17 September 2021

Nick Lee, Chair and Members of the Wilton Inland Wetlands Commission Town of Wilton/ Town Hall 238 Danbury Road Wilton, CT 06897

RE: 141 Danbury Road, Wilton CT

The 141 Danbury Road application consists of approximately 4.28 acres adjacent to the Norwalk River. The applicant seeks to convert the warehouse style office building and large surface parking lot (extending to the river's edge) into a 173 unit (317 bedroom) apartment complex. The application claims to restore and expand functions to the current insignificant habitat and non-existent riparian buffer. To this end, the applicant proposes "significant" planting of the 100-foot upland review area that includes a 300± foot, point to point, river's edge perimeter, or estimated 360 feet if you consider the natural characteristics of the meandering edge of the Norwalk River. The applicant admits that the impervious surface will increase overall, but claims that the "significant improvements to the river buffer and new stormwater drainage system will dramatically improve water quality leaving the site," to empty directly into the Norwalk River. The applicant implies that their riparian buffer plan will also enhance the current non-existent wildlife habitat, by providing "food, nesting, and cover for local and migrating wildlife." The application adds, that their riparian buffer restoration will "provide outdoor, passive recreation space for residents."

The Norwalk River is a significant habitat. In 2019 the Wilton Bulletin reported on a CT Department of Energy and Environmental Protection (DEEP) fish count of the Norwalk River that revealed significant diversity and abundance of fish swimming and spawning in the Norwalk River. Such counts and water quality monitoring activities are invaluable for sustaining the fisheries, and recreational fishing, that the Norwalk River supports. Of special interest is the return of the American Eel, whose numbers are increasing since Norwalk River restoration projects began to restore natural flow and increase habitat complexity. This same article cautioned about persistent challenges to the Norwalk River including control of impervious surfaces stormwater runoff containing contaminants (i.e., chemical and microbiological), and increased water temperatures and reduced oxygen levels due to impoundments and restricted water flow. Significant, well designed, thoughtfully complex riparian vegetative buffers with healthy mycorrhizal soils can sequester nutrients, and absorb microorganisms known to impair water and compromise fisheries and wetland habitat (Chen et al., 2019). Additionally, predicted changes to climate in our area will result in more frequent storm surges (see attached EPA 430-F-16-009), and these surges can quickly overwhelm poorly designed stormwater management systems based on legal yet outdated design models that underestimate the frequency of future storm surges. The consequence of overwhelming dated pervious stormwater systems, is flashes of dirty surface water pouring directly into rivers with insignificant riparian buffers.

The buffer restoration proposed here will do little, in my opinion, to increase and sustain local wildlife such as birds and small mammals. A couple of rows of shrubs and trees along the river edge, coupled with lookout platforms, that are inviting to human traffic and pets, will not build a riparian buffer that will attract or sustain wildlife, or a wildlife corridor, but will provide opportunity for unintended personal debris, invasive microorganisms and biologicals, and secondary pollution (thermal, light, noise) proximity to the river's edge that otherwise would not be there. In my expert opinion, I find the proposed restoration of the riparian buffer too narrow and too manicured. Only 30 feet in the south west corner of the property where the buffer is approximately 80 feet wide may function as an effective riparian vegetative buffer. This represents approximately 8% of the estimated 360-foot-long Norwalk River edge

owned by this applicant. Connecticut fisheries and EPA data driven research recommend 100ft riparian buffer widths with substantial mycorrhizal soils to support safe fisheries, restore healthy habitats, and control stormwater runoff and erosion. A wider, less managed, more complex riparian buffer throughout the length of the river's edge, as well as restricted access to the water's edge, will greatly improve the wildlife and habitat restoration objectives of this application, as well as increase the preventive maintenance and management of stormwater runoff into the river due to storm surges predicted in the next decade. This improved riparian buffer will also help prevent snow melt, rich in sand and salt, from unintentionally impairing the Norwalk River. Climate change models for Connecticut predict greater precipitation in the winter and spring, suggesting that greater than expected snowmelt should be anticipated. In anticipation of this snow melt, snow storage should be stored furthest from the river's edge and in deeper/more recessed basins than currently planned.

Respectfully submitted,

Theodora Pinou, Ph.D.

 Inland Wetlands and Watercourses Act (IWWA): Connecticut General Statues (CGS) Sections 22a-36 through 22a-45

CH 440: Wetlands and Watercourses: Sec. 22a-36. Inland wetlands and watercourses. Legislative finding. The inland wetlands and watercourses of the state of Connecticut are an indispensable and irreplaceable but fragile natural resource with which the citizens of the state have been endowed. The wetlands and watercourses are an interrelated web of nature essential to an adequate supply of surface and underground water; to hydrological stability and control of flooding and erosion; to the recharging and purification of groundwater; and to the existence of many forms of animal, aquatic and plant life. Many inland wetlands and watercourses have been destroyed or are in danger of destruction because of unregulated use by reason of the deposition, filling or removal of material, the diversion or obstruction of water flow, the erection of structures and other uses, all of which have despoiled, polluted and eliminated wetlands and watercourses. Such unregulated activity has had, and will continue to have, a significant, adverse impact on the environment and ecology of the state of Connecticut and has and will continue to imperil the quality of the environment thus adversely affecting the ecological, scenic, historic and recreational values and benefits of the state for its citizens now and forever more. The preservation and protection of the wetlands and watercourses from random, unnecessary, undesirable and unregulated uses, disturbance or destruction is in the public interest and is essential to the health, welfare and safety of the citizens of the state. It is, therefore, the purpose of sections 22a-36 to 22a-45, inclusive, to protect the citizens of the state by making provisions for the protection, preservation, maintenance and use of the inland wetlands and watercourses by minimizing their disturbance and pollution; maintaining and improving water quality in accordance with the highest standards set by federal, state or local authority; preventing damage from erosion, turbidity or siltation; preventing loss of fish and other beneficial aquatic organisms, wildlife and vegetation and the destruction of the natural habitats thereof; deterring and inhibiting the danger of flood and pollution; protecting the quality of wetlands and watercourses for their conservation, economic, aesthetic, recreational and other public and private uses and values; and protecting the state's potable fresh water supplies from the dangers of drought, overdraft, pollution, misuse and mismanagement by providing an orderly process to balance the need for the economic growth of the state and the use of its land with the need to protect its environment and ecology in order to forever guarantee to the people of the state, the safety of such natural resources for their benefit and enjoyment and for the benefit and enjoyment of generations yet unborn.





Beneficial Services of Arbuscular Mycorrhizal Fungi – From Ecology to Application

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Arbuscular mycorrhiza (AM) is the most common symbiotic association of plants with microbes. AM fungi occur in the majority of natural habitats and they provide a range of important ecological services, in particular by improving plant nutrition, stress resistance and tolerance, soil structure and fertility. AM fungi also interact with most crop plants including cereals, vegetables, and fruit trees, therefore, they receive increasing attention for their potential use in sustainable agriculture. Basic research of the past decade has revealed the existence of a dedicated recognition and signaling pathway that is required for AM. Furthermore, recent evidence provided new insight into the exchange of nutritional benefits between the symbiotic partners. The great potential for application of AM has given rise to a thriving industry for AM-related products for agriculture, horticulture, and landscaping. Here, we discuss new developments in these fields, and we highlight future potential and limits toward the use of AM fungi for plant production.

Keywords: arbuscular mycorrhiza, symbiosis, abiotic stress, plant growth, plant protection, plant nutrition, soil structure. Glomeromycota

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INTRODUCTION

If an innovation spreads globally, becomes adapted to a multitude of diverse applications and persists over eons of time, it can be considered a great success. This is certainly the case for arbuscular mycorrhiza (AM). AM is thought to have a monophyletic origin in the Ordovician, approximately 480 Mio years ago (Redecker et al., 2000; Delaux, 2017), and it is found in the majority of land plants in most taxa and virtually all ecological niches (Read, 2002; Wang and Qiu, 2006). Most land plants are facultative symbionts, i.e., they profit from AM fungi, but can also live without them, although at considerable fitness costs (see below). However, some plant species have turned to obligate parasites on the AM fungus, i.e., they became fully dependent on fungal nutrition and lost photosynthetic capacity (mycoheterotrophs) (Graham et al., 2017). On the other end of the scale, some plant taxa, e.g., the *Brassicacea* and *Chenopodiaceae*, became asymbiotic, i.e., they lost the capacity to interact with AM fungi and evolved alternative strategies to meet their nutritional needs (Brundrett, 2004).

Arbuscular mycorrhiza symbiosis is thought to be a largely promiscuous association between >100,000 plant species and a few 100 AM fungal morphotypes, which have long been regarded as the equivalent of species. However, due to the relatively few distinctive morphological features of AM fungi (primarily associated with spores), and due to their essentially asexual mode of propagation, the traditional species concept is problematic in the context of AM fungi. AM fungi have never been shown to form sexual stages or to mate, however, they can undergo hyphal fusion (anastomoses) and exchange genetic material, thereby reshuffling their genomes and generating

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new genetic diversity in the absence of classical meiosis and recombination (Chagnon, 2014). Anastomosis depends on genetic relatedness, hence this feature could potentially be used as an additional criterion to define taxonomic units besides spore morphotypes.

With the advent of large scale sequencing approaches, AM fungal taxonomy and systematics rose to a new level (Spatafora et al., 2016). Results obtained with these modern tools indicate that the diversity of AM fungi has been underestimated (Husband et al., 2002; Öpik et al., 2006, 2013; Lee et al., 2013). Hence, the true number of AM fungal species, including genetically and functionally distinct "cryptic species" that cannot be distinguished by morphometric parameters (Munkvold et al., 2004; Rosendahl, 2008; Chen et al., 2018; Savary et al., 2018), may exceed current estimates by orders of magnitudes. The fact that recent results have documented unprecedented genetic variability even within one AM fungal species at a given site (Chen et al., 2018) points to the fact that the peculiar genetics and mode of reproduction of AM fungi impede with systematics and nomenclature in AM fungi.

ORIGIN AND EVOLUTION OF AM

Recent evidence indicates that the evolution of early plants from non-photosynthetic eukaryotes occurred in a freshwater environment by engulfment and domestication of a photosynthetic cyanobacterium (which subsequently evolved to the chloroplasts) (Ponce-Toledo et al., 2017). Hence, plants are the result of an endosymbiosis that was successful enough to allow them to radiate through most aquatic environments. Which innovations allowed plants to subsequently conquer the dry land masses of the continents? Some of the obvious adaptations required for the colonization of this new environment include protection against high radiation, a water-impermeant cuticle, and water-conductive vascular systems. However, an equally important innovation was required to allow plants to acquire water and nutrients from the substrate in the absence of specialized absorptive organs such as roots, which only evolved later (Brundrett, 2002). Conceivably, fungal symbioses were instrumental to allow the colonization of land by descendants of freshwater algae (Bidartondo et al., 2011; Delwiche and Cooper, 2015; de Vries and Archibald, 2018).

Although associations with AM fungi may not have been the first fungal symbiosis of early land plants (Field et al., 2015), recent evidence suggests that the advent of AM in the early land plants was a unique event, hence, AM appear to be a monophyletic innovation that may have enabled the rapid colonization of the continents by vascular plants (Delaux, 2017). Thus, it is conceivable that early rootless plants engaged in various kinds of fungal associations, as they are still observed today in early-diverging plant lineages (Read et al., 2000), and that roots coevolved with AM in the vascular plants (Brundrett, 2002). AM fungal associations were so successful that still the majority of land plants in most ecological niches (except for aquatic environments) engage in this symbiotic association.

MECHANISMS INVOLVED IN INTRACELLULAR ACCOMMODATION OF AM FUNGI

The very long evolutionary history of AM symbiosis of more than 400 Mio years (Redecker et al., 2000; Heckman et al., 2001; Schüssler et al., 2001), and the involvement of plant-derived and fungal signaling molecules that promote AM (Gutjahr and Parniske, 2013), suggests a high degree of adaptation and genetic/metabolic coordination between mycorrhizal partners. Indeed, formation of AM requires a dedicated signaling pathway starting with the root-borne signal strigolactone, which is exuded to stimulate AM fungal activity (Akiyama et al., 2005; Besserer et al., 2006; Kretzschmar et al., 2012). AM fungi subsequently secrete lipochito-oligosaccharides, which are perceived by the plant and activate a signal transduction pathway that is shared with root nodule symbiosis and therefore is known as the common symbiosis signaling pathway (CSSP), which has been elucidated in great detail in recent years (Harrison, 2012; Gutjahr and Parniske, 2013). In the light of the very low host specificity in AM, the involvement of a bidirectional exchange of symbiosis signals challenges our current understanding of communication between the partners, since it would require either many alternative signals for each potential partner, or few signals that can be recognized by a wide range of potential partners.

While central questions related to recognition and infection remain open, a rich body of microscopic evidence shows that at later stages the interaction has a very high degree of coordination at the cellular level. The most impressive examples are the formation of an infection structure (prepenetration apparatus; PPA) that allows cellular invasion (Genre et al., 2005, 2008), and the formation of the intracellular arbuscules that serve as nutritional interface between the partners (Harrison, 2012; Gutjahr and Parniske, 2013). Although the molecular-genetic basis of PPA formation is elusive, PPAs are thought to be a prerequisite for AM fungal infection of host roots, and to require signaling through the CSSP (Genre et al., 2005). Establishment of AM is associated with a fundamental reprogramming of the host cells including the activation of hundreds of genes (Liu et al., 2003; Güimil et al., 2005; Hohnjec et al., 2005; Fiorilli et al., 2009; Gomez et al., 2009; Guether et al., 2009; Breuillin et al., 2010; Gaude et al., 2012; Tromas et al., 2012; Hogekamp and Küster, 2013; Calabrese et al., 2017), of which some are expressed primarily or exclusively in cells with arbuscules. Although these genes are thought to be required for intracellular accommodation of the fungus, and for coordination of symbiotic functions, their molecular and cellular function has been elucidated only in few cases (see below).

NEW PARADIGMS IN THE EXCHANGE OF BENEFITS IN AM SYMBIOSIS

The finely branched fungal arbuscules (Figure 1A), and the surrounding peri-arbuscular membrane of the host (Figure 1B),

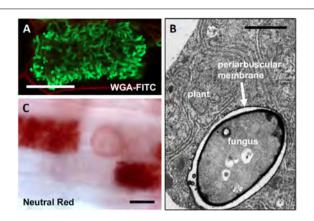


FIGURE 1 | Characteristics of AM fungal arbuscules. **(A)** Arbuscules are highly branched hyphal structures that nearly fill the cortex cells of the host. Green staining of fungal structures with wheat germ agglutinin (WGA) coupled to fluorescein isothicocyanate (FITC), red staining of the cell wall with propidium iodide (from Kretzschmar et al., 2012). **(B)** Transmission electron micrograph of a colonized host cell with an arbuscular branch (fungus), surrounded by the periarbuscular membrane. **(C)** A colonized root stained with Neutral Red which accumulates in acidic compartments, in this case the space between the periarbuscular membrane and the fungal cell wall [compare with **(B)]**. Size bars, 20 μ m in **(A,C)**; 1 μ m in **(B)**.

represent a considerably increased contact surface (also known as symbiotic interface) between the two partners, which has been estimated to correspond to a multiple of the entire cell surface (Alexander et al., 1989). In addition, the symbiotic interface is acidified (Figure 1C) to energize nutrient transport across the fungal plasma membrane and the periarbuscular membrane (Guttenberger, 2000; Krajinski et al., 2014; Wang et al., 2014). Therefore, cells with arbuscules are ideally suited for nutrient exchange. Indeed, the plant host expresses many symbiosis-specific nutrient transporters that are thought to mediate mineral nutrient uptake from the AM fungus (Rausch et al., 2001). The best-characterized example is a symbiotic phosphate transporter (PT) that is expressed exclusively in cells with arbuscules (MtPT4 in Medicago truncatula; OsPT11 in rice) (Harrison et al., 2002; Yang et al., 2012). Phylogenomic analysis of MtPT4 and its orthologs in other land plants suggests that the AM-related phosphate uptake pathway represents an early evolutionary innovation that became conserved after the advent of the angiosperms (Vigneron et al., 2018). Phosphate delivery is among the most important benefits for the host in AM symbiosis (Karandashov and Bucher, 2005), and the collective information suggests that the arbuscules are the site of transfer of phosphate from the fungus to the plant (MacLean et al., 2017).

The induction of many other mineral nutrient transporters in mycorrhizal roots (Wang et al., 2017), and the fact that mycorrhizal plants contain increased amounts of various mineral nutrient elements (Clark and Zeto, 2000; George, 2000) suggest that nutrient elements such as nitrogen, sulfur, and microminerals such as copper and zinc may also be transferred via the arbuscules. However, for most AM-induced predicted nutrient transporters, the expression pattern, protein localization, and function remain to be established.

Interestingly, AM-related pathways can also stimulate plant growth and physiology in nutrient-independent ways. For example, mycorrhizal plants show enhanced photosynthetic capacity (Boldt et al., 2011). More strikingly, the overexpression of a petunia strigolactone transporter (PDR1), which is involved in AM signaling (Kretzschmar et al., 2012), is sufficient to improve root and shoot growth in the absence of AM fungi (Liu et al., 2018). Thus, AM and its signaling can potentially increase plant growth in yet unexplored ways that are more related to plant developmental programs than to plant nutrition.

As a reward for its symbiotic services, the AM fungus receives fixed carbon from the plant. In analogy to plant pathogen interactions, carbon transfer has long been thought to proceed in the form of carbohydrates (in particular hexoses). Indeed, a large body of evidence has demonstrated that AM fungi can take up and utilize sugars, but only under symbiotic conditions in the roots (Roth and Paszkowski, 2017). Recently, the surprising discovery that two AM fungal genomes lack a fatty acid synthase complex (Wewer et al., 2014; Tang et al., 2016) has raised the question how AM fungi may generate their abundant lipid reserves in spores and vesicles (Rich et al., 2017b). Intriguingly, the plant host induces several components of fatty acid biosynthesis and processing in mycorrhizal roots indicating that AM fungi may also receive fatty acids besides sugars. Indeed, recent evidence has demonstrated that AM fungal lipids are, at least partially, derived from the plant host (Bravo et al., 2017; Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017; Brands et al., 2018).

The supply of lipids to AM fungi involves host genes encoding enzymes of fatty acid biosynthesis, a glycerol-3-phosphate acyl transferase (GPAT) that generates a monoacylglycerol (MAG) intermediate and a pair of ATP-binding cassette transporters of the G-type (ABCGs) that form a heterodimeric transporter in the peri-arbuscular membrane (Zhang et al., 2010). These elements resemble components required for the generation and secretion of the lipid precursor for the extracellular lipid polyester cutin, suggesting that the two pathways may share a common evolutionary origin in early land plants (Rich et al., 2017b). The AM-specific transcription factor REQUIRED FOR ARBUSCULAR MYCORRHIZA1 (RAM1) in the host is responsible for induction of many of the genes required for a functional AM, including the GPAT RAM2 and the ABCGs STUNTED ARBUSCULE (STR) and STR2 (Park et al., 2015; Rich et al., 2015; Pimprikar et al., 2016; Luginbuehl et al., 2017; Rich et al., 2017a). However, many aspects of lipid transfer to AM fungi remain to be elucidated.

SIGNIFICANCE OF AM FOR PLANTS IN NATURAL HABITATS

How much a plant benefits from AM fungal colonization depends to a large degree on the environmental conditions. In most natural environments, which are characterized by mineral nutrient deficiency and various abiotic stress conditions, mycorrhizal plants are thought to have a selective advantage over non-mycorrhizal individuals of the same species. Thus, AM can

potentially promote intraspecific competitiveness and selectively favor mycorrhizal plants. Conceivably, this is the reason why AM symbiosis has prevailed over very long periods of evolutionary time in most land plant taxa.

A complication arises due to the fact that plants can have several different AM fungal partners, and vice versa, each fungal mycelium can infect several host plants of the same or different species. The resulting common mycorrhizal networks (CMNs) add an additional level of complexity to the analysis of benefits in mycorrhizal interactions (Jakobsen and Hammer, 2015). A strongly interconnected plant community can potentially gain stability because weaker individuals could profit from mineral nutrient supply from the CMN at the expense of stronger plants that entertain the CMN. In this way, the stronger plants indirectly benefit less competitive plants, thereby attenuating competition among plant individuals. Such "underground socialism" has been envoked particularly in cases where seedlings grew better when they were connected to a CMN that had been established by older plants, a phenomenon known as facilitation (van der Heijden and Horton, 2009). However, the effects of CMN on seedlings are highly context-dependent and vary with the involved species. In some cases, AMF can even increase intra- or interspecific competition, hence, the effects of CMN cannot be generalized. In the most extreme version of the theme, achlorophyllous plants obtain all their resources, including carbon, from CMN, thereby parasitizing—indirectly—on other plants that supply the network with their carbon (Bidartondo et al., 2002). While this represents an extreme nutritional strategy that emerged only in a minority of land plants, there are many intermediate examples of plants that obtain part of their carbon from mycorrhizal fungi (mixotrophy), a condition that has likely been the transitional evolutionary phase from autotrophy to mycoheterotrophy (Bidartondo, 2005; Selosse et al., 2017).

FUNCTIONAL SPECIFICITY IN AM INTERACTIONS

The variability of the effects of AM fungi on their hosts (see above) indicates that certain combinations are beneficial for the plant, whereas others are neutral or even negative. Conversely, AM fungal proliferation and sporulation are highly dependent on plant host identity (Bever, 2002). These findings suggest a certain degree of functional specialization in AM interactions. Indeed, a systematic combinatorial study on mycorrhizal benefits employing a large panel of plant and fungal species from different geographical locations showed that the mycorrhizal growth response (MGR; defined as the difference between the weights of mycorrhizal vs. non-mycorrhizal plants) ranged from -50% to +50% growth promotion, with almost half of the combinations resulting in growth depression (Figure 2) (Klironomos, 2003). The mutualistic potential did not correlate with phylogenetic patterns in either partner, indicative of adaptive mechanisms independent from lineage. Interestingly, combinations of partners isolated from the same location performed better, indicative of co-adaptation. Conceivably, combinations of good mutualists enjoy positive bidirectional

feedback that results in progressive mutual adaptation of the most effective mutualistic combinations (Kiers and Denison, 2008), although the interaction shows very little host specificity at the level of infection (see above). In agreement with functional specialization, soils with a diverse AM fungal flora can support more diverse plant communities than if only one or few AM fungi are present (van der Heijden et al., 1998). This finding is compatible with a scenario in which each plant species requires a suitable AM fungal partner. Thus, despite the very low host specificity of AM under laboratory conditions, functional specialization within the AM fungal community shapes the level of the biodiversity and productivity of plant communities.

EFFECTS OF AM FUNGI ON PLANT DEFENSE AND DISEASE RESISTANCE

Mycorrhizal roots often exhibit very intense fungal colonization, both intercellularly and intracellularly, that can reach more than 90% total root length. This observation has led Dangeard to coin the genus name Rhizophagus (greek for "root eater"), based on the initial assumption that mycorrhizal roots were colonized by an aggressive pathogen (Dangeard, 1900). We now know that most plants can potentially profit from AM fungal colonization (depending on the right fungal partner and the environmental conditions), but it is still a mystery how plants can tolerate such high degrees of colonization without mounting a defense response, given that fungi in general (including AM fungi) contain and release many molecular signals (e.g., chitin oligomers) that can be recognized by plants, and that have shown to trigger defense responses in various plant species (Wan et al., 2008; Boller and Felix, 2009). It has therefore been proposed that AM involves the suppression of defense. Indeed, plant mutants defective in genes required for symbiotic signaling and AM establishment (see above) often show characteristic defense responses upon infection by AM fungi, indicating that these fungi have potent signaling molecules that trigger defense, and that these mechanisms are suppressed during normal AM development. Pathogens usually produce inhibitors of defense (known as effectors), and recently, numerous effectors where also predicted to occur in the genomes of AM fungi (Sedzielewska Toro and Brachmann, 2016; Kamel et al., 2017). However, only very few of them have been functionally analyzed (Kloppholz et al., 2011).

Although defense mechanisms in the host have to be attenuated to allow AM fungal infection and colonization of the roots, general defense needs to remain active to cope with rhizospheric pathogens. Indeed, general disease resistance of mycorrhizal plants is not decreased. In contrast, mycorrhizal plants often exhibit increased disease resistance (Borowicz, 2001; Pozo and Azcon-Aguilar, 2007; Jung et al., 2012; Cameron D.D. et al., 2013). Experiments with split root systems revealed that this effect is often systemic, i.e., the entire plant is protected against pathogens. This can involve generally improved plant health due to better nutrition, or a systemic induction of the defense status, known as systemic acquired resistance (SAR). In addition, mycorrhizal plants may be prepared to react faster and

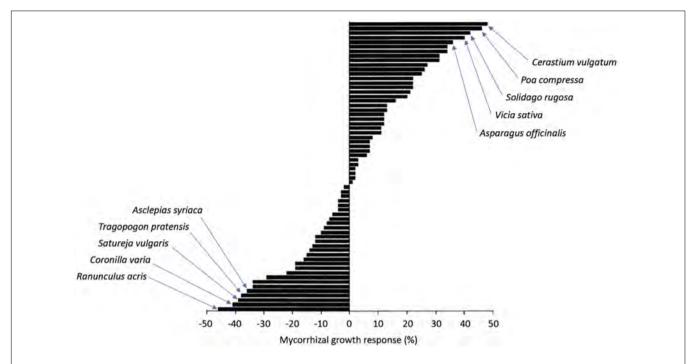


FIGURE 2 | Mycorrhizal growth response (MGR) depends on the symbiotic partners. Various plants were inoculated with *G. etunicatum*. After 16 weeks of coculture, the dry weight of the host plants was determined and the percent change relative to the non-mycorrhizal controls (referred to as MGR) was calculated. MGR ranged between –50% and +50% change in dry weight. The 10 most responsive plants (five positive, five negative) are indicated (modified from Klironomos, 2003).

stronger to pathogen attack, a phenomenon known as induced systemic resistance (ISR), or priming (Conrath et al., 2006). These protective effects of AM are of great interest for sustainable strategies of plant protection (Solaiman et al., 2014). Although priming is a systemic phenomenon, AM fungi are primarily employed to protect plants from soil-borne pathogens (Cameron D.D. et al., 2013; Jung et al., 2012). In addition, AM fungi, or other microbes associated with their mycelium, can directly interfere with rhizospheric pathogens either by the release of antimicrobial compounds, or by direct competition for space and resources. Although the potential of AM fungi for plant protection is widely acknowledged, it should be noted that in certain cases, mycorrhizal crops have no benefits from AM, or may even exhibit reduced growth and fitness (Jacott et al., 2017) (see also above). It is tempting to speculate that this phenomenon may be related to breeding programs that targeted traits related to shoot architecture and yield, while root-related traits were ignored. While this does not necessarily prevent plants from becoming infected, it may have interfered with the regulatory mechanisms that ensure optimal metabolic coordination of both partners.

SIGNIFICANCE OF AM IN THE MAJOR CLIMATIC ZONES AND IN MANAGED ECOSYSTEMS

Arbuscular mycorrhiza fungi have been observed in virtually all major ecosystems worldwide (Öpik et al., 2006), from

arctic regions (Varga et al., 2015), to tropical forests (Lovelock et al., 2003), from the deserts in the arabic peninsula (Al-Yahya'ei et al., 2011) to the high himalayans (Liu et al., 2011). While some AM fungal isolates show only restricted distribution in natural communities, others appear to be true cosmopolitans (Rosendahl et al., 2009). Whether this reflects natural distribution, or transport by human activity is unclear. In addition, some cosmopolitan species may in fact represent genetically differentiated species complexes that cannot be distinguished by morphological criteria. The occurrence of truly cosmopolitan AM fungal species (Rosendahl et al., 2009) suggests that these fungi are extremely adaptable, both, in terms of environmental conditions, and in terms of a wide host range. Since AM fungi play an instrumental role in the protection against abiotic stresses such as nutrient starvation (see above), heat (Bunn et al., 2009), and drought (Augé, 2001; Ruiz-Sanchez et al., 2011; Rapparini and Penuelas, 2014; Chitarra et al., 2016), they can benefit their hosts in the wild and in agriculture (Wu, 2017). Consequently, AM fungi are thought to have a great impact in natural environments (Read, 2002; Smith and Read, 2008; van der Heijden et al., 2015), as in managed conditions in agriculture, horticulture, and forestry (see below).

REDUCTION OF SOIL EROSION AND NUTRIENT LEACHING BY AM

An important service of AM fungi in natural as well as in agricultural contexts is the beneficial alteration of soil structure

(Leifheit et al., 2014). The dense hyphal network of the highly ramified AM fungal mycelium creates a three-dimensional matrix that enmeshes and crosslinks soil particles without compacting the soil. A soil glycoprotein was identified as an additional important agent in the stabilization of soil aggregates (Rillig, 2004; Singh et al., 2013). It is referred to as glomalin, because it is thought to be produced by AM fungi. Glomalin is not a defined gene product or chemically homogenous molecular species, rather, it is a soil fraction that is defined by its extractability and immuno-reactive properties (Rillig, 2004). Glomalin and glomalin-related soil proteins (GRSPs) have recently seen a renaissance in the literature, however, their origin and function are far from clear. Nevertheless, they represent an important determinant of soil quality and a very stable carbon sink with estimated half-life times in the range of several years up to decades (Rillig et al., 2001). GRSPs can account for a significant fraction of total organic soil carbon (2-5%), and since they protect other forms of organic carbon from degradation by increased soil particle aggregation, they may contribute significantly to sequestration of carbon in the soil (Rillig et al., 2001; Wilson et al., 2009). Taken together, the hyphal network of AM fungi, and their promoting effects on plant growth and root system development (Gutjahr and Paszkowski, 2013) protects the soil from erosion by wind and water.

The collective effects of AM fungi on soil qualities also results in higher water retention capacity, which benefits plant growth in addition to improved nutrient supply. The benefits of AM fungi are particularly critical for plants in dry sandy soils in arid regions. These soils often show low fertility and are highly vulnerable to erosion by wind and rain. In such cases, plantings with mycorrhizal plants can be a sustainable way to counteract erosion and improve soil fertility (see below).

Apart from the improved soil structure, AM fungi reduce nutrient leaching from the soil (Cavagnaro et al., 2015). Nutrient leaching is a serious problem since it results in loss of soil fertility and pollution of ground water and surface water (rivers, lakes). Intact ecosystems exhibit a good nutrient retention capacity due to efficient adsorption and retention of nutrients by roots and soil microorganisms (including AM fungi). However, agricultural soils are by definition disturbed by agricultural practice (in particular plowing), and they receive large amounts of fertilizer, mainly N, P, K. These, in particular the highly mobile nitrate, are prone to be washed out from the soil due to the lack of a good nutrient retention system (Cameron K.C. et al., 2013).

The beneficial effects of AM fungi against nutrient leaching operate at different levels. First, improved soil structure (see above) allows for increased nutrient sequestration to the microand macro-aggregates in mycorrhizal soil, second, AM fungi take up nutrients from the soil solution (Clark and Zeto, 2000; George, 2000), and final, mycorrhizal soils exhibit better retention capacity of the soil solution (see above) (Querejeta, 2017), thereby benefitting at the same time the availability of nutrients and water to the plant. A detailed documentation of the beneficial effect of AM fungi on plants under drought stress was reported for tomato (Bitterlich et al., 2018). Reduced leaching from mycorrhizal soils has been documented in particular for P and N, but it conceivably also involves other mineral nutrients.

Taken together, AM fungi integrate the nutrient fluxes in the soil by generating closed nutrient cycles, thereby promoting long-term soil fertility (Cavagnaro et al., 2015).

COMMERCIAL USE OF AM FUNGI

The multiple benefits of AM have raised opportunities for their commercial application. Consequently, the AM-related markets grew considerably during the past decades, with increasing numbers of actors, products and market volume (Vosatka et al., 2008). However, due to the fact that most of the AM-related industry consists of privately owned relatively small firms, public information about the dynamics of market shares are scarce. Hence, we carried out a systematic survey on the number of firms producing and selling AMF products in Europe and worldwide, and we assessed the number of their products as key figures in the market

The results show that since the 1990s, the number of companies selling mycorrhizal products has increased considerably. On a global scale, the main players are located in North America, Europe, Asia, and Latin America. In the domain of the Americas, the main markets include United States, Canada, Mexico, Brazil, Argentina, Colombia, and Chile. The Asia region is mainly dominated by India, followed by China. The Indian market itself has seen an outstanding growth rate during the last decade. One of the reasons is the promotion of mycorrhiza-based bio-stimulants by the Indian government and the actions from organizations such as The Energy and Resources Institute (TERI1). In general, the AMF businesses are smalland medium-sized firms producing for the local and regional markets. However, there are some exceptions of larger companies from the United States, Canada, Germany, Italy, Czech Republic, United Kingdom, and Spain that export their products to various geographical regions.

The European market represents one of the leading markets for mycorrhizal bio-stimulants. In Europe itself, the number of firms producing and selling AMF-products has increased from less than 10 firms in the late 1990s, to more than 75 firms in 2017 (Figure 3). Most of the European companies are found in Germany, Italy, Spain, the United Kingdom, France, The Netherlands, Czech Republic, Austria, Belgium, Estonia, and Switzerland (Figure 4). The largest domains of application include gardening and landscaping, horticulture, agriculture, forestry, golf courses (in particular greens), recultivation of degraded land, roof plantings, soil remediation, and research (Figure 5). In terms of retail prices for hobby and semiprofessional users, the average price per plant ranges between 10 and 50 cents. The cost of mycorrhizal inoculation for professional uses at an agricultural scale is considerably lower, with an estimated investment of 135 \$ per hectare in the case of potato in the United States (Hijri, 2016). Apart from pure AM fungal inocula, many products include mixed fungal inocula, sometimes in combination with ectomycorrhizal fungi or with plant growth promoting rhizobacteria.

¹http://www.teriin.org

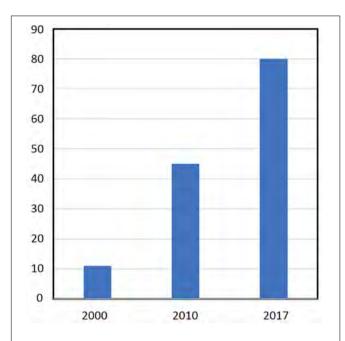


FIGURE 3 I Increase in the number of companies in the European AM market. A survey on the number of firms selling AM inocula in Europe was determined by an internet surveyed. Based on the year of foundation, the number of firms was determined for three time points (year 2000, 2010, 2017).

APPLICATION OF AM TO AGRICULTURAL AND HORTICULTURAL CROPS

With the multiple benefits that AMF confer to their hosts, they hold great promise for application in crop production under various conditions. Most agricultural crops are hosts for AMF and can therefore potentially benefit from inoculation with AMF. Indeed, many studies have shown that application of commercial AMF inoculum benefits crops under agricultural conditions (Weber, 2014). Numerous studies have shown that AMF can increase plant health and yield (Mäder et al., 2000; Rouphael et al., 2015; Hijri, 2016). AMF support plant nutrition by absorbing and translocating mineral nutrients beyond the depletion zones of plant rhizosphere (see above) and induce changes in secondary metabolism leading to improved nutraceutical values. In addition, AMF interfere with the phytohormone balance of host plants, thereby influencing plant development (bioregulators) and inducing tolerance to soil and environmental stresses (bioprotector) (Rouphael et al., 2015). One important aspect of this is the promotion of root system development (Gutjahr and Paszkowski, 2013).

Since the production and application of AM fungal inoculum is relatively labor-intensive, AM application is particularly interesting for high-value crops, e.g., in horticulture, and for the adaptation of cuttings and micro-propagated plantlets in nurseries (AzconAguilar and Barea, 1997; Jeffries et al., 2003; Kleinwächter et al., 2008; Maronek et al., 2011). A large part of the horticultural plant production involves sterile micropropagation

in vitro. A critical point of development of plantlets generated in this way is the transfer to soil (weaning) that can cause large losses (Schubert and Lubraco, 2000). Inoculation with AMF of micropropagated fruit trees at transplant improves growth and nutrient uptake during the weaning stage, yielding plants of larger size and improved commercial characteristics (Lovato et al., 1992; Cordier et al., 1996; Schubert and Lubraco, 2000). AM fungi can accelerate this transition and improve the health of the plantlets (Vestberg et al., 2002), thereby rendering plant production more profitable. A good example for such an application are apple and peach cuttings that grow stronger with AM fungal inoculum (Schubert and Lubraco, 2000; Balla et al., 2008).

Arbuscular mycorrhiza inoculation can also be profitable in plant production at a large agricultural scale. A particularly well documented case is a large meta-analysis of potato production in 231 field trials in Europe and North America, which showed a significant increase in tuber production after inoculation with the commercial strain R. irregularis (DAOM 197198) (Hijri, 2016). Interestingly, in all these field trials, the farmers themselves carried out the application and evaluation under their respective conventional agricultural practice (including application of pesticides and fertilizers). This approach caused the experimental conditions to be heterogeneous, and the experimental design did not involve replicate plots or randomization. However, the large number of field sites provide robustness to the results, which were remarkably positive. Interestingly, a general beneficial effect was observed independent of location, soil type, experimentor and the details of farming practice (Hijri, 2016). The average yield increase in these 231 field trials amounted to 3.9 tons/ha, representing 9.5% of total crop yield. With an estimated threshold for profitability of 0.67 tons/ha increased yield, nearly 80% of all trials were more profitable thanks to AMF application. This impressive meta-analysis suggests that farmers of potato, and perhaps other crops, can realize significantly increased revenue thanks to AM. In addition, AMF application can allow to decrease the amount of fertilization without a decrease in yield, thereby further increasing profitability. In conclusion, such large-scale trials provide more robust results than more controlled greenhouse or small-scale trials.

Although the application of AM in horticulture and agriculture has great potential, the effectiveness and success of AMF on extended field applications depend to a large degree on external conditions that need to be taken into account. Factors such as plowing and high fertilizer application (in particular P) interfere with AMF abundance and colonization (Douds and Millner, 1999; Mäder et al., 2000; Grant et al., 2005; Hartmann et al., 2015). Other factors that affect AMF symbiosis include the use of specific biocides and cropping with non-host plants (e.g., Brassicaceae, Chenopodiaceae) (Njeru et al., 2015). In addition, for every crop, the best corresponding AM fungus should be selected (Rouphael et al., 2015), because AM fungi can provide diverse benefits (growth, stress resistance etc.), and not in each combination of plant and fungus, the trait of interest (e.g., growth) is necessarily positively influenced (Klironomos, 2003) (see above).

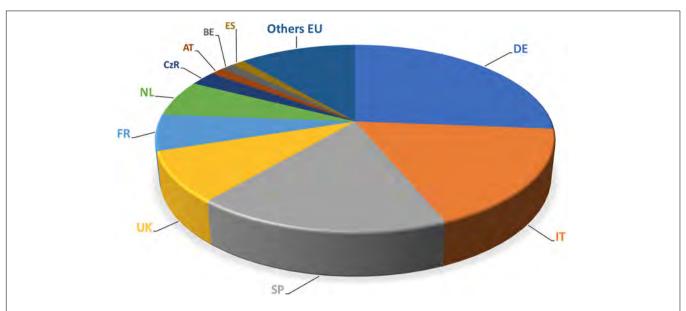


FIGURE 4 | Main players in the AM market in the European Union. The number of companies selling AM inocula is expressed in relation of their location of the main house. Main producer countries are Germany (DE), Italy (IT), Spain (SP), the United Kingdom (UK), France (FR), and the Netherlands (NL).

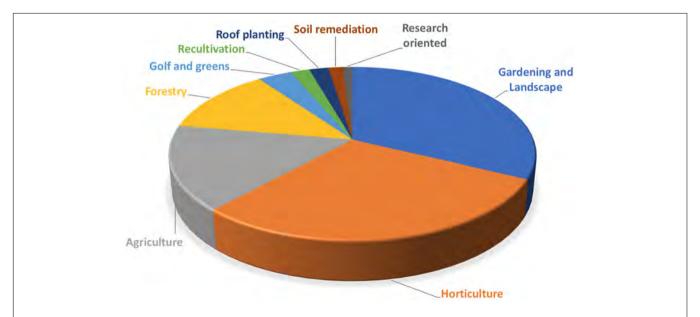


FIGURE 5 | Main domains of application of AM products. The number of products of European firms was determined for each domain of application. Main fields of application are gardening and landscaping, horticulture, agriculture, and forestry.

POTENTIAL FOR THE USE OF AM FUNGI FOR RENATURATION, REFORESTATION, AND LANDSCAPING

Renaturation and afforestation are measures to stabilize degraded and eroding surfaces. In particular in arid regions, young trees are very vulnerable to abiotic stresses (drought, heat, nutrient starvation), in particular at early stages until they have established a deep root system that allows them to access ground water reserves. This critical phase can be overcome with mycorrhizal

inoculation of the trees before planting. For example, the Moroccan argan tree, the fruits of which are used to prepare the precious argan oil (El Abbassi et al., 2014), are endangered in their original areas of distribution due to overuse (Lybbert et al., 2011), despite their protection as UNESCO biological reservation². Argan reforestation requires that young plantlets raised in nurseries are planted out, and that they quickly adapt to the dry climate of the native range of these trees. Mycorrhizal inoculation significantly increases the growth and health of

²http://www.unesco.org

young argan trees, thereby increasing their fitness and survival after planting (Sellal et al., 2017).

A similar case is represented by the use of a mixture of indigenous AM fungi for the inoculation of young Cypress trees (*Cupressus atlantica*) (Ouahmane et al., 2007). In this study, only AM fungi isolated from the natural site of *C. atlantica* were used, thereby increasing the chances to employ fungi that are well adapted to drought and to *C. atlantica*, and avoiding to introduce new AM fungal species with unpredictable effects on the local environment. AM inoculation not only increased plant growth, but also increased survival of the trees in the dry native conditions. This latter point is perhaps even more important than the growth promotion, because it renders reforestation efforts more sustainable.

Another interesting example is stabilizing sand dunes by planting of the drought-tolerant mesquite tree (*Prosopis juliflora*), which increases mycorrhizal communities in sand dunes (Moradi et al., 2017). On the other hand, the mesquite trees profit from AM colonization (Solis-Dominguez et al., 2011). Hence, AM symbiosis can be a critical component in strategies to protect vulnerable sandy soils against erosion, and to improve their fertility.

CAN AM FUNGI PROMOTE BIOREMEDIATION OF CONTAMINATED SOILS?

During the last decades, the potential of plants has been explored to reduce the contamination of soils polluted by organic compounds or heavy metals, and AM fungi could potentially play a central role in such strategies (Leyval et al., 2002; Turnau et al., 2006; Khade and Adholeya, 2007; Sheoran et al., 2010). Thanks to their mineral-scavenging capacities, and with their protective role against abiotic stress, AM fungi can potentially promote plant growth in contaminated soils, a capacity commonly referred to as bioremediation (Leyval et al., 2002; Göhre and Paszkowski, 2006). They can do so in two ways: they can either accumulate and sequester toxic metal ions, thereby protecting their host from the pollutant (Weissenhorn et al., 1995; Diaz et al., 1996; Gonzalez-Chavez et al., 2004), or they can deliver them to the host just like essential mineral nutrients such as Cu and Zn, resulting in heavy metal accumulation in the host. In the first case, plant production can be enabled in polluted substrate, with minimal contamination of the crop. In the second case, the plants can be harvested and destroyed to reduce the heavy metal load of the site (phytoextraction) (Burns et al., 1996; Khan et al., 2000). Of course, both approaches require heavy-metal-tolerant AM fungi, and phytoextraction in addition requires highly tolerant host plants that can cope with toxic heavy metals, and at the same time yield large shoot biomass in order to accumulate significant amounts of heavy metals.

To date numerous laboratory studies have been carried out to explore the potential of AM in bioremediation of the soil, however, only few field studies have addressed the applicability of this approach to large scale conditions (Burns et al., 1996; Adriano et al., 2004; Chibuike, 2013). Worldwide, there are only

few companies offering AM fungal products for bioremediation. Some of the obstacles include the fact that most heavy metal-accumulating plants are rather small, and some are not host plants for AM fungi (e.g., the crucifer *Thlaspi*). In addition, AM colonization is often reduced by high pollution.

CAN AM FUNGI BE BRED FOR IMPROVED SYMBIOTIC TRAITS?

Given the promising features, but also the limitations of AM fungi for application in plant production, it would be a great asset if they could be bred for higher efficiency in the respective context of interest. Classical breeding (by crossing and selection in the progeny of variants with new desired features) is currently impossible with AM fungi owing to their particular genetic constitution (Sanders and Croll, 2010). Their syncytial nature and their purely clonal propagation, as well as the absence of recognizable sexual stages prevents forward and reverse genetic approaches such as mutant screening, transformation, crossing, genetic mapping etc.. However, the mycelia of AM fungi can fuse by a process known as anastomosis, which allows for the exchange of genetic material (incl. nuclei) between the two syncytia (Giovannetti et al., 1999).

"Crossing" of AM fungi by anastomosis, and subsequent culturing of AM fungal progeny can generate new genotypes with new symbiotic features (Angelard et al., 2010; Angelard and Sanders, 2011). In particular, new beneficial mycorrhizal traits can result from this kind of breeding scheme (Angelard et al., 2010). However, anastomosis is only possible between compatible AM fungal isolates of the same, or perhaps closely related species (de Novais et al., 2017), indicating that it requires genetic compatibility factors. Based on these findings, AM fungi could potentially be bred for improved symbiotic traits by systematic genetic reshuffling between divergent (but compatible) AM fungal isolates, followed by screening for the most beneficial new strains among AM fungal segregants. This screening should be performed with each host plant of interest, thus allowing to identify the best-suited AM fungal segregant for each target host species. Such combinations could subsequently further evolve by continued selection for improved AM fungal descendants with more beneficial effects on the host plant. Such changes can emerge surprisingly quickly, possibly driven by genetic drift among the heterogeneous nucleotypes of the expanding syncytial AM fungal mycelium (Angelard et al., 2014).

FUTURE OPPORTUNITIES AND CHALLENGES FOR AMF

Inspite of its growing trend, the current market for mycorrhizal products remains far from its full potential. Apart from technical issues, challenges for AM fungal products in the coming years include (i) political and regulatory constraints; (ii) quality assurance and product efficacy; and (iii) customer awareness and acceptance.

In terms of regulations and policies, the current market for mycorrhizal products has, to date, remained relatively unrestricted by political forces. In Europe, there is no unifying regulation covering (and controlling) the manufacture, use or movement of mycorrhizal fungal products (Vosatka et al., 2008). Depending on their intended use, AM fungal products could be registered in the market in three different categories: as bioprotectants, as biofertilizers, or as biostimulants. The registration has to be performed according to the national regulations of each EU state member. In some cases (e.g., France and Belgium), the regulatory process is quite complex and expensive. Such regulations result in limitations and market entry barriers for AM fungal products (Vosatka et al., 2008). In this regard, the European Parliament is currently evaluating the establishment of an equitable EU market for biostimulants. The key elements considered by the EU to establish a single-market include: defining biostimulants and defining the boundary with plant protection products; the requirement to develop safety criteria and harmonized standards, in particular for microorganisms, and promoting a circular economy with the efficient use of plants and plant extracts. A single harmonized market for biostimulants will support EU farmers to become more competitive and participate in developing sustainable agriculture with a reduced impact on the environment.

In relation to product quality, given the lack of regulatory bodies to set the quality parameters, AMF producers rely on self-imposed quality standards to ensure best practice in production (Vosatka et al., 2008). In Europe, for instance, the main AMF producers have agreed on the use of a protocol proposed by Gianinazzi-Pearson et al. (1985) to define quality of AMF products. This protocol is known as the "most probable number" (MPN) and serves to determine the presence or absence of AMF in a dilution series, with the results interpreted as a probability estimate of propagule number from a statistical table. Even though the assay is indirect (absolute numbers of propagules are not measured), it has the advantage of providing a single number that can be compared directly with other tests in the same assay. However, other qualitative parameters should also be taken into account, in particular richness of inoculum (number of spores or propagules/ml) and infectivity, i.e., the capacity of the inoculum to establish mycorrhizal symbiosis. Finally, not every combination of a plant and an AM fungus is beneficial (see above), hence, it is advisable to test different AM inocula for each crop of interest to identify optimal combinations of plant and AMF.

In general, product quality and efficiency are still areas that require further attention. The appropriate dosage, or propagule density, for a given market sector is not yet formalized and it leaves scope for the marketers to set these values. The aforementioned constraints open a window of opportunity for

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the research community in order to assist producers and the market in defining what should be the minimum treatment standard (Vosatka et al., 2008). Other critical challenges for the AM market are customer awareness and acceptance. Although the use of biostimulants and biofertilizers is growing in popularity, the use of traditional chemical fertilizer products remains as the most common practice among farmers. In this respect, AMF producers are focusing their efforts to establish relevant case studies and field trials to demonstrate and prove the benefits of AMF in agriculture and horticulture. Larger organisms contributing to the promotion of biostimulants include the The European Biostimulant Industry Council (EBIC³), and the International Mycorrhiza Society (IMS⁴).

OUTLOOK

Arbuscular mycorrhizal fungi promote many aspects of plant life, in particular improved nutrition, better growth, stress tolerance, and disease resistance. In addition, the hyphal networks of AM fungi improve soil characters such as soil particle aggregation thereby improving the resistance of soil toward erosion by wind and water. Finally, AM fungi decrease nutrient leaching from the soil, thereby contributing to the retention of nutrients in the soil, and decreasing the risks of contamination of ground water. These multiple benefits of AM fungi translate into significant ecological services in natural contexts. The promises for agriculture have been clearly documented for certain crops, in particular potato, however, many applications have still to be developed, which requires significant investment in research and development of AM fungal inocula suited for additional crops.

AUTHOR CONTRIBUTIONS

MC, MA, LB, and EN contributed significant parts of the text. DR was the main coordinator and wrote a large part of the manuscript.

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³ http://www.biostimulants.eu

⁴http://mycorrhizas.org

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- **Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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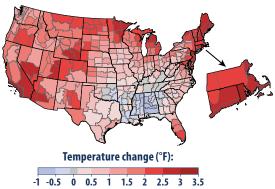


What Climate Change Means for Connecticut

Connecticut's climate is changing. The state has warmed two to three degrees (F) in the last century. Throughout the northeastern United States, spring is arriving earlier and bringing more precipitation, heavy rainstorms are more frequent, and summers are hotter and drier. Sea level is rising, and severe storms increasingly cause floods that damage property and infrastructure. In the coming decades, changing the climate is likely to increase flooding, harm ecosystems, disrupt farming, and increase some risks to human health.

Our climate is changing because the earth is warming. Since the late 1700s, people have increased the amount of carbon dioxide in the air by 40 percent. Other heat-trapping green-house gases are also increasing. These gases have warmed the surface and lower atmosphere of our planet about one degree during the last 50 years. Evaporation increases as the atmosphere warms, which increases humidity, average rainfall, and the frequency of heavy rainstorms in many places—but contributes to drought in others.

Greenhouse gases are also changing the world's oceans and ice cover. Carbon dioxide reacts with water to form carbonic acid, so the oceans are becoming more acidic. The surface of the ocean has warmed about one degree during the last 80 years. Warming is causing snow to melt earlier in spring, and mountain glaciers are retreating. Even the great ice sheets on Greenland and Antarctica are shrinking. Thus the sea is rising at an increasing rate.



Rising temperatures in the last century. Connecticut has warmed twice as much as the rest of the contiguous 48 states. Source: EPA, Climate Change Indicators in the United States.

Increasing Temperature and Changing Precipitation Patterns

Rising temperatures and shifting rainfall patterns are likely to increase the intensity of both floods and droughts. Average annual precipitation in the Northeast increased 10 percent from 1895 to 2011, and precipitation from extremely heavy storms has increased 70 percent since 1958. During the next century, average annual precipitation and the frequency of heavy downpours are likely to keep rising. Average precipitation is likely to increase during winter and spring, but not change significantly during summer and fall. Rising temperatures will melt snow earlier in spring and increase evaporation, and thereby dry the soil during summer and fall. So flooding is likely to be worse during winter and spring, and droughts worse during summer and fall.



In 2011, Hurricane Irene filled the Connecticut River with muddy sediment as a result of erosion upstream. Heavy storms are becoming more common as a result of climate change. Credit: NASA.

Sea Level Rise, Wetland Loss, and Coastal Flooding

Rising sea level erodes wetlands and beaches and increases damage from coastal storms. Tidal wetlands are inherently vulnerable because of their low elevations, and shoreline development prevents them from migrating inland onto higher ground. Human activities such as filling wetlands have destroyed about one third of New England's coastal wetlands since the early 1800s. Wetlands provide habitat for many bird species, such as osprey and heron, as well as several fish species. Losing coastal wetlands would harm coastal ecosystems and remove an important line of defense against coastal flooding.

Coastal cities and towns will become more vulnerable to storms in the coming century as sea level rises, shorelines erode, and storm surges become higher. Storms can destroy coastal homes, wash out highways and rail lines, and damage essential communication, energy, and wastewater management infrastructure.



Coastal marshes in Old Saybrook and nearby properties are at risk from sea level rise. © James G. Titus; used by permission.

Ecosystems and Agriculture

Changing the climate threatens ecosystems by disrupting relationships between species. Wildflowers and woody perennials are blooming—and migratory birds are arriving—sooner in spring. Not all species adjust in the same way,

however, so the food that one species needs may no longer be available when that species arrives on its migration. Warmer temperatures allow deer populations to increase, leading to a loss of forest underbrush, which makes some animals more vulnerable to predators. Rising temperatures also enable invasive species to move into areas that were previously too cold.

Climate change may also pose challenges for agriculture: Warmer temperatures cause cows to eat less and produce less milk. That could reduce the output of Connecticut's \$70-million dairy industry, which provides 13 percent of the state's farm revenue. Some farms may be harmed if more hot days and droughts reduce crop yields, or if more flooding and wetter springs delay their planting dates. Other farms may benefit from a longer growing season and the fertilizing effect of carbon dioxide.

Human Health

Changes in temperature and precipitation could increase the incidence of acute and chronic respiratory conditions such as asthma. Higher temperatures can increase the formation of ground-level ozone (smog), a pollutant that can contribute to respiratory problems. Rising temperatures may also increase the length and severity of the pollen season for plants such as ragweed—which has already been observed in other regions. Certain people are especially vulnerable, including children, the elderly, the sick, and the poor.

The risk of some diseases carried by insects may also increase. The ticks that transmit Lyme disease are active when temperatures are above 45°F, so warmer winters could lengthen the season during which ticks can become infected or people can be exposed to the ticks. Higher temperatures would also make more of New England warm enough for the Asian tiger mosquito, a common carrier of West Nile virus. The number of cases may or may not increase, depending on what people do to control insect populations and avoid insect bites.

The sources of information about climate and the impacts of climate change in this publication are: the national climate assessments by the U.S. Global Change Research Program, synthesis and assessment products by the U.S. Climate Change Science Program, assessment reports by the Intergovernmental Panel on Climate Change, and EPA's *Climate Change Indicators in the United States*. Mention of a particular season, location, species, or any other aspect of an impact does not imply anything about the likelihood or importance of aspects that are not mentioned. For more information about climate change science, impacts, responses, and what you can do, visit EPA's Climate Change website at www.epa.gov/climatechange.

Curriculum Vitae THEODORA PINOU

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Revised: March 2021

PRINCIPAL AREAS OF INTEREST:

Ecology and Evolutionary Biology of Vertebrates; Sea Turtle Epibionts; Science Education.

EDUCATION

Secondary Science Teacher Certification – 2004; Alternative Route to Certification, Biology, Connecticut Department of Higher Education. (ED125)

Ph.D. in Biology - 1993; New York University, Department of Biology.

M. Phil. - 1991; New York University, Department of Biology.

M. S. in Biology - 1988; New York University, Department of Biology.

B. A. - 1986; New York University, Department of Biology.

Bronx High School of Science, Bronx, NY, 1978-1982.

PROFESSIONAL EXPERIENCE

Academic

- Biology Department Chairman & Full Professor, Western Connecticut State University, Department of Biological and Environmental Sciences, 8/2020 present.
- -MS in Integrative Biological Diversity Program Coordinator, Western Connecticut State University, Department of Biological and Environmental Sciences, 6/2019 present.
- H. G. Dowling Herpetological Collection Faculty Curator, Western Connecticut State University, Department of Biological and Environmental Sciences, 6/2015 present.
- -Full Professor, Western Connecticut State University, Department of Biological and Environmental Sciences, 8/14 present.
- -Associate Professor, Western Connecticut State University, Department of Biological and Environmental Sciences, 8/09 –8/14.
- -Assistant Professor, Western Connecticut State University, Department of Biological and Environmental Sciences, 8/04 8/09.
- -Curatorial Affiliate in Vertebrate Zoology, Yale University, Peabody Museum of Natural History, 12/04 Present. (nominated and elected by museum curators).
- -Connecticut Science Center Fellow, Connecticut Science Center, Hartford CT, 8/08 2012.
- -Elected President, Sigma Xi WCSU Chapter, Danbury CT, 5/12 5/2018; 5/2020 current.
- -Elected Associate Director, Sigma Xi, Northeast Region, 9/17 Current

Science Education

- -Science Partner, Barnard Environmental Magnet School, New Haven, CT, 04/03-06/19.
- -Teacher Education and Mentoring Program (TEAM). Certified Mentor by the Connecticut Department of Education, June 2011.
 - -National Council for Accreditation of Teacher Education Programs (NCATE/CAEP),

National Science Teachers Association (NSTA) Reviewer for Science SPA's, Fall 2007 – 2019.

-Beginning Educator Support Certified. Certified by the Connecticut Department of Education to mentor new science teachers. August, 2005.

-Cyber- Scientist Program Developer. A Professional Development Program for inservice and pre-service science teachers, 2003 - 2010.

TEACHING EXPERIENCE

Western Connecticut State University

- (Bio100) Concepts of Biology lecture and lab (non-majors lab science course).
- (Bio107) Scientific Inquiry in the Field.
- (Bio104) Introductory Biology for Majors and Lab.
- (Bio204) Vertebrate Zoology (lab course for majors)
- (Bio 560) Herpetology (graduate course including lecture, lab, and associated field trips).
 - (Bio 480) Undergraduate Group Research Course.
- (Hon198) Debating Sustainable Agriculture. An Institute On Science for Global Policy (ISGP) partnership.
 - (ED442) Methods in Teaching Secondary Science Education.

GRADUATE STUDENTS

Western Connecticut State University

- -Stacy Evans. (2014, MA), "A comparative morphometric analysis of *Stomatolepas elegans* Costa, 1838 and *S. praegustator* Pilsbry, 1910 (Cirrepedia: Balanomorpha: Coronuloidea)."
- -Molly O'Leary. (2014, MA), "A comparison of hard and soft edges and their impact on endangered species."
- -Christine Lener. (2010, MA), "Sea Turtle Epibionts as an indicator of foraging grounds of the Loggerhead Sea Turtle (*Caretta caretta*) in Casey Key, Florida."
- -Diane Murphy, (2011, MA), "Habitat utilization and seasonal movements of a small population of box turtles (*Terrapene c. carolina*) at the northeastern periphery of their range in Dutchess Co., NY."

Yale University/ University of Crete

-Georgia Mantziou. 2006, Ph.D. "Phylogeography and population genetics of *Mauremys rivulata* (Chelonia: Geoemydidae)." Thesis committee member.

AWARDS AND EXTERNAL SUPPORT

Awards

- -Excellence in Teaching Award, The National Society of Leadership and Success (WCSU chapter), 5/2014.
 - -Norton Mezvinsky Trustees Research Awards (WCSU & System Level Award), 3/10
- -Ernest A.Lynton Award for the Scholarship of Engagement for Early Career Faculty, New England Resource Center for Higher Education, Nomination, 4/09.
 - -14 Initiative, Connecticut Science Center Fellow, 8/08

Research and Scholarship Support

National Oceanic and Atmospheric Administration (NOAA)

- Finding Our Way: Building a College Ready STEM Pipeline for At-Risk High School Youth Through Watershed Stewardship. #NA19NMF0080088 Award Amount \$306,200.00. PI-Pinou, 8/2019 10/23.
- Finding Our Way: An Experiential Watershed Learning Program for Middle School Children and their families: #NA16NMF0080003 Award Amount \$300,178.00. PI- Pinou, 4/2016

-12/2019.

Connecticut Department of Transportation (DOT)

- Edge effects of road development on the northern most range of the protected slimy salamander (*Plethodon glutinosus*). Pinou invited by Parsons Environmental Engineering Co. and The Connecticut Science Center to Co-PI, **Award** - \$25,000.00 over 5 years. 1/2010 - 2015.

National Oceanic and Atmospheric Administration (NOAA)

- Student Opportunity for Learning Grant to establish a summer camp for middle school students in collaboration with Danbury Public Schools: #NA10NES4400005 Award Amount \$250,000. PI – Pinou, 5/09 – 3/13.

Connecticut State Department of Education, Bureau of Teaching and Learning

-Mathematics and Science Partnership Grant (MSP), Award - \$250,000 over 3 years. Pinou invited to Co-PI with Danbury Public Schools, 1/2010 – 2011.

Endowment for Applied Water Quality Research

-Water Research Foundation, Award - \$45,557.00. Improving the water quality in Lake Kenosia, CT. PI-Pinou, 1/2010 - 2011.

State of Connecticut, Department of Higher Education (DHE)

-Teacher Partnership Quality Grant (TQP), Award - \$125,000.00. WestConn Institute for Science Teacher Research (WISTR). PI-Pinou, 4/06 – 6/07.

Private and Corporate Sponsors

- **-Praxair-Linde- \$5,000.** To support Graduate Student Research Training Fellowship. PI-Pinou, 01/20
- -Goldring Family Foundation, Award \$10,000.00. To support Terrapin Tracking program. Coordinator- Pinou, 05/19 -2020.
- **-Praxair Corporation- \$7,500.00.** To support STEM College Readiness programing in middle school children and their families. PI Pinou, 11/2018
- -Goldring Family Foundation, Award \$7,500.00. To support students examining relationship between post-migration patterns and genetic diversity of Olive Ridley turtles in Mexican Pacific as well as investigate the barnacle diversity associated with these turtles. PI-Pinou, 11/12 11/14.
- -Goldring Family Foundation, Award \$7,500.00. To support WCSU Great Hollow Interns. Intern Coordinator- Pinou, 05/15.
- -Center for Technological Literacy, Hofstra University, Award \$5000.00. Start-up funding to develop STEM Leadership in Public Schools. PI-Pinou, 3/09 7/09.
- -- Riverhead Foundation for Marine Research and Conservation, Award --
- **\$15,000.00.** Project Migration ARGOS Satellite Transmission Time. PI- Pinou, 7/08 7/10.
- **-Wooster School, Award \$20,000.00.** Project Migration Satellite Transmitter Sponsorship. PI- Pinou, 7/08 -7/10.
- -Crane Co. Foundation, (\$10,360.00). Project Migration Satellite Transmitter Sponsorship. PI- Pinou, 6/08 6/10.

INTERNAL FUNDING AND SUPPORT

Scholarship

WestConn Faculty Development Funds

-Total Award - \$3,200.00 over 5 years to improve instruction.

CSU/AAUP University Research Grants

- Total Award - \$65,000 over 14 years to study sea turtle behavior and their interspecies relationships.

ATTENDANCE AT INVITED WORKSHOPS

- -NOAA B-Wet Northeast Regional Meeting, October, 2019, Portland, ME.
- US Department of Education MSP Regional Conference, February, 2010, San Diego,

CA.

- NSF NOYCE Teacher Scholarship Program Proposal Writing Workshop, NSF, January 2009, Atlanta, Georgia.
- NSF MSTP STEM Symposium, Hofstra University, March 5-7, 2009, Singer Island, Florida.
- ATLAST Item Writing Workshop, Horizon Research, May 15-16, 2009, Chapel Hill, North Carolina.

SYMPOSIA WORKSHOPS ORGANIZED

- 2021 Sigma Xi Northeast Region Research Conference, WCSU, Danbury, CT, 17 April.
- 2020 Joint Meetings of Ichthyologists and Herpetologists (JMIH), Norfolk, Virginia. *Exemplary Practices in Herpetological Education;* July 20- 26. (Special Invitation).
- 2020 Northeast Natural History Conference, Stamford, CT. *Herpetology Ecology*; April 18-19. (Special Invitation).
- -2020 Science At Night, Danbury, CT. *What Do We Want From Our Lakes?* September 28th, October 26st, November 30th. https://www.wcsu.edu/biology-msbiodiversity/upcoming-events/event-recordings/
- -2019 Science At Night, Danbury, CT. What Do We Want From Our Lakes? September 30th, October 21st, November 25th.
- -2020 Science At Night, Danbury, CT. What Do We Want From Our Lakes? September 28th, October 26st, November 30th
- 2017 Annual Symposium on Sea Turtle Biology and Conservation, Las Vegas, NV. Future Trends in Sea Turtle Epibiont Research; April 15-20. (http://internationalseaturtlesociety.org/workshops/)

PODCASTS & MEDIA PRESENTATIONS

- Academic Minute - *Teaching the Environment*; October 27, 2020. (https://www.insidehighered.com/audio/2020/10/27/teaching-environment).

INVITED SEMINARS

- Cape Eleuthera Institute *Why study snakes on Cape Eleuthera, Bahamas*?; December 2019.
- New England B-Wet Regional Meeting. Gulf of Maine Research Institute, Portland, Maine *Finding Our Way*; October 29-30, 2019.
 - -State of the Candlewood Lake Conference. Sherman, CT April 22, 2018.
- Western Connecticut State University & Candlewood Lake Authority *Triploid Carp Project*; April 2017.
- Beardsley Zoo Evening Lecture Series, CT *Sea Turtle Conservation in Costa Rica*; June, 2016
- The New York Turtle and Tortoise Society, NY *Uncovering Marine Biodiversity Through Sea Turtle Conservation*; October, 2015.
- Western Connecticut State University, Department of Biological and Environmental Sciences Research Seminar, CT *Turtles and Their Barnacles. What to save first?* April, 2013.
- Framingham State University, MA *Uncovering Marine Biodiversity Through Sea Turtle Conservation*; February, 2012.
- Central Connecticut State University *Sea Turtle Conservation and Marine Health*; December, 2008.
 - University of Guadalajara (CUCBA) Sea Turtle Epibionts; October, 2007.
- University of Puerto Rico at Mayaguez *Using Technology to Understand Biodiversity*; March 2007.

- Western Connecticut State University, Sigma-XI Research Seminar *Snake Evolution and Systematics*; March, 2006.
- Western Connecticut State University, Science at Night Lecture Series *Conservation of Sea Turtles and the Organisms that Live on Them*; April, 2005.
- Yale Peabody Museum of Natural History, O. C. Marsh Lecture Series *Sea Turtle Conservation in Mexico*; March, 2005.

RESEARCH COLLABORATIONS

International

Wetlands Conservation and Island Biodiversity

- Petros Lymberakis, Ph. D., Natural History Museum of Crete-University of Crete, Greece. Topic: Island Biodiversity and Conservation.
- Aris Parmakelis, Ph.D., Department of Ecology and Taxonomy, University of Athens, Greece: Topic: Genomic Evolution of Marine Organisms.

Marine Conservation and Community Stewardship

- Ildefonso Enciso, Research Scientists in Marine Ecosystems, University of Guadalajara, CUCBA.
- Frank Paladino and Nathan Robinson, Leatherback Trust, Playa Grande, Costa Rica. Cape Eleuthera Institute, Eleuthera, Bahamas. Topic: Sea Turtle Conservation in the eastern Pacific Ocean.

National

Herpetology and Conservation

- -Eric Lazo-Wasem, Yale University, Peabody Museum of Natural History, Division of Invertebrate Zoology. Topic: Systematics of sea turtle epibionts.
- -Gisella Caccone, Ph. D., Yale University, Department of Ecology and Evolutionary Biology & Director of the YIBS Laboratory for Molecular Systematics and Conservation Genetics. Topic: Reptilian phylogenetics, molecular systematics, and conservation.

Resource Management and Stewardship

- -Rob DiGiovanni, Director, Atlantic Marine Conservation Society, Hampton Bays, NY. Topic: Sea turtle migration.
- Mark Howarth, Director, Candle Wood Lake Authority, Danbury, CT. Topic: Citizen science and Lake stewardship.
- -Ed Faison, Ecologist, Highstead Arboretum, Redding, CT. Topic: A survey of amphibians and reptiles found in Highstead Arboretum.

PUBLICATIONS

(#Student Co-Authors, ## Teacher Co-Authors)

Journal Article Preparation

Articles Under Review

Pinou, T., *Plisko, C. & LaBanca, F. [2020]. Informal Learning improves Science Motivation and Self-efficacy. Journal of STEM Outreach [under review].

Published Journal Articles

- **Pinou, T.**, Pitzrick, M., Rahaman A., Young, M.A. 2021. Letter to the Editor. JMBE. 22(1): DOI:https://doi.org/10.1128/jmbe.v22il.2199.
- Hoefer S., Robinson N.J., Mills S., **Pinou T.** 2021. What the dead tell us about the living: using roadkill to analyze diet and endoparasite prevalence in two Bahamian snakes. Copeia. Ichthyology & Herpetology 109(3):685-690.
- Hoefer S., Robinson N.J., **Pinou T.** 2021. Size matters Size matters: Sexual dimorphism in the pelvic spurs of the Bahamas Boa (*Chilabothrus strigilatus strigilatus*). Herpetology Notes 14:201-203.

- Reynolds H., **Pires J., **Lalier N., **Brewster J. & **Pinou T**. 2021. Mud as a phenomenon to model energy flow and chemical processes. Science Scope. March/April: 40-45.
- Robinson N., *Deguzman K., Bonacci-Sullivan L., DiGiovanni R., **Pinou T**. 2020. Do rehabilitated sea turtles resume typical migratory behaviors? Satellite tracking juvenile loggerhead, green, and kemp's ridley turtles in the northeastern USA. Endangered Species Research 43: 133-143, DOI.org/10.3354/esr01065.
- Monette M., **Pires J., **Lalier N., **Brewster J. & **Pinou T**. 2020. Migrating beyond the classroom: Enriching STEM education with family-based, self-directed learning. Science and Children. 2020-Jan-S&C-F-2040.R1. (Accepted)
- Robinson N.J., *Lazo-Wasem E., Butler B.O., Lazo-Wasem E.A., Zardus J.D., **Pinou, T**. 2019. Spatial distribution of epibionts on olive ridley sea turtles at Playa Ostional, Costa Rica. PLoS ONE 14(9): e0218838. https://doi.org/10.1371/journal.pone.0218838.
- Majewska, R., Bosak, S., Frankovich, T.A., Ashworth, M.P., Sullivan, M.J., Robinson, N.J., Lazo-Wasem, E. A., **Pinou, T.**, Nel, R., Van de Vijver, B. 2019. Six new epibiotic *Proschkinia* (Bacillariophyta) species and new insights into the genus phylogeny. The European Journal of Phycology, 54(4): 609-631. DOI: 10.1080/09670262.2019.1628307.
- **Pinou, T.**, Domenech, F., Lazo-Wasem, E., Majewska, R., Pfaller, J. B., Zardus, Robinson, N. J. 2019. Standardizing Sea Turtle Epibiont Sampling: Outcomes of the Epibiont Workshop at the 37th International Sea Turtle Symposium. Marine Turtle Newsletter 157:22-32.
- **Pinou, T.,** Prunier, R., *Bresson, M., Padilla, I. E., Perez, JF.J., Trejo, A., DiGiovanni, R. A. and N.J. Robinson. 2018. Repeated sampling adds to the genetic diversity of *Lepidochelys olivacea* (Eschscholtz 1829) olive ridley sea turtle. Journal of Natural History 52: 2899-2917, DOI:10.1080/00222933.2018.1557755.
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- **Pinou, T.** (2017). Book Review. *Ecoