonized plants had both arbuscules and vesicles (Figures 3.9 & 3.10). However, average mycorrhizal colonization levels found within plant roots differed between species. Interactions were observed between the plant species and the soil-vegetation level which had significant effects on all AM colonization response variables (Table 3.2). Although the proportion of *S. hermaphrodita* AM root colonization was significantly greater ($p < 0.0001$) than the proportion of *P. australis* AM root colonization, the magnitude of the difference between the two species depended on the location. Mean hyphal colonization of *S. hermaphrodita* (60.373% ± 2.500%) was approximately 10 times higher than the mean hyphal colonization of *P. australis* roots (5.707% ± 2.369%). However, the relative hyphal colonization among both species differed across locations in which the proportion of *S. hermaphrodita* root hyphal colonization was significantly higher ($p < 0.0001$) in pure *P. australis* soils (89.731% ± 5.184%), than the proportion of *S. hermaphrodita* root hyphal colonization in pure *S. hermaphrodita* soils (41.409% ±

5.954%) (Figure 3.8). In *P. australis* roots, AM colonization was low overall, but root hyphal colonization was significantly lower in pure *P. australis* soils (1.553% ± 5.098%) compared to the proportion of *P. australis* root hyphal colonization in moderately dense *S. hermaphrodita* soils (9.858% ± 5.175%) (Figure 3.8).

Arbuscular colonization also differed among species and interactions were observed between the plant species and the soil-vegetation level which had significant effects ($p < 0.0001$) on the arbuscular colonization response variable (Table 3.2). Mean arbuscular colonization of *S. hermaphrodita* (39.391% ± 2.585%) was approximately 35 times higher than the mean arbuscular colonization of *P. australis* roots (1.096% ± 2.426%). However, the relative arbuscular colonization among both species differed across locations in which the proportion of *S. hermaphrodita* root arbuscular colonization was significantly higher in pure *P. australis* soils (66.594% ± 5.283%), compared to the proportion of *S. hermaphrodita* root arbuscular colonization in pure *S. hermaphrodita* soils (20.738% ± 6.189%) (Figure 3.8). *P. australis* roots exhibited consistently low proportion of arbuscular colonization throughout all locations; however, arbuscular colonization was highest in pure *S. hermaphrodita* soils (1.539% ± 6.099%) and lowest in pure *P. australis* soils (0.353% ± 5.175%) (Figure 3.8).

Vesicular colonization differed among species and interactions were also observed between the plant species and the soil-vegetation level which had significant effects ($p < 0.01$) on the vesicular colonization response variable (Table 3.2). Although mean vesicular colonization of *S. hermaphrodita* roots (12.702% ± 1.863%) was significantly higher ($p < 0.0001$) than the mean vesicular colonization of *P. australis* roots (0.182% ± 1.726%), the magnitude of the difference between the two species depended on the location. The relative vesicular colonization among both species differed across locations, in which the proportion of *S. hermaphrodita* root vesicular colonization was significantly higher in soils obtained from within intermediate vegetation areas (22.310% ± 3.734%), compared to the proportion of *S. hermaphrodita* root vesicular colonization in pure *S. hermaphrodita* soils (6.479% ± 4.472%), and pure *P. australis* soils (9.707% ± 3.728%) (Figure 3.8). In *P. australis* roots, vesicular colonization was

consistently very low throughout all locations, however, the proportion of *P. australis* root vesicular colonization was highest in moderately dense *S. hermaphrodita* soils (0.565% ± 3.723%), and lowest in pure *P. australis* soils (0.018% ± 3.623%) (Figure 3.8).

Additionally, the transect blocking factor had a significant effect ($p < 0.01$) on all AM colonization response variables (Table 3.2). Specifically, there was a significant difference in AM colonization among the five transects with the southernmost transect producing less root colonization compared to the northernmost transect. Significant reductions in root colonization response variables including, hyphal colonization (47%), arbuscular colonization (68%), and vesicular colonization (78%) were observed between the northernmost transect and the southernmost transect.

3.3.3 Soil nutrient composition correlations

A principal component analysis (PCA) was performed to clarify the relationship between the soil nutrient variables, plant performance and fungal colonization for each species. The PCA resulted in three principal components cumulatively explaining 57.4% of the total variance. The amount of variation that each plant and environmental trait explains, was represented through loadings on the PCA. PC 1 explained a gradient in soil characteristics in which soil pH and Ca content were negatively correlated to Mg, K, Mn, and total P content (Table 3.4). PC 2 explained a gradient in soil nutrient characteristics related to both *S. hermaphrodita* and *P. australis* performance and AM colonization. On this axis *S. hermaphrodita* total biomass and hyphal colonization as representatives of plant performance and AM colonization respectively, were positively correlated to soil moisture, NO_3-, available P content, and negatively correlated to *P. australis* total biomass, hyphal colonization, soil NH_4+ and total N content (Figure 3.11). PC 3 accounted for a gradient in the remaining soil nutrient characteristics in which soil Na was negatively correlated to Zn content (Figure 3.11) (Table 3.4).

Multiple regression analyses indicated that root hyphal colonization of *S. hermaphrodita* was positively correlated to total biomass ($R^2 = 0.3087$; $p < 0.01$) (Figure S3.1), and soil moisture ($R^2 = 0.2245$; $p < 0.05$) (Figure S3.2) and negatively correlated to total N content ($R^2 = 0.2727$; $p < 0.01$)

(Figure S3.3). Root hyphal colonization of *P. australis* was negatively correlated to soil moisture (R^2 = 0.1831; $p < 0.05$) (Figure S3.2), available P (R^2 = 0.1578; $p < 0.05$) (Figure S3.4), and NO_3- content (R^2 = 0.2293; $p < 0.05$) (Figure S3.5). No significant relationships were observed between *S. hermaphrodita* or *P. australis* total biomass or root colonization and other environmental variables (Table 3.3).

3.4 Discussion

In the present study, our examination of the interaction between the endangered *S. hermaphrodita* and the invasive *P. australis* provided no evidence to support the idea according to which the invasion success of *P. australis* can be attributed to allelopathic or phytotoxic alterations of soil chemistry and microbiotic properties. Previous research has shown that *P. australis* is capable of exhibiting allelopathic exclusion in many ways, including through the active release or indirect microbial release of inhibitory phenolic compounds such as gallic acid (Rudrappa et al., 2007; Rudrappa et al., 2009; Bains et al., 2009), the release of phytotoxic tissue extracts resulting from decomposition (Uddin et al., 2012; Uddin et al., 2014a), and also through the release of compounds produced by *P. australis* associated endophytes (White et al., 2018). By releasing these compounds into the surrounding soil, *P. australis* demonstrated the capacity to directly or indirectly affect the rhizosphere and soil chemistry, in turn enhancing its invasive potential by inhibiting the germination and growth of native species (Bains et al., 2009; Uddin et al., 2012; Uddin et al., 2014a; Uddin et al., 2014b; Uddin et al., 2017; White et al., 2018).

Since further identification of specific phytotoxic phenolic compounds released directly or indirectly by *P. australis* needs to be completed, little is known about what effect frozen storage may have on the microbial populations and possible phenolic compounds present within the soils collected for this study. Although previous studies indicated that gallic acid can be rapidly degraded in non-sterile soil (Weidenhamer and Romeo, 2004; Weidenhamer et al., 2013), other studies reported that some compounds are resistant to degradation (Sosa et al., 2010); may break down into more toxic compounds (Krogh et al., 2006), or may persist in the soil and remain phytotoxic after plant decomposition (Bains et

al., 2009; Uddin et al., 2012; Uddin et al., 2014b). It is based on this knowledge that we expected the inhibited performance of *S. hermaphrodita* plants grown in soils associated with *P. australis.*

Interestingly, the performance of both species was lowest when grown in soils collected within their own stands and highest when grown in soils collected from within their competitor stands. Since it is widely accepted that invasive *P. australis* is highly adaptable and can rapidly colonize new areas (Saltonstall, 2002; Belzile et al., 2010; Kettenring and Mock, 2012; Soares et al., 2016), it is not surprising that *P. australis* performance would not be negatively affected by a new soil. However, the significantly increased performance of both species in the competitor's soils suggest that both species may act to facilitate the other's expansion into new locations. The overall positive impact that *P. australis* associated soils had on *S. hermaphrodita* plants was particularly interesting since we expected dense field grown stands of *P. australis* would significantly alter the soil biota and chemistry, making it inhospitable for the native *S. hermaphrodita* growth. Our findings are not compatible with previous reports that soil pH is lowered by the release of phenolics through root exudation or decomposition in *P. australis* invaded areas (Armstrong and Armstrong, 1999; Uddin et al., 2014b; Uddin and Robinson, 2018). Changes in soil pH at TCA were not correlated with plant performance. The soil pH and positive association results observed between the soil-vegetation level and *S. hermaphrodita* performance (Figure 3.4), suggest that *P. australis* is not allelopathically or phytotoxically altering the belowground environment to competitively inhibit the growth of *S. hermaphrodita* at TCA.

In addition to plant performance, AMF colonization was also not (allelopathically) inhibited by *P. australis*. Limited information is available about the AMF colonization status of both investigated plant species, however, *P. australis* colonization levels supported well established knowledge of AMF - plant relationships. Comparable to previous reports of *P. australis* colonization (Oliveira et al., 2001; Dolinar and Gaberščik, 2010), overall colonization levels were low (Figure 3.8). Additionally, *P. australis* results in PC score 2 supported reports that AMF colonization is typically negatively correlated with soil moisture (Stevens and Peterson, 1996; Oliveira et al., 2001) (Figure 3.11) and available phosphorus levels

(Figure S3.2 & Figure S3.4 since the main benefit of AMF symbioses is to improve plant acquisition of water and nutrients with an emphasis on phosphorus (Khan, 1975; Harley and Smith, 1987; Bolan, 1991).

Allelopathic exclusion of native species through interference of AMF colonization has been previously attributed to invasive species, including *P. australis* (Roberts and Anderson, 2001; Uddin et al., 2017). However, our results are not supportive of such findings because AMF colonization of *S. hermaphrodita* was significantly higher in *P. australis* dominated soils (Figure 3.8) suggesting that *P. australis* did not allelopathically alter the soil in such a way to interfere with *S. hermaphrodita's* relationship with AMF. Surprisingly, *S. hermaphrodita* results in PC score 2 does not coincide with typical relationships between plants and AMF, since the colonization of *S. hermaphrodita* roots was positively correlated to soil moisture and available phosphorus levels (Figure 3.11). Due to the absence of a control treatment with sterile soil, it is impossible to determine the extent of the relationship between *S. hermaphrodita* and AMF. However, because of the overall high levels of colonization throughout the soil-vegetation locations and the positive correlation observed between AMF and total plant biomass (Figure S3.1), it is unlikely that AMF form a parasitic relationship with *S. hermaphrodita*, and our results may suggest that *S. hermaphrodita* is an obligate mycotroph that relies on symbioses with AMF to survive (Johnson et al., 1997; Koide, 2010).

Our results are not in agreement with the previous work suggesting that *P. australis* allelopathically excludes native species like *S. hermaphrodita* during the invasion process. Instead, our findings support the potential for soil nutrient enrichment to influence the performance and invasion process of *P. australis* (Uddin and Robinson, 2018). The general decline in root – shoot ratios for each species between pure *S. hermaphrodita* soils and pure *P. australis* soils in addition to the results of the PCA provide insight into how the plants respond to their soil environments. The generally high root – shoot ratios observed in *S. hermaphrodita* dominated soils (Figure 3.5) suggests that these potentially nutrient poor soils are driving both species to allocate more resources to their roots (Tilman, 1985).

Conversely, the lower root – shoot ratios observed in the *P. australis* dominated soils suggests that necessary nutrients are high, and both species can allocate more resources to their shoots (Tilman, 1985).

Although the average root – shoot ratio for *P. australis* plants indicates that self-dominated soils may promote its growth, the plant performance in these soils was significantly reduced, particularly for measures of root performance. Our results support previously documented findings that *P. australis* allocates relatively more to searching for soil resources than other plants (Uddin and Robinson, 2018) and that the relationship between *P. australis* root – shoot ratio and nutrient availability may not be linear. *Sida hermaphrodita* performance, however, did follow typical trends since performance was significantly increased in *P. australis* soils where root – shoot ratio was lower. The presence of several soil nutrients was examined during this study, however, the nutrients associated with plant performance and colonization were most applicable to the overall research goal. PC score 2 accounted for 17.5% of the overall variation with *S. hermaphrodita* total biomass, *S. hermaphrodita* hyphal colonization, soil moisture, NO_3- and available phosphorus positively loaded and *P. australis* total biomass, *P. australis* hyphal colonization, total N and NH_4+ negatively loaded. Given that wetland plants including *P. australis* are typically limited by either nitrogen or phosphorus supply (Koerselman and Meuleman, 1996; Romero et al., 1999), the observed relationships between nitrogen and phosphorus levels may be responsible for the investigated plant performance responses. The increased performance of *P. australis* in *S. hermaphrodita* dominated soils, where total N and NH_4+ levels are high, agree with previous reports that *P. australis* growth is nitrogen limited (Romero et al., 1999; Rickey and Anderson, 2004). Additionally, *P. australis* has been observed to have high plasticity towards its N source (Tylova-Munzarova et al., 2005; Munzarova et al., 2006) and although it's capable of utilizing NO_3-, it has also been suggested to have a preference or affinity for NH_4+ similarly to other wetland plants (Romero et al., 1999; Cedergreen and Madsen, 2003; Tylová et al., 2008). Conversely, limited information is available about *S. hermaphrodita's* specific nutrient requirements, however, NPK fertilization has been documented to increase *S. hermaphrodita* agricultural yield (Nabel et al., 2016; Nabel et al., 2017). Furthermore, biomass

yield of cultivated *S. hermaphrodita* was documented to increase when treated with phosphorus in comparison to nitrogen (Borkowska et al., 2009). These results, in addition to the observed performance increase of *S. hermaphrodita* in *P. australis* soils where available P is high, may suggest that *S. hermaphrodita* performance is phosphorus limited. Further examination needs to be completed to determine the potential and extent of *S. hermaphrodita's* phosphorus limitation in addition to its dependency for AMF colonization.

Based on the nutrient limitations for both species, our results surprisingly suggest that the soils dominated by *S. hermaphrodita* or *P. australis* may promote the growth of their competitor. Numerous ecological factors, including negative frequency dependent selection, stabilizing niche differences, relative fitness differences, and competitive exclusion could lead to species coexistence or competition (Chesson, 2000; Suding et al., 2005; Adler et al., 2007; Suttle et al., 2007; Adler et al., 2010; HilleRisLambers et al., 2012; Yenni et al., 2017). Specifically, we wanted to assess the potential for *P. australis* to impact the performance of *S. hermaphrodita* through competitive belowground allelopathic or phytotoxic soil modifications. Interestingly, our results provided no evidence for a negative belowground interaction between the investigated plant species. Positive effects of soil nutrients on plant performance are not surprising since ecological theory states that plant invasion depends on resource availability (Davis et al., 2001). Resource availability however, in addition to other gradients in the abiotic environment, can impact the balance between competition and facilitation in species interactions (Callaway and Walker, 1997; Callaway, 1998; Maestre et al., 2009). In general, in conditions where there is low abiotic stress that permit the rapid acquisition of resources, competition between species increases. Alternatively, increased abiotic stress causes plant interactions to shift from competitive to facilitative, as the neighboring plants buffer one another from extremes in the environment (Bertness and Callaway, 1994; Callaway and Walker 1997; He et al., 2013). Although we expected only competitive interactions to occur between the investigated species, the results observed in this study indicate instead that these species may be facilitating limited soil nutrients for one another potentially due to severe unknown

physical stressors present at TCA (e.g., water table level, temperature, salinity). Environmental conditions were not measured during this study which would define stress gradients that the species were subjected to at TCA, so further examination of physical conditions will need to be completed in order to confirm this facilitative interaction or determine if a different mechanism of coexistence may take place between the two species. Additionally, due to the observed increased shoot growth of *P. australis* in comparison to *S. hermaphrodita* during the experiment, we expect that light availability may be an additional factor that could impact the outcome of the interaction between both species. Plants were grown in individual soil cores during this study which prevented any above ground competition. Although *S. hermaphrodita* displayed increased performance in *P. australis* dominated soils, in natural settings, the presence of dense and fast-growing *P. australis* stands may reduce the extent of the facilitation interaction by increasing light stress for the slower growing *S. hermaphrodita*. The higher nitrogen availability in *S. hermaphrodita* soils may then enable further *P. australis* invasion and shift plant competition from belowground to aboveground to outcompete the *S. hermaphrodita* stands present (Minchinton and Bertness, 2003; Sillman and Bertness, 2004). Although further research is needed to define the interaction between these species and confirm whether shading by *P. australis* is the main competitive factor limiting *S. hermaphrodita* species success, our research provides new information that *P. australis* may not be as competitive as previously believed, and that physical factors may impact its ability to threaten native species.

3.5 Acknowledgements

This research was supported by the Species at Risk Research Fund grant awarded by the Ontario Ministry of Natural Resources and Forestry in 2018 to 2019. We are grateful to the Grand River Conservation Authority for access to the Taquanyah Conservation Area. We also thank Dr. Rebecca Rooney and Dr. Eric Snyder for their valuable suggestions regarding the experimental design, statistical analyses and editing as well as the numerous students and research assistants including Kayla Salive, Rebekah Hamp, and Rachel Tzeckas for their assistance with field sample collection and plant harvest. The views expressed in this publication are the views of the authors and do not necessarily reflect the views of the Province of Ontario.

3.6 Tables and Figures

Table 3.1: Output of two-way ANOVA used to assess differences between the two plant species, five transects, and the five soil-vegetation levels (Location), in addition to the interaction between species and location and their effect on various measures of plant performance. Response variables tested were shoot biomass (SB), root biomass (RB), total biomass (TB), root-to-shoot biomass ratio (R/S), shoot project area (SPA), root project area (RPA), root average diameter (RAD), and root length (RL). To meet ANOVA assumptions, square-root transformations were applied to response variables, including shoot biomass, total biomass, shoot project area, root project area, and root average diameter. Log transformations were applied to remaining response variables, including root biomass, root/shoot biomass ratio and root length. Soil cores were given one week to acclimate to greenhouse conditions prior to when seedlings were planted. Plants were harvested and measured 12 weeks after planting. Significance level of $p < 0.05$ was used for all statistical tests and significant effects are indicated with an asterisk (*).

	Species			**Transect**			**Location**			**Species x Location**		
	Df	**F**	**P**	**Df**	**F**	**P**	**Df**	**F**	**P**	**Df**	**F**	**P**
SB (g)	1/149	42.27	<0.0001*	4/149	1.855	0.123	4/149	0.490	0.743	4/149	3.598	0.0082*
RB (g)	1/136	96.90	<0.0001*	4/136	2.851	0.027*	4/136	0.746	0.577	4/136	3.959	0.0046*
TB (g)	1/136	78.86	<0.0001*	4/136	1.361	0.251	4/136	0.588	0.677	4/136	3.137	0.0170*
R/S	1/135	56.47	<0.0001*	4/135	0.949	0.438	4/135	1.646	0.217	4/135	0.959	0.4329
SPA (cm^2)	1/137	42.46	<0.0001*	4/137	2.173	0.076	4/137	0.573	0.687	4/137	5.578	0.0004*
RPA (cm^2)	1/136	124.81	<0.0001*	4/136	1.462	0.218	4/136	0.265	0.896	4/136	2.377	0.0555
RAD (mm)	1/136	55.46	<0.0001*	4/136	0.724	0.577	4/136	2.979	0.084	4/136	0.528	0.7151
RL (cm)	1/136	151.60	<0.0001*	4/136	1.209	0.310	4/136	0.340	0.847	4/136	3.864	0.0054*

Table 3.2: Output of two-way ANOVA used to assess differences between the two plant species, five transects, and the five soil-vegetation levels (Location), in addition to the interaction between species and location and their effect on various measures of root AM colonization. Response variables tested were hyphal colonization (HC), arbuscular colonization (AC), and vesicular colonization (VC). To meet ANOVA assumptions, square-root transformations were applied to all AM colonization response variables. Soil cores were given one week to acclimate to greenhouse conditions prior to when seedlings were planted. Plants were harvested and roots were stained and assessed for colonization 12 weeks after planting. Significance level of $p < 0.05$ was used for all statistical tests and significant effects are indicated with an asterisk (*).

	Species			**Transect**			**Location**			**Species x Location**		
	Df	**F**	**P**	**Df**	**F**	**P**	**Df**	**F**	**P**	**Df**	**F**	**P**
HC (%)	1/151	379.81	<0.0001*	4/151	4.023	0.004*	4/151	0.553	0.700	4/151	10.514	<0.0001*
AC (%)	1/151	281.73	<0.0001*	4/151	4.894	0.001*	4/151	1.663	0.214	4/151	7.139	<0.0001*
VC (%)	1/151	155.48	<0.0001*	4/151	3.860	0.005*	4/151	2.320	0.120	4/151	3.487	0.0095*

Table 3.3: Correlations matrix for soil characteristics, related to average plant performance, and AM colonization levels for both *S. hermaphrodita* and *P. australis*. Both *S. hermaphrodita* and *P. australis* plant performance and AM colonization levels are represented by total biomass (STB or PTB) and root hyphal colonization (SHC or PHC) respectively. Various soil characteristics consist of soil moisture (H_2O), soil pH, and soil nutrients including Calcium (Ca), Sodium (Na), Magnesium (Mg), Potassium (K), Manganese (Mn), Zinc (Zn), available Phosphorus (P), total Phosphorus (TP), Ammonium (NH_4+), Nitrate (NO_3-), and total Nitrogen (TN). *S. hermaphrodita* and *P. australis* plants were harvested and assessed for AM colonization after 12 weeks of growth. Soil composition measurements were completed by the University of Guelph Soil and Nutrient Laboratory. A principal component analysis was used to determine trends between *S. hermaphrodita* and *P. australis* performance, fungal colonization and soil characteristics. Multiple linear regression analyses were used to determine if soil characteristics influenced plant performance and AM colonization. Significant correlations ($p < 0.05$) are indicated with an asterisk (*).

	STB	SHC	PTB	PHC	Ca	Na	Mg	K	Mn	Zn	pH	H2O	P	TP	NH4+	NO3-	TN
STB	1.0000																
SHC	0.4294*	1.0000															
PTB	-0.2242	-0.2591	1.0000														
PHC	-0.0301	-0.0371	0.1365	1.0000													
Ca	-0.2790	-0.0406	-0.1105	-0.0307	1.0000												
Na	-0.1195	0.0144	0.0728	0.4167	0.0205	1.0000											
Mg	0.1205	-0.1009	0.1320	0.3158	-0.6655*	0.4320*	1.0000										
K	0.3162	0.0530	0.0903	0.0218	-0.8736*	-0.1153	0.5497*	1.0000									
Mn	0.2616	-0.0603	0.1423	-0.1314	-0.8686*	-0.2045	0.6561*	0.7998*	1.0000								
Zn	-0.1453	-0.2205	0.2271	-0.0031	-0.3402	-0.3196	-0.0917	0.3932	0.2877	1.0000							
pH	-0.0689	0.0819	-0.1463	0.0370	0.3936	0.1568	-0.4025*	-0.4985*	-0.5493*	-0.4217*	1.0000						
H2O	0.0593	0.4062*	-0.1959	-0.2245	0.0085	0.0743	-0.1252	-0.0845	-0.0583	-0.2434	0.1458	1.0000					
P	0.1699	0.3214	0.0124	-0.2626	-0.4869*	-0.1279	-0.0552	0.4711	0.3184	0.3225	-0.0548	0.4043*	1.0000				
TP	0.1595	-0.1120	0.0087	0.0684	-0.6196*	0.0311	0.6635*	0.5594	0.6026*	-0.0469	-0.1517	-0.1491	0.1679	1.0000			
NH4+	0.0319	0.0252	-0.0736	-0.0041	-0.0276	-0.0772	0.0836	0.1544	0.0572*	-0.1624	-0.0143	-0.2596	-0.1363	0.4218*	1.0000		
NO3-	0.0787	0.3219	-0.0357	-0.3262	0.2727	-0.1700	-0.5035*	-0.1309	-0.2258	0.0043	-0.0626	0.4143*	0.3034	-0.6243*	-0.2301	1.0000	
TN	-0.1886	-0.4246*	0.0103	0.0336	0.2577	-0.2772	-0.1381	-0.1480	-0.0155	0.1061	-0.3291	-0.4450*	-0.5644*	-0.0542	0.3184	-0.0805	1.0000

Table 3.4: Loadings of the principal component analysis used to clarify the relationship between the evaluated plant performance, fungal colonization and various soil nutrient variables for both *S. hermaphrodita* and *P. australis*. Principal components (PC) 1-3 cumulatively explained 57.4% of the total variance. PC 1 explained a gradient in soil characteristics: increasing Magnesium (Mg), Potassium (K), Manganese (Mn), total Phosphorus (TP), and decreasing soil pH and Calcium (Ca). PC 2 explained a gradient in soil nutrient characteristics related to plant performance and AM colonization: increasing *S. hermaphrodita* total biomass (STB), *S. hermaphrodita* root hyphal colonization (SHC), soil moisture (H_2O), available Phosphorus (P), Nitrate (NO_3-), and decreasing *P. australis* total biomass (PTB), *P. australis* hyphal colonization (PHC), Ammonium (NH_4+), and total Nitrogen (TN). PC 3 explained a gradient in the remaining soil nutrient characteristics: increasing Sodium (Na) and decreasing Zinc (Zn).

	PC 1	**PC 2**	**PC 3**
Variation Explained (%)	26.6	17.5	13.3
STB	0.2735	0.3902	0.1808
SHC	-0.0602	0.6561	0.3010
PTB	0.1635	-0.2265	-0.1920
PHC	0.0783	-0.4195	0.4046
Ca	-0.9319	-0.2024	-0.0254
Na	-0.0308	-0.1595	0.7092
Mg	0.7658	-0.2424	0.4407
K	0.8971	0.1955	-0.1188
Mn	0.9067	0.0942	-0.1617
Zn	0.3367	0.0145	-0.6743
pH	-0.5422	0.0781	0.4628
H_2O	-0.1665	0.7035	0.2224
P	0.3537	0.7524	-0.1127
TP	0.7565	-0.1962	0.3006
NH_4+	0.1710	-0.3235	0.0760
NO_3-	-0.4012	0.6062	-0.3600
TN	-0.1053	-0.6483	-0.4861

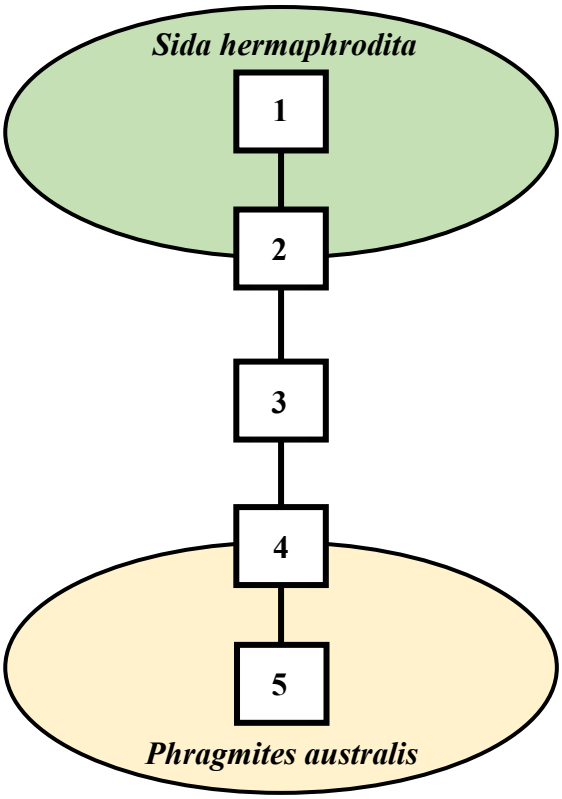

Figure 3.1: Experimental layout of each of the five transects where soil cores were collected. Each transect included five 1 m x 1 m quadrats placed in locations of different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands. The vegetation levels included locations of high-density *S. hermaphrodita* (1), moderately dense *S. hermaphrodita* (2), an area of intermediate vegetation (3), a location of moderately dense *P. australis* (4), and a location of high-density *P. australis* (5). Two soil cores were obtained from the corners of each quadrat and one additional soil core was obtained from the centre of each quadrat to examine soil nutrient contents.

Figure 3.2: Photographs taken at TCA of quadrats placed within locations of different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands. Five 1 m x 1 m quadrats were placed within locations of high-density *S. hermaphrodita* (A), moderately dense *S. hermaphrodita*, intermediate vegetation (B), moderately dense *P. australis* and high-density *P. australis* (C). Two soil cores were obtained using a split-core sampler from the corners of each quadrat to be subsequently sown with either *S. hermaphrodita* or *P. australis* seeds to assess the effects of potential soil alterations on plant performance. One additional soil core was obtained from the centre of each quadrat to examine soil nutrient contents.

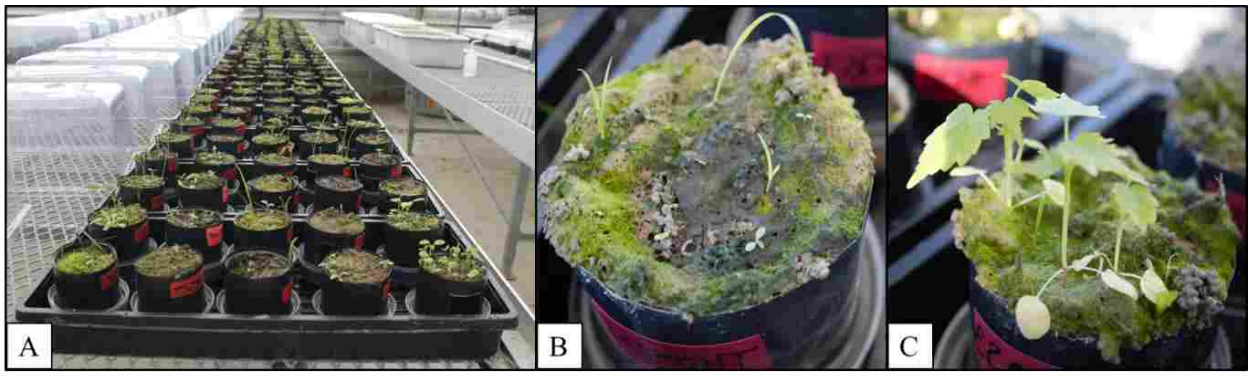

Figure 3.3: Experimental soil core set up in the rooftop greenhouse at the Waterloo Centre for Cold Regions and Water Science. 200 Soil cores were obtained from different locations between adjacent *S. hermaphrodita* and *P. australis* stands at TCA. Each core was individually wrapped in black polyethylene film and placed in polystyrene dome lids to serve as water basins (A). The two soil cores obtained from each location were sown with either 3 pre-germinated *P. australis* seeds (B) or 3 pre-germinated *S. hermaphrodita* seeds (C) in order to examine how plant performance is affected by potential alterations in soil conditions resulting from *P. australis* interactions. Plants were removed from soils following 12 weeks of growth and plant performance and AMF colonization measurements were taken.

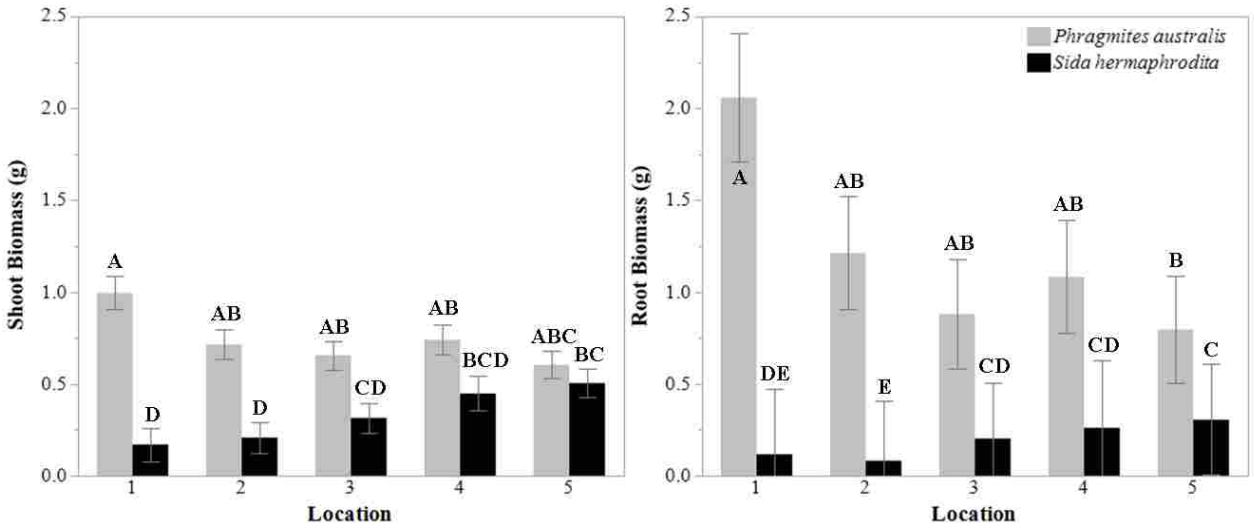

Figure 3.4: Plant performance of *S. hermaphrodita* (Black) and *P. australis* (Grey) seedlings planted in soils collected from different vegetation levels between adjacent *S. hermaphrodita* and *P. australis* stands. The vegetation levels included locations within dense *S. hermaphrodita* stands (1), moderately dense *S. hermaphrodita* (2), intermediate vegetation (3), moderately dense *P. australis* (4) and within dense *P. australis* stands (5). The interaction between plant species and the soil-vegetation level (location) and their effect on shoot biomass (left) and root biomass (right) as representatives of plant performance were assessed using a two-way analysis of variance (ANOVA). To meet ANOVA assumptions, square-root or log transformations were applied to shoot biomass or root biomass response variables respectively and differences in plant performance among species and across location levels were determined using Student's T multiple comparisons. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

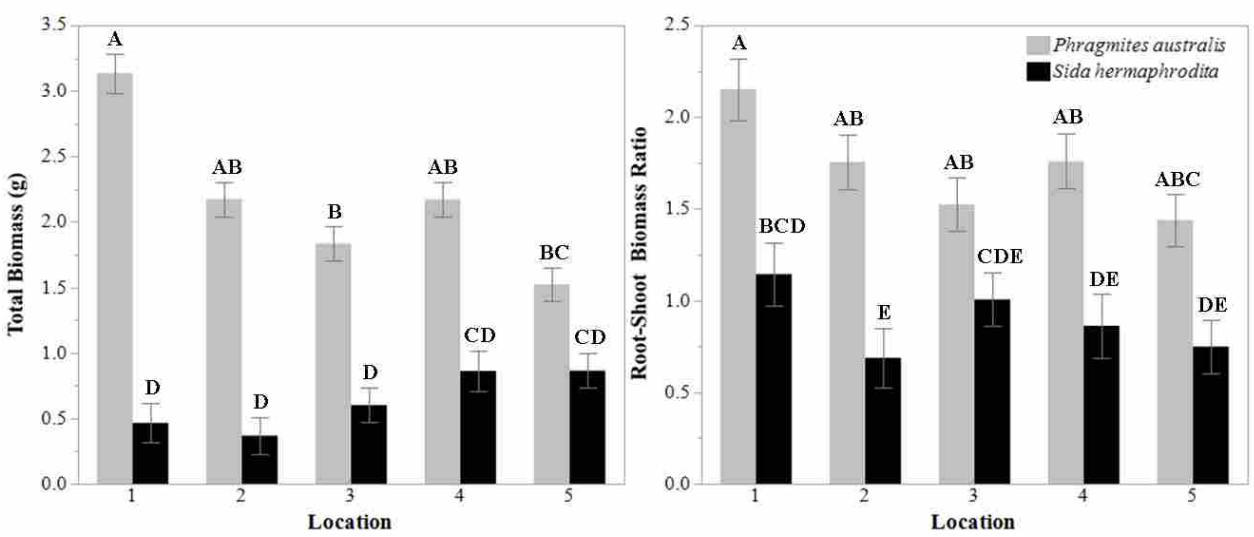

Figure 3.5: Plant performance of *S. hermaphrodita* (Black) and *P. australis* (Grey) seedlings planted in soils collected from different vegetation levels between adjacent *S. hermaphrodita* and *P. australis* stands. The vegetation levels included locations within dense *S. hermaphrodita* stands (1), moderately dense *S. hermaphrodita* (2), intermediate vegetation (3), moderately dense *P. australis* (4) and within dense *P. australis* stands (5). The interaction between plant species and the soil-vegetation level (location) and their effect on total biomass (left) and root - shoot biomass ratio (right) as representatives of plant performance were assessed using a two-way analysis of variance (ANOVA). To meet ANOVA assumptions, square-root or log transformations were applied to total biomass or root - shoot biomass ratio response variables respectively and differences in plant performance among species and across location levels were determined using Student's T multiple comparisons. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

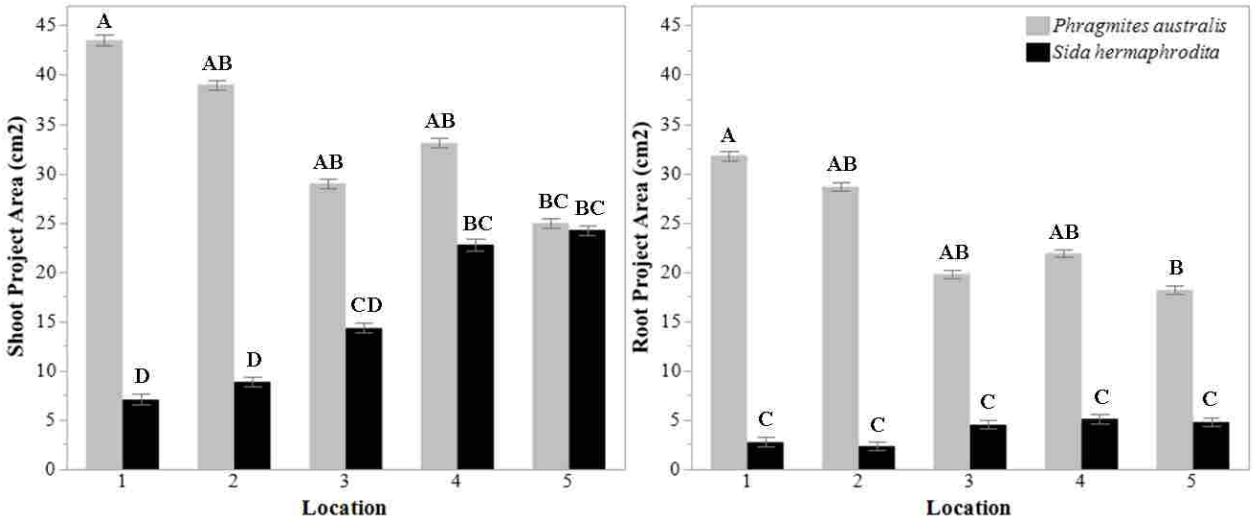

Figure 3.6: Plant performance of *S. hermaphrodita* (Black) and *P. australis* (Grey) seedlings planted in soils collected from different vegetation levels between adjacent *S. hermaphrodita* and *P. australis* stands. The vegetation levels included locations within dense *S. hermaphrodita* stands (1), moderately dense *S. hermaphrodita* (2), intermediate vegetation (3), moderately dense *P. australis* (4) and within dense *P. australis* stands (5). The interaction between plant species and the soil-vegetation level (location) and their effect on shoot project area (left) and root project area (right) as representatives of plant performance were assessed using a two-way analysis of variance (ANOVA). To meet ANOVA assumptions, square-root transformations were applied to response variables and differences in plant performance among species and across location levels were determined using Student's T multiple comparisons. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

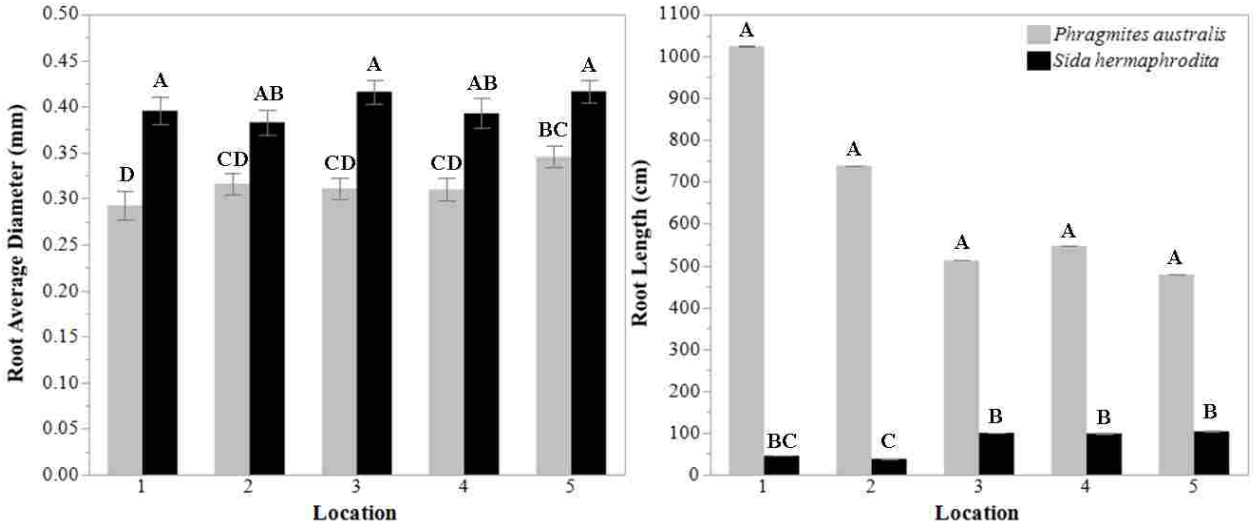

Figure 3.7: Plant performance of *S. hermaphrodita* (Black) and *P. australis* (Grey) seedlings planted in soils collected from different vegetation levels between adjacent *S. hermaphrodita* and *P. australis* stands. The vegetation levels included locations within dense *S. hermaphrodita* stands (1), moderately dense *S. hermaphrodita* (2), intermediate vegetation (3), moderately dense *P. australis* (4) and within dense *P. australis* stands (5). The interaction between plant species and the soil-vegetation level (location) and their effect on root average diameter (left) and root length (right) as representatives of plant performance were assessed using a two-way analysis of variance (ANOVA). To meet ANOVA assumptions, square-root or log transformations were applied to root average diameter or root length response variables respectively and differences in plant performance among species and across location levels were determined using Student's T multiple comparisons. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

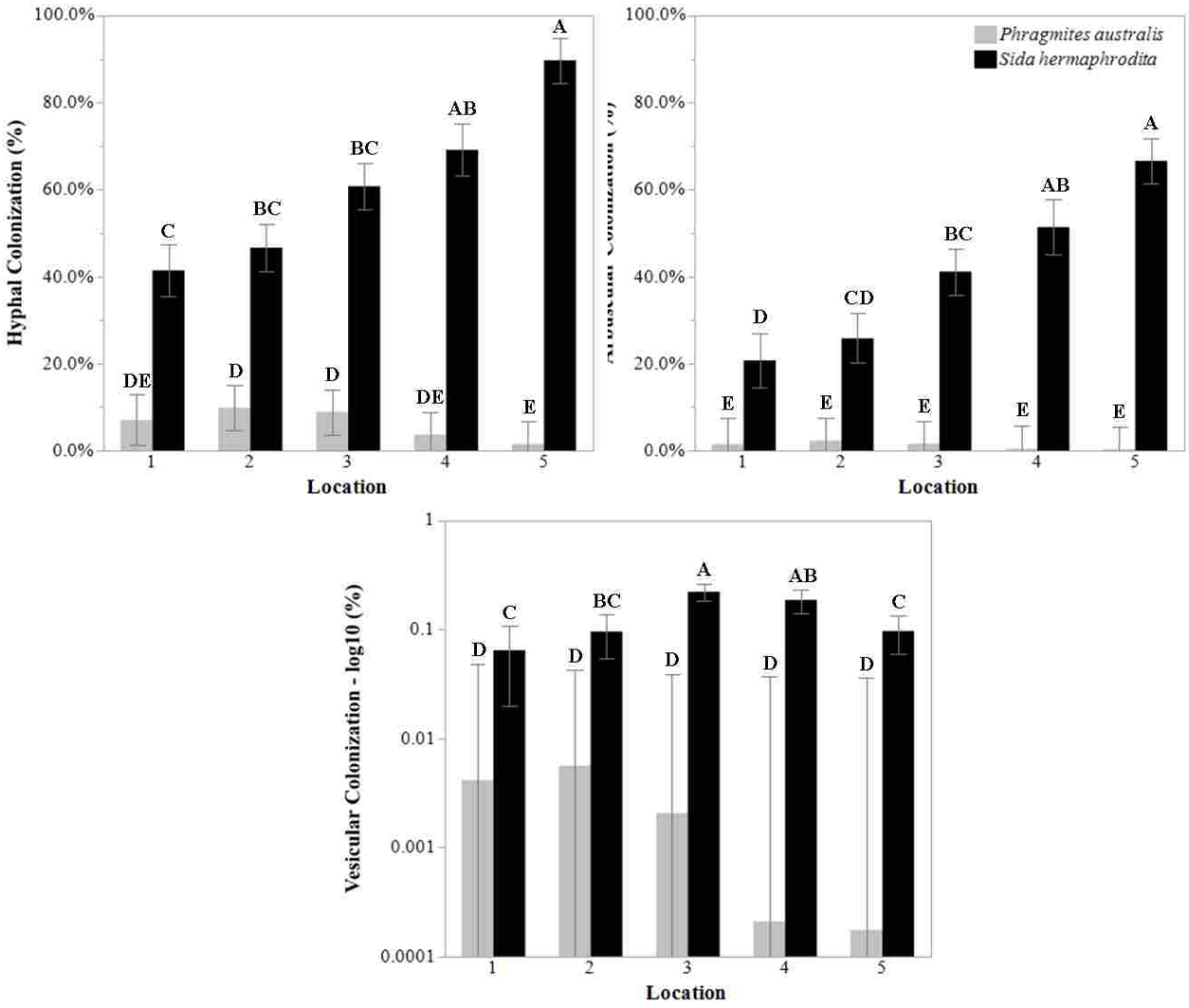

Figure 3.8: Root AMF colonization of *S. hermaphrodita* (Black) and *P. australis* (Grey) seedlings planted in soils collected from different vegetation levels between adjacent *S. hermaphrodita* and *P. australis* stands. The vegetation levels included locations within dense *S. hermaphrodita* stands (1), moderately dense *S. hermaphrodita* (2), intermediate vegetation (3), moderately dense *P. australis* (4) and within dense *P. australis* stands (5). The interaction between plant species and soil-vegetation level (location) and their effect on hyphal colonization (left), arbuscular colonization (right), and vesicular colonization(bottom) as representatives of root AMF colonization were assessed using a two-way analysis of variance (ANOVA). To meet ANOVA assumptions, square-root transformations were applied to response variables and differences in plant performance among species and across location levels were

determined using Student's T multiple comparisons. Vesicular colonization is presented on a log scale to better display low levels of root colonization in *P. australis* roots. Bars represent means ± standard error and bars with the same letters are not significantly different ($p < 0.05$).

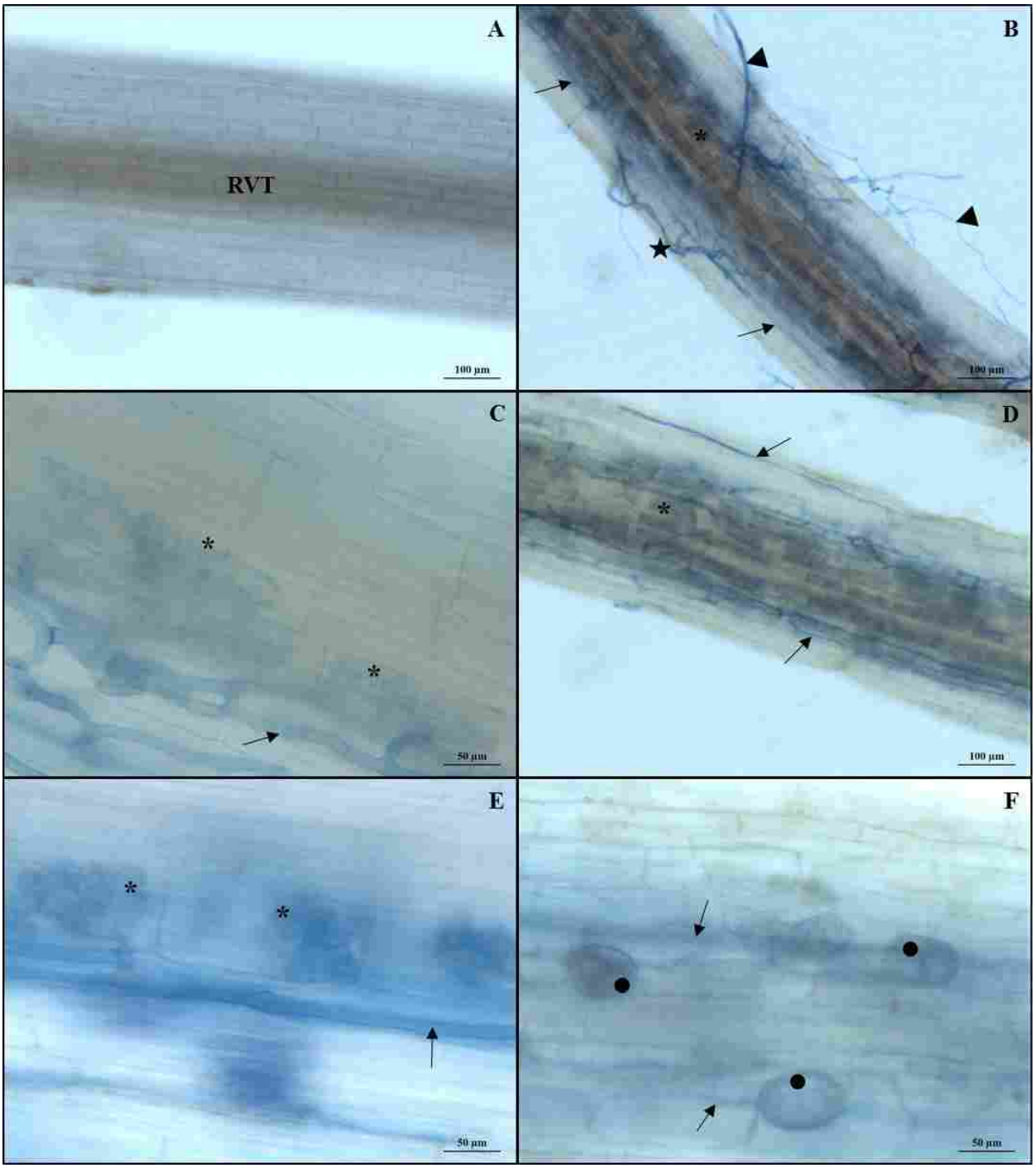

Figure 3.9: Cleared and stained roots of *S. hermaphrodita* grown in soils collected from within different vegetation categories between adjacent *S. hermaphrodita* stands and *P. australis* stands. Root samples were cleared in a 10% KOH solution, stained with a 5% ink in vinegar solution and AMF colonization was quantified using a modified version of the magnified intersections method (McGonigle et al., 1990)

A: Un-colonized area of a *S. hermaphrodita* root with the Root Vascular Tissue (RVT) running through the centre of the root. B-F: Areas of *S. hermaphrodita* roots colonized with blue stained AMF structures. B: Heavily colonized root section exhibiting extra-radicular hyphae (arrowhead) surrounding the root and an area where the hypha entered the epidermal cells (star) and formed intra-radicular hyphae (arrow) within the cortex of the root and numerous arbuscules (asterisk). C-E: Root sections exhibiting intra-radicular hyphae (arrow) within the cortical tissue, running parallel to the root vascular tissue and numerous arbuscules (asterisk). F: Root section exhibiting intra-radicular hyphae (arrow) and numerous vesicles (circle).

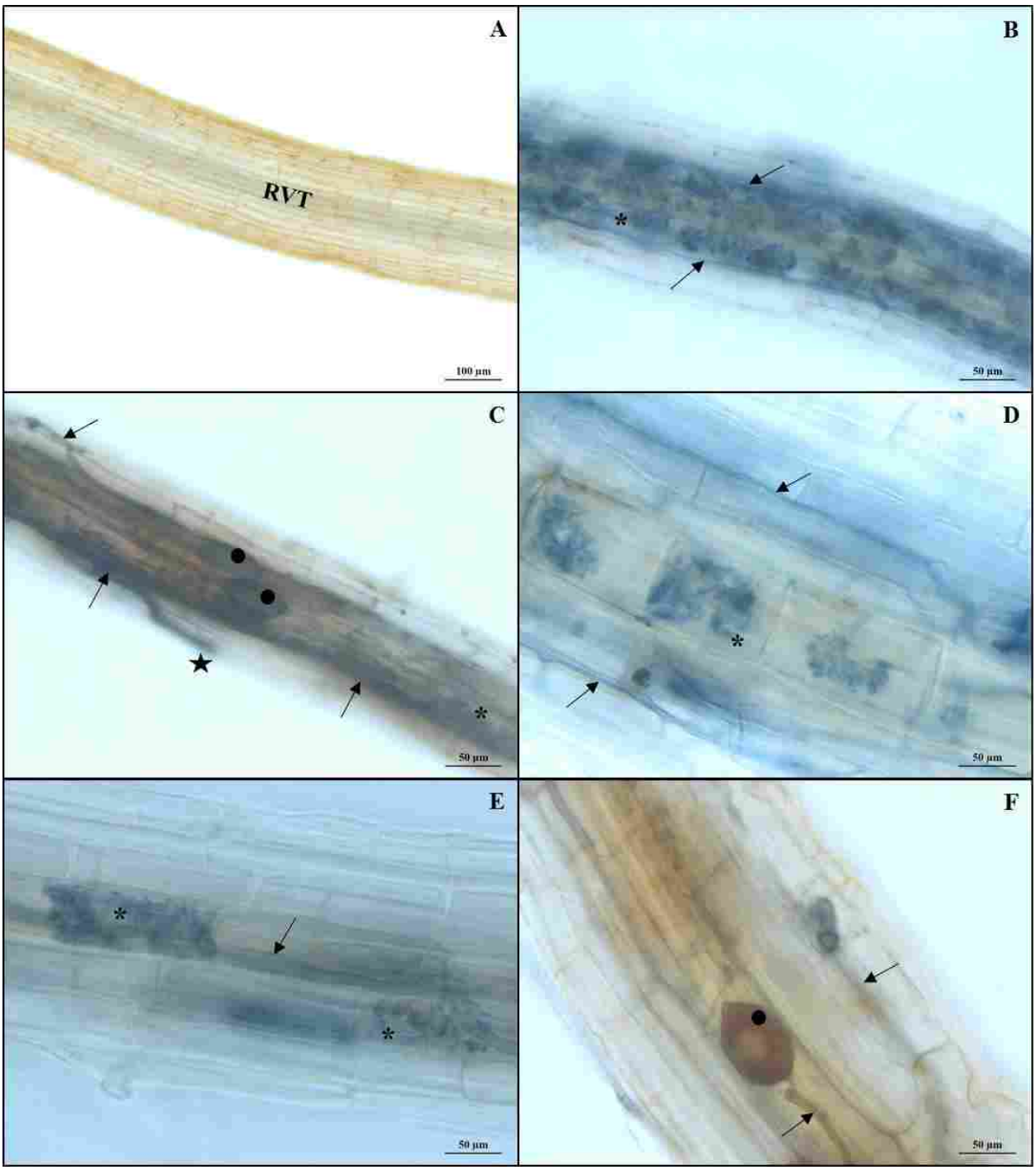

Figure 3.10: Cleared and stained roots of *P. australis* grown in soils collected from within different vegetation categories between adjacent *S. hermaphrodita* stands and *P. australis* stands. Root samples were cleared in a 10% KOH solution, stained with a 5% ink in vinegar solution and AMF colonization was quantified using a modified version of the magnified intersections method (McGonigle et al., 1990)

A: Un-colonized area of a *P. australis* root with the Root Vascular Tissue (RVT) running through the centre of the root. B-F: Areas of *P. australis* roots colonized with blue stained AMF structures. B: Heavily colonized root section exhibiting intra-radicular hyphae (arrow) within the cortex of the root and numerous arbuscules (asterisk). C: Root section exhibiting an area where the hypha entered the epidermal cells (star) and formed intra-radicular hyphae (arrow) within the cortex of the root and form numerous arbuscules (asterisk) and vesicles (circle). D-E: Magnified root sections exhibiting intra-radicular hyphae (arrow) within the cortical tissue, running parallel to the root vascular tissue and numerous arbuscules (asterisk). F: Root section exhibiting intra-radicular hyphae (arrow) and a vesicle (circle).

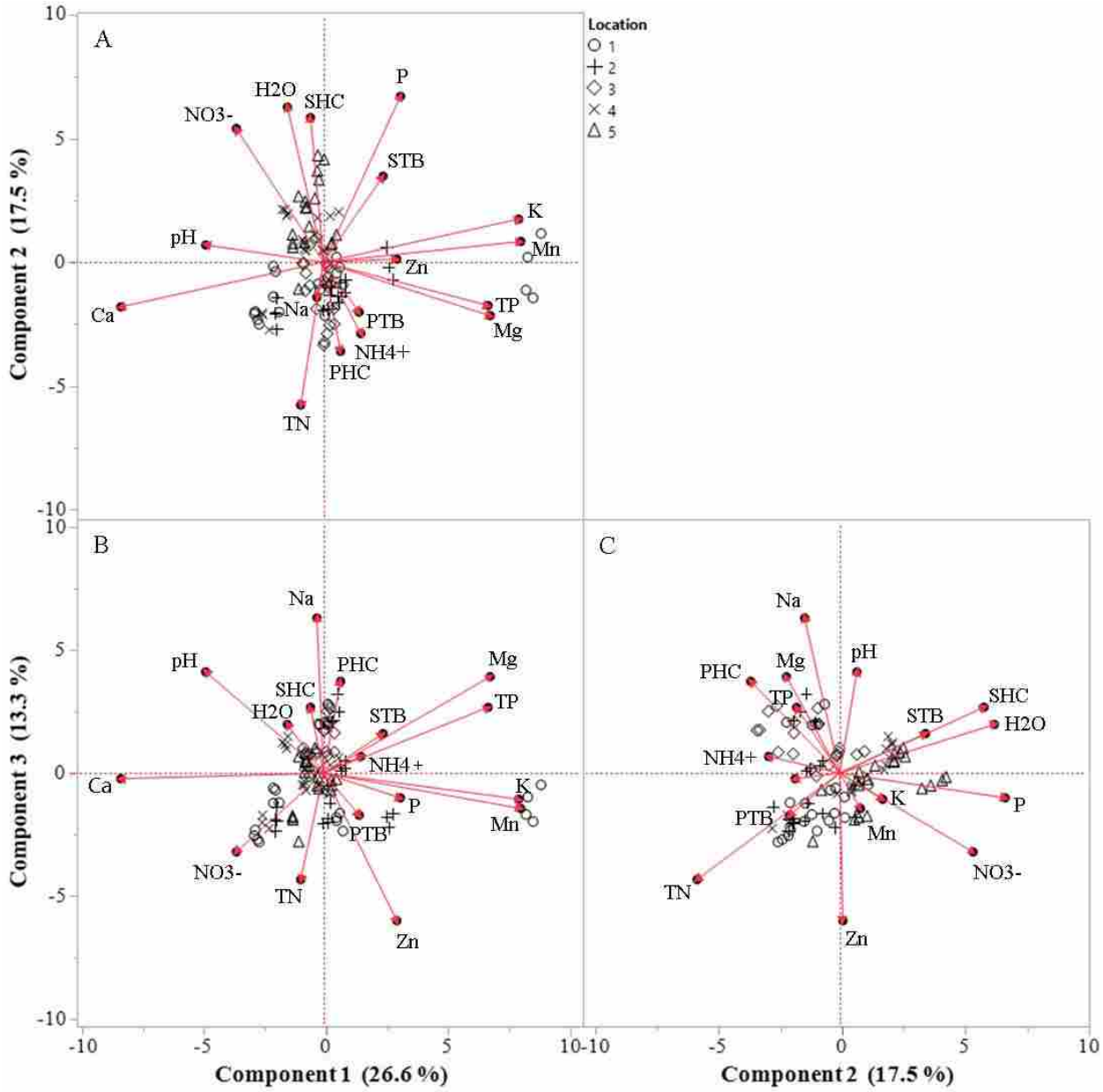

Figure 3.11: A: Bi-plot of principal component 1 against principal component 2 from PCA used to determine trends soil nutrient characteristics, average plant performance and AM root colonization of both *S. hermaphrodita* and *P. australis* plants. B: Bi-plot of principal component 1 against principal component 3. C: Bi-plot of principal component 2 against principal component 3. Amount of variation explained by each component is indicated on each axis. PC1 explained a gradient in soil characteristics: increasing Magnesium (Mg), Potassium (K), Manganese (Mn), total Phosphorus (TP), and decreasing soil

pH and Calcium (Ca). PC 2 explained a gradient in soil nutrient characteristics related to plant performance and AM colonization: increasing *S. hermaphrodita* total biomass (STB), *S. hermaphrodita* root hyphal colonization (SHC), soil moisture (H_2O), available Phosphorus (P), Nitrate (NO_3-), and decreasing *P. australis* total biomass (PTB), *P. australis* hyphal colonization (PHC), Ammonium (NH_4+), and total Nitrogen (TN). PC 3 explained a gradient in the remaining soil nutrient characteristics: increasing Sodium (Na) and decreasing Zinc (Zn).

3.7 Supplemental Information

Table S3.1: Classification of all species identified within the first quadrat of each of the five transects set up at TCA where soil was to be obtained within a high-density *S. hermaphrodita* stand. Plants were identified using the species key by Dickinson et al. (2004). Status as introduced (I) or native (N), and physiognomy (Phys) was determined from the USDA PLANTS database. Percent vegetation cover (PVC) of each species identified within the 1 m x 1 m quadrat is presented.

Transect	Family	Genus, Species	Common Name	I/N	Phys	PVC
1	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	20%
	Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	20%
	Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Garlic Mustard	I	Forb/Herb	5%
2	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	15%
	Juglandaceae	*Juglans nigra* L.	Black Walnut	N	Tree	<5%
3	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	20%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	<5%
4	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	30%
	Asteraceae	*Cirsium arvense* (L.) Scop.	Creeping Thistle	I	Forb/Herb	<5%
5	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	20%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	10%
	Asteraceae	*Cirsium arvense* (L.) Scop.	Creeping Thistle	I	Forb/Herb	<5%

Table S3.2: Classification of all species identified within the second quadrat of each of the five transects set up at TCA where soil was to be obtained within a moderate-density *S. hermaphrodita* stand. Plants were identified using the species key by Dickinson et al. (2004). Status as introduced (I) or native (N), and physiognomy (Phys) was determined from the USDA PLANTS database. Percent vegetation cover (PVC) of each species identified within the 1 m x 1 m quadrat is presented. Unidentified species are indicated by UN.

Transect	Family	Genus, Species	Common Name	I/N	Phys	PVC
1	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	5%
	Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	70%
	Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Garlic Mustard	I	Forb/Herb	<5%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	<5%
	Asteraceae	*Arctium minus* Bernh.	Lesser Burdock	I	Forb/Herb	10%
	Rosaceae	*Geum urbanum* L.	Avens	I	Forb/Herb	5%
2	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	10%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	30%
3	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	5%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	30%
	Asteraceae	*Sonchus arvensis* L.	Field Sow Thistle	I	Forb/Herb	<5%
	Asteraceae	*Tussilago farfara* L.	Coltsfoot	I	Forb/Herb	5%
	Juglandaceae	*Juglans nigra* L.	Black Walnut	N	Tree	<5%
4	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	10%
	Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	20%
	Asteraceae	*Symphyotrichum lanceolatum* (Willd.) G.L. Nesom	White Panicle Aster	N	Forb/Herb	<5%
5	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	10%
	Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	20%

	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	30%
	Rosaceae	*Geum urbanum* L.	Avens	I	Forb/Herb	5%
	Asteraceae	*Cirsium arvense* (L.) Scop.	Creeping Thistle	I	Forb/Herb	<5%
	Dipsacaceae	*Dipsacus fullonum* L.	Fuller's Teasel	I	Forb/Herb	<5%
	Asteraceae	*Taraxacum officinale* F.H. Wigg.	Common Dandelion	I/N	Forb/Herb	<5%
	Salicaceae	Salix sp.	UN	UN	Tree	<5%

Table S3.3: Classification of all species identified within the third quadrat of each of the five transects set up at TCA where soil was to be obtained within an area of intermediate vegetation. Plants were identified using the species key by Dickinson et al. (2004). Status as introduced (I) or native (N), and physiognomy (Phys) was determined from the USDA PLANTS database. Percent vegetation cover (PVC) of each species identified within the 1 m x 1 m quadrat is presented. Unidentified species are indicated by UN.

Transect	Family	Genus, Species	Common Name	I/N	Phys	PVC
1	Malvaceae	*Sida hermaphrodita* (L.) Rusby	Virginia Mallow	N	Forb/Herb	<5%
	Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	20%
	Asteraceae	*Arctium minus* Bernh.	Lesser Burdock	I	Forb/Herb	<5%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	60%
	Apiaceae	*Daucus carota* L.	Queen Anne's Lace	I	Forb/Herb	<5%
	Asteraceae	*Tussilago farfara* L.	Coltsfoot	I	Forb/Herb	10%
	Asteraceae	*Sonchus arvensis* L.	Field Sow Thistle	I	Forb/Herb	<5%
2	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	70%
3	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	15%
	Apiaceae	*Daucus carota* L.	Queen Anne's Lace	I	Forb/Herb	15%
	Asteraceae	*Sonchus arvensis* L.	Field Sow Thistle	I	Forb/Herb	5%
	Rosaceae	*Geum urbanum* L.	Avens	I	Forb/Herb	30%
	Asteraceae	*Symphyotrichum novae-angliae* (L.) G.L. Nesom	New England Aster	N	Forb/Herb	<5%
	Asteraceae	*Symphyotrichum lanceolatum* (Willd.) G.L. Nesom	White Panicle Aster	N	Forb/Herb	<5%
	Poaceae	Poaceae sp.	UN	UN	Graminoid	70%
4	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	50%
	Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Garlic Mustard	I	Forb/Herb	15%
5	Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	5%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	10%
	Asteraceae	*Symphyotrichum novae-angliae* (L.) G.L. Nesom	New England Aster	N	Forb/Herb	<5%

	Poaceae	Poaceae sp.	UN	UN	Graminoid	80%
	Salicaceae	Salix sp.	UN	UN	Tree	5%

Table S3.4: Classification of all species identified within the fourth quadrat of each of the five transects set up at TCA where soil was to be obtained within a moderate-density *P. australis* stand. Plants were identified using the species key by Dickinson et al. (2004). Status as introduced (I) or native (N), and physiognomy (Phys) was determined from the USDA PLANTS database. Percent vegetation cover (PVC) of each species identified within the 1 m x 1 m quadrat is presented.

Transect	Family	Genus, Species	Common Name	I/N	Phys	PVC
1	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	10%
	Poaceae	*Phalaris arundinacea* L.	Reed Canary Grass	N	Graminoid	10%
	Asteraceae	*Arctium minus* Bernh.	Lesser Burdock	I	Forb/Herb	40%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	20%
	Asteraceae	*Tussilago farfara* L.	Coltsfoot	I	Forb/Herb	15%
2	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	10%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	60%
	Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Garlic Mustard	I	Forb/Herb	30%
3	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	30%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	30%
4	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	10%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	30%
	Asteraceae	*Symphyotrichum lanceolatum* (Willd.) G.L. Nesom	White Panicle Aster	N	Forb/Herb	<5%
5	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	60%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	10%
	Dipsacaceae	*Dipsacus fullonum* L.	Fuller's Teasel	I	Forb/Herb	10%

Table S3.5: Classification of all species identified within the fifth quadrat of each of the five transects set up at TCA where soil was to be obtained within a high-density *P. australis* stand. Plants were identified using the species key by Dickinson et al. (2004). Status as introduced (I) or native (N), and physiognomy (Phys) was determined from the USDA PLANTS database. Percent vegetation cover (PVC) of each species identified within the 1 m x 1 m quadrat is presented.

Transect	Family	Genus, Species	Common Name	I/N	Phys	PVC
1	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	30%
	Asteraceae	*Arctium minus* Bernh.	Lesser Burdock	I	Forb/Herb	<5%
	Asteraceae	*Solidago canadensis* L.	Canadian Goldenrod	N	Forb/Herb	20%
2	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	70%
3	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	80%
	Balsaminaceae	*Impatiens capensis* Meerb.	Spotted Touch-Me-Not	N	Forb/Herb	<5%
4	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	90%
	Brassicaceae	*Alliaria petiolata* (M. Bieb.) Cavara & Grande	Garlic Mustard	I	Forb/Herb	10%
5	Poaceae	*Phragmites australis* (Cav.) Trin. Ex Steud.	Common Reed	I	Graminoid	90%
	Balsaminaceae	*Impatiens capensis* Meerb.	Spotted Touch-Me-Not	N	Forb/Herb	<5%

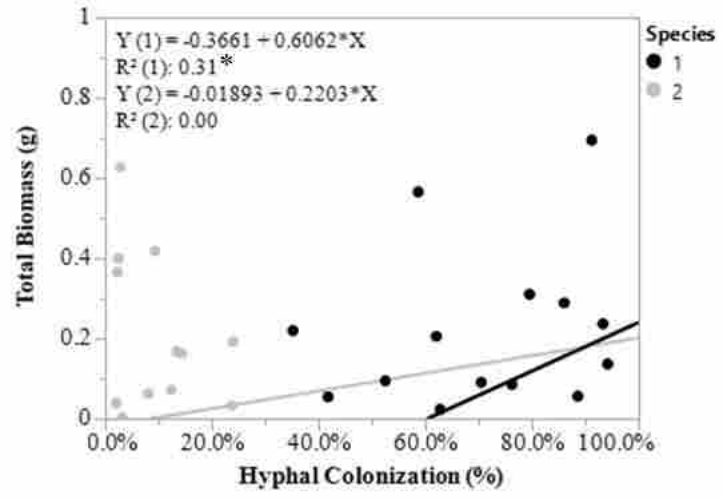

Figure S3.1: Linear regression to assess relationship between root hyphal colonization and total biomass of *S. hermaphrodita* (Species 1) and *P. australis* (Species 2) plants grown in soils collected within different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands. Asterisk (*) indicate significant correlation ($p < 0.01$).

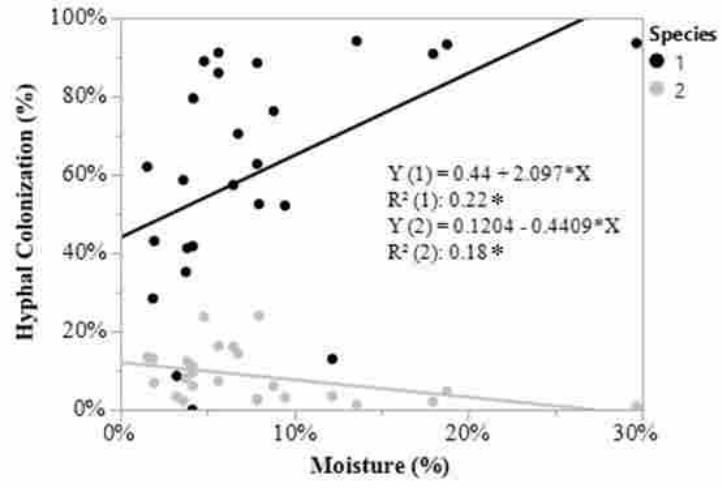

Figure S3.2: Linear regression to assess relationship between soil moisture and root hyphal colonization of *S. hermaphrodita* (Species 1) and *P. australis* (Species 2) plants grown in soils collected within different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands. Asterisk (*) indicate significant correlation ($p < 0.05$).

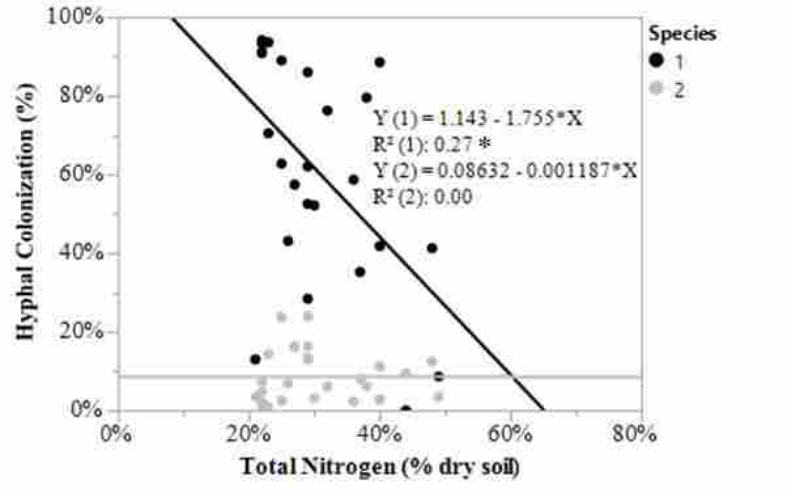

Figure S3.3: Linear regression to assess relationship between soil total nitrogen content and root hyphal colonization of *S. hermaphrodita* (Species 1) and *P. australis* (Species 2) plants grown in soils collected within different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands. Asterisk (*) indicate significant correlation ($p < 0.01$).

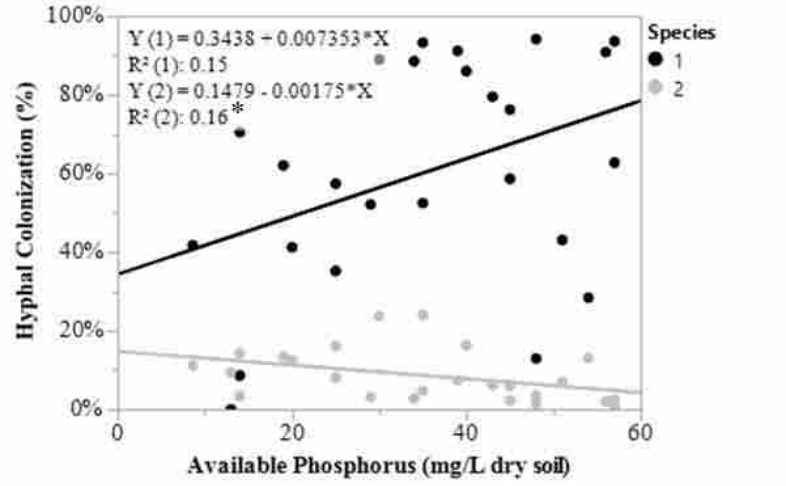

Figure S3.4: Linear regression to assess relationship between soil available phosphorus content and root hyphal colonization of *S. hermaphrodita* (Species 1) and *P. australis* (Species 2) plants grown in soils collected within different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands. Asterisk (*) indicate significant correlation ($p < 0.05$).

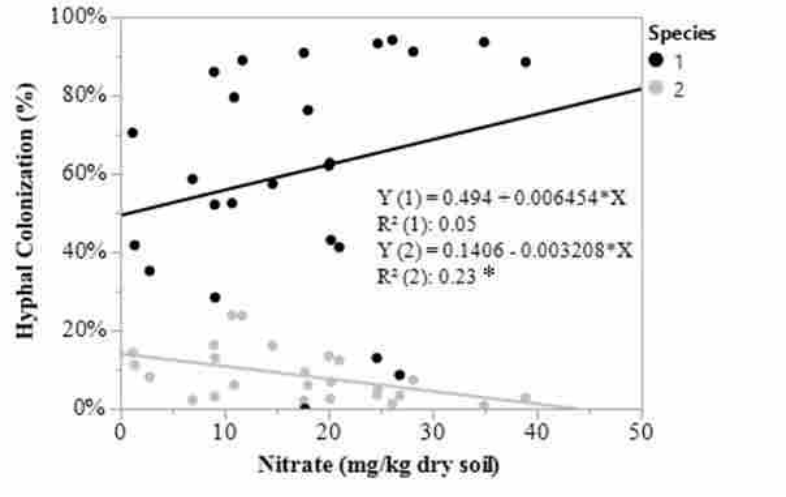

Figure S3.5: Linear regression to assess relationship between soil nitrate content and root hyphal colonization of *S. hermaphrodita* (Species 1) and *P. australis* (Species 2) plants grown in soils collected within different vegetation categories between adjacent *S. hermaphrodita* and *P. australis* stands. Asterisk (*) indicate significant correlation ($p < 0.05$).

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Chapter 4: General discussion

4.1 Summary of main research findings

The overarching goal of this study was to examine the reciprocal interaction between the endangered *S. hermaphrodita* and the invasive *P. australis* in order to determine how each species impacts the performance of the other. The specific objectives were to (1) determine how seedling performance and AMF root colonization of *S. hermaphrodita* in the field relates to the presence/absence of *P. australis*; (2) determine how putative chemical compounds and microorganisms present within the soils associated with *S. hermaphrodita* and *P. australis* affect the performance and mycorrhizal colonization of both plants. To complete this study, field surveys were performed during the growing seasons of 2016, 2017, and 2018 at the Taquanyah Conservation Area (TCA) in Haldimand County ON, and a greenhouse study was conducted in 2017-2018 at the Centre for Cold Regions and Water Science in Waterloo ON. The main findings were as follows:

1) During field assessments, both *S. hermaphrodita* seedling emergence and mortality increased significantly ($p < 0.001$) throughout the three years of sampling. The change in *P. australis* proximity level had anomalous effects on *S. hermaphrodita* seedling performance, since average *S. hermaphrodita* seedling emergence in the intermediate *P. australis* proximity level was significantly higher ($p < 0.05$) than the average seedling emergence in the close and far proximity levels. However, change in *P. australis* proximity level did not have a significant effect on *S. hermaphrodita* seedling mortality.

   Symbioses between *S. hermaphrodita* and arbuscular mycorrhizal fungi was confirmed in 2016, however, *P. australis* proximity did not have any significant effects on the various measures of *S. hermaphrodita* seedling AMF colonization during the assessments completed in 2016 and 2018.
2) During the greenhouse study, both *S. hermaphrodita's* and *P. australis'* performance differed significantly ($p < 0.0001$), in which in general, *P. australis* significantly outperformed *S.*

hermaphrodita. However, the magnitude of the difference between species depended on the location. Significant reductions ($p < 0.05$) in in general plant performance between pure *S. hermaphrodita* soils and pure *P. australis* soils were observed for *P. australis* plants whereas general plant performance was improved for *S. hermaphrodita* plants.

Symbioses between AMF and both species were observed, and levels of AM colonization were significantly higher ($p < 0.0001$) in *S. hermaphrodita* roots than in *P. australis* roots. Similarly, the magnitude of the difference between species depended on the location, in which the general proportion of AM colonization in *S. hermaphrodita* roots was significantly increased ($p < 0.0001$) between pure *S. hermaphrodita* soils and pure *P. australis* soils, while the proportion of AM colonization in *P. australis* roots was reduced.

Following soil nutrient analyses, positive correlations between *S. hermaphrodita* AM colonization, total biomass, and soil moisture were observed. Negative correlations between *S. hermaphrodita* AM colonization and total nitrogen content were also observed in addition to negative correlations between *P. australis* AM colonization, soil moisture, available phosphorus and soil nitrate content.

4.2 Main conclusions

Phragmites australis has been labelled a highly competitive species that is threatening ecosystems around the world, and its invasion has been attributed, among others, to its ability to allelopathically condition soils to interfere with neighbouring plant performance and their beneficial microbiotic symbioses (Rudrappa et al., 2007; Jordan et al., 2008; Uddin et al., 2012; Uddin et al., 2014; Uddin et al., 2017; Crocker et al., 2017). Through our results quantifying its interaction with the endangered *Sida hermaphrodita*, we found no evidence for *P. australis'* allelopathic soil conditioning capacity, since *S. hermaphrodita* seedling germination and emergence was improved in closer proximities to *P. australis* stands. In fact, *S. hermaphrodita* plants were shown to grow better in soils extracted from pure *P. australis* stands than in soils retrieved from within their own stands.

Our research provided the first empirical evidence that *S. hermaphrodita* can form relationships with arbuscular mycorrhizal fungi and interestingly, *S. hermaphrodita* AMF root colonization was also promoted in pure *P. australis* soils. Additionally, our results contribute to the growing research confirming that *P. australis* can also form symbioses with AMF and that its mycorrhization is negatively correlated with moisture levels (Oliveira et al., 2001).

Due to the observed inverse promotion of plant performance and AMF colonization of both species in their competitor's soil, coupled with soil nutrient analyses, we believe *P. australis* is not as strictly competitive as previously suggested. Under competitive interactions, where there is low abiotic stress, both species would rapidly acquire any available resources to outcompete the other (Bertness and Callaway, 1994; Callaway and Walker 1997; He et al., 2013). The improved performance results of both species in our greenhouse study reflect instead traits associated with a belowground facilitative interaction between *P. australis* and *S. hermaphrodita*. During this study, both species appeared to facilitate limiting soil nutrient availability for each other and subsequently promote the growth of their opponent to potentially alleviate the pressures of an unknown abiotic stress present at TCA. We suspect it is because of this unknown stress, that there has been an increase in *S. hermaphrodita* population size and density over the past 6 years, since *P. australis* has not been vigorous enough to fully displace *S. hermaphrodita,* resulting in their co-existence at TCA.

Although our results suggest *S. hermaphrodita* and *P. australis* are coexisting, and that belowground conditions are not excluding neighbouring species, we believe changes to aboveground conditions are primarily responsible for limiting *S. hermaphrodita's* performance. Due to the observed high seedling mortality throughout TCA and the improved *S. hermaphrodita* performance in *P. australis* soils where aboveground competition was absent, we can infer that interception of light by other neighbouring species is the most detrimental factor impacting *S. hermaphrodita* seedling growth and the subsequent dispersal of this endangered species. Furthermore, we propose that this competitive exclusion of light is the key mechanism responsible for *P. australis'* invasion success.

Through this research we contribute to an improved understanding of the biology and ecology related to both *S. hermaphrodita* and *P. australis* and provide valuable insight into factors impacting the outcome of interactions between species. Our research has proved that *S. hermaphrodita* has the capacity to increase its distribution, and by defining *S. hermaphrodita's* aboveground threat, we can make suggestions for conservation efforts (see below) to mediate this threat and help promote the further vegetative and seedling expansion of *S. hermaphrodita*, in hopes of restoring its native distribution previously taken over by invasive species like *P. australis*.

4.3 Integration and collaboration

The field of ecology is inherently integrative as it overlaps with different fields of biology and draws on techniques from other branches of science to examine and explain life processes, the abundance, distribution and adaptations of different organisms as well as the interactions between different organisms and their biotic and abiotic environments. In doing so, ecologists seek to understand how all these interactions impact the dynamic functioning of ecosystems on a much larger scale. As an ecology study, this research required an integrative approach since it encompassed aspects of botany, mycology, microbiology, soil chemistry, and conservation biology. Through the combination of each field, the predominant goal of this research was to explain the interaction between two organisms; *Sida hermaphrodita* and *Phragmites australis*. Since *S. hermaphrodita* and *P. australis* are not the sole inhabitants of TCA, it was necessary that other factors and relationships were included within the scope of this project. Additional abiotic factors including light availability and soil characteristics as well as biotic factors including interactions with arbuscular mycorrhizal fungi and other plant species were taken into consideration in order to fully explain the reciprocal interaction between both species. The integrative approach of this ecological research has helped improve the understanding of the life processes of both species and define the key aboveground detrimental impacts responsible for the success of invasive species like *P. australis*. Furthermore, our findings provide us with direction that can be applied to conservation initiatives used to mitigate the negative impacts of invasive species and restore the native

biodiversity of natural ecosystems like TCA and other areas where native species like *S. hermaphrodita* are threatened by *P. australis* invasion.

Throughout the completion of this project, this research has also incorporated information obtained by other researchers and has involved several valuable collaborations. This work has been part of an ongoing Species at Risk (SAR) research project for the Ministry of Natural Resources and Forestry (MNRF) involving monitoring, protecting and managing the *S. hermaphrodita* population at TCA. Dominic Smoluch, a previous researcher for Dr. Mihai Costea and Dr. Kevin Stevens in the biology department at Wilfrid Laurier University, was the first to begin examining plant community structure at TCA in 2014. Some of Dominic's field work at TCA guided the sampling design of the field work in this project since in 2014, he established the 28 permanent 1 m x 1 m quadrats at various locations surrounding existing *S. hermaphrodita* stands, that were surveyed yearly during this study for vegetation cover and to examine *S. hermaphrodita* seedling performance in relation to *P. australis* proximity. Additionally, he also helped establish the GPS protocol for delimiting the boundaries of existing *S. hermaphrodita* stands and estimating the total stem density of *S. hermaphrodita* at TCA. We used his results from the 2014 boundary delimitation and stem density estimates to compare with our estimates collected in 2016 and 2018 to describe the *S. hermaphrodita* population growth at TCA (Chapter 2).

Through this SAR project, our research has involved the collaboration with Dr. Rebecca Rooney (Assistant Professor, Department of Biology, University of Waterloo) and a member of her wetland ecology lab, Courtney Robichaud (PhD. Candidate, Department of Biology, University of Waterloo). Through Dr. Rooney's support of invasive species management and SAR protection, they examined the carbon assimilation and leaf nutrients of both *S. hermaphrodita* and *P. australis* to assess how both species may impact the photosynthetic capacity of one another. As previously mentioned, their results complement the light interference assumption from our research and will be later combined into a manuscript.

Throughout the duration of this SAR study, continuous collaboration has also been necessary between the Dr. Stevens and Dr. Costea labs and associates at the Grand River Conservation Authority (GRCA) including Anthony Zammit (Aquatic and Terrestrial Ecologist, GRCA) and Lindsay Campbell (Restoration Specialist, GRCA). Access to perform field assessments at TCA was made possible through them and they provided insight into any potential impacts on plant populations resulting from human disturbance (e.g. cutting, spraying, A.T.V. trampling) or animal disturbance (e.g. flooding ensuing from beaver dams). Our results from yearly monitoring of plant community structure and *S. hermaphrodita* population density have been shared to provide updates to changes in habitat characteristics and SAR abundance, and subsequently assist with determining any need for population management on the GRCA property. Additionally, reports have been submitted to the MNRF including data collected by GRCA collaborators and the results obtained from our research. Ongoing research in our labs has focussed on examining different SAR including *S. hermaphrodita* and *Ammannia robusta* Heer & Regel (Scarlet Ammannia; Lythraceae), and through collaboration with MNRF associates like Dr. Eric Snyder (Plant SAR Specialist, Ministry of Environment, Conservation and Parks), our research has addressed knowledge gaps surrounding these SAR and impacts from invasive species, and also contributed to meeting recovery actions outlined by Government Response Statements.

4.4 Future directions and conservation implications

Chapter 1 addressed the need for expanding the knowledge surrounding the endangered *S. hermaphrodita* and the invasive *P. australis* in order to define the interaction between both species, so that appropriate management practices may be undertaken. Our research examined the belowground soil conditioning potential of *P. australis* in order to verify previous reports that it can allelopathically inhibit neighbouring species (Rudrappa et al., 2007; Bains et al., 2009; Uddin et al., 2014; Crocker et al., 2017). The results presented in both chapters 2 and 3 provided no evidence to corroborate our initial soil modification hypothesis; however, additional chemical analysis of soils collected at TCA would be valuable to further substantiate our results. Since previous studies have reported the presence of

allelopathic phenolic compounds within *P. australis* soils (Rudrappa et al., 2007; Rudrappa et al., 2009; Uddin et al., 2012; Uddin et al., 2014), it would be beneficial to perform further assessments of TCA soils recently extracted from *P. australis* stands as well as soils kept under frozen storage. By determining whether phenolic compounds are in fact absent, this analysis would help confirm our results that belowground allelopathic modification is not the principal mechanism responsible for *P. australis* invasion.

Also, through our assessments of relationships between both plant species and beneficial AMF, our results support the limited information regarding *P. australis'* mycorrhizal status (Oliveira et al., 2001). As for *S. hermaphrodita* however, the consistently high levels of AMF colonization observed in its roots provide incentive for further investigation. As discussed in chapter 3, our *S. hermaphrodita* colonization results do not coincide with other frequently observed AMF relationships (Khan, 1975; Harley and Smith, 1987; Bolan, 1991) since colonization levels were positively correlated with soil moisture and available phosphorous levels. Furthermore, because we observed positive correlations between AMF root colonization and *S. hermaphrodita* performance, we suspect that this AMF interaction is not parasitic. However, due to the complexity of AMF associations and the absence of a sterile control treatment from our study, we could not determine the stability of *S. hermaphrodita's* AMF relationship. Further inspection into *S. hermaphrodita's* performance with and without AMF colonization as well as the response to different levels of soil fertility, would be beneficial in expanding the knowledge on *S. hermaphrodita's* AMF reliance and the conditions that best support its growth.

Additionally, based on the performance results observed throughout our study, we postulated that *P. australis* and *S. hermaphrodita* may engage in a belowground facilitative interaction whereby limiting nutrients are shared between both species to alleviate the pressures of an unknown abiotic stressor at TCA. Although our study provides support for the stress-gradient hypothesis which states that gradients in the abiotic environment can impact the balance between competition and facilitation in species interactions (Callaway and Walker, 1997; Callaway, 1998), unfortunately our study was not designed to

specifically test this hypothesis (Maestre et al., 2009). To validate our results, a re-evaluation of the interaction between *S. hermaphrodita* and *P. australis* would be beneficial in which all abiotic environmental conditions at TCA such as light intensity, water availability, salinity, soil temperature and air temperature are also accounted for. By incorporating additional abiotic stress gradients into the study, we would be able to further characterize the environmental conditions at TCA, identify the unknown source of severe abiotic stress and either confirm that this stressor is impacting the outcome of the belowground facilitative interaction observed between *S. hermaphrodita* and *P. australis* or determine whether coexistence through frequency dependent selection (Chesson 2000; HilleRisLambers et al., 2010) may be taking place between these two species at TCA.

Lastly, the main outcome of our research has emphasized a need for examining aboveground competition at TCA. Our research has outlined that *P. australis'* ability to monopolize light is probably key to its invasion success and that competitive exclusion of light is likely the main factor impeding *S. hermaphrodita's* performance. Based on our results, we recommend that additional examination into aboveground competition for light would be the most valuable in explaining the interactions between *S. hermaphrodita* and invasive species like *P. australis* as well as establishing appropriate management practices to combat these unfavourable interactions. Focussing on *P. australis* specifically, various control methods have been suggested including mowing, burning, drainage, and herbicide application (Mal and Narine, 2004). Derr (2008), identified that mowing and chemical application of the herbicide glyphosate (N-(Phosphonomethyl)glycine) was effective in reducing *P. australis* populations by approximately 90% one year after the application (Derr, 2008). In the fall of 2018, a similar management treatment of trampling and herbicide spraying was applied by GRCA land managers to *P. australis* stands surrounding *S. hermaphrodita* stands at TCA. Although vegetation surveys and population estimates have not been completed this year to confirm, the regrowth of *P. australis* stems this growing season was surprisingly limited (Figure 4.1). Through personal observation, it was apparent that areas surrounding the cold-water stream that were densely populated by *P. australis* monocultures last year, were now

occupied by other species including *Solidago canadensis* L., *Impatiens capensis* Meerb., and *Cirsium arvense* (L.) Scop. (Figure 4.2). Additionally, *S. hermaphrodita* growth was also evident by the appearance of new vegetative shoots and seedlings in areas previously occupied by *P. australis* (Figure 4.3). This new growth outside of *S. hermaphrodita* stands appears very promising for the recovery of *S. hermaphrodita* populations and we believe the continued culling of *P. australis* stands would be beneficial in improving the distribution of this endangered species. By continuously applying these management techniques to areas occupied by invasive species like *P. australis*, in addition to removing the dead litter resulting from the treatments, the positive feedbacks exploited by monoculture-forming invasive species would be disrupted and light availability would be substantially improved for native species and their seedlings (Holdredge and Bertness, 2011). Furthermore, counteracting the aboveground effects of invaders like *P. australis* would be beneficial in the global recovery of endangered species like *S. hermaphrodita* and the restoration of native biodiversity into invaded ecosystems.

4.5 Figures

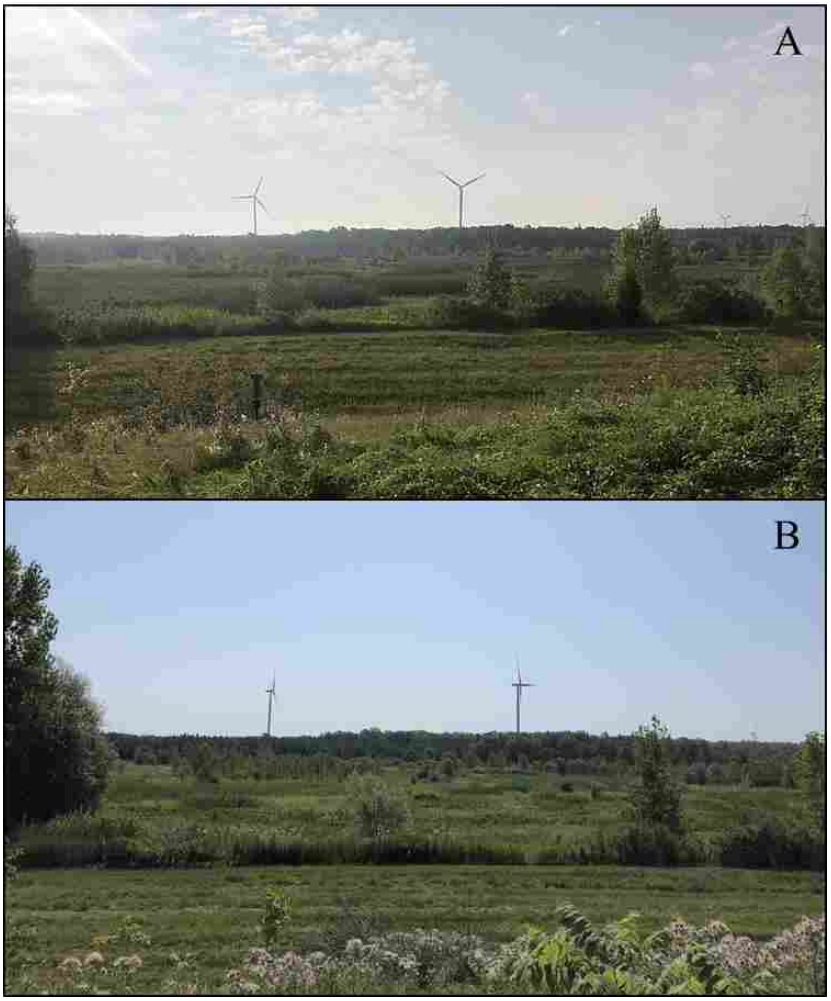

Figure 4.1: Photographs taken at Taquanyah Conservation Area during consecutive growing seasons depicting changes in overall vegetation. In August 2018, large areas of TCA were occupied by dense and expanding monocultures of *P. australis* (A). Following management treatments applied in the fall of 2018 by members of the GRCA, *P. australis* stands were no longer dominant in July 2019, and a variety of plant species were observed colonizing the previously invaded areas (B).

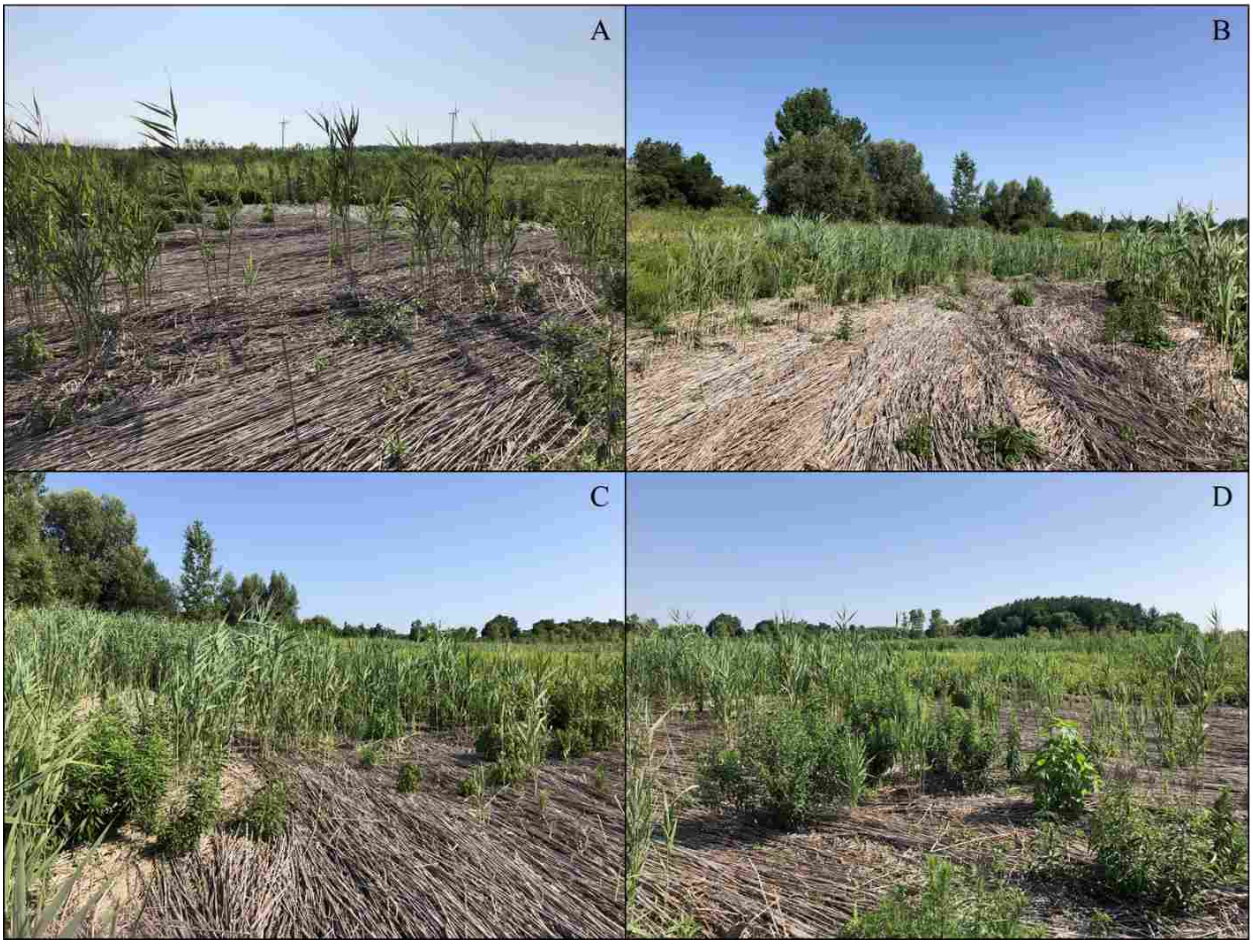

Figure 4.2: Photographs taken at TCA in July 2019 of areas where trampling and spraying treatments were applied to *P. australis* stands by GRCA land managers in the fall of 2018. All images (A-D) depict the remaining dead *P. australis* litter resulting from the 2018 treatment. Obvious reductions in *P. australis* stem regrowth was apparent in treatment areas (A) when compared to the regrowth at periphery areas where a barrier was maintained to protect *S. hermaphrodita* stands (B). Treatments appeared promising for habitat restoration of *P. australis* dominated areas due to the growth of new species including *S. canadensis*, *I. capensis*, and *S. hermaphrodita* where *P. australis* treatment was applied (C & D).

Figure 4.3: Photographs taken at TCA in July 2019 depicting *S. hermaphrodita* growth in areas where *P. australis* treatment was applied during the fall of 2018. Vegetative and reproductive expansion of *S. hermaphrodita* stands into areas previously occupied by *P. australis* was evident due to the growth of new *S. hermaphrodita* seedlings (C & D) as well as the growth of new vegetative shoots outside the *S. hermaphrodita* stands (A, B, E).

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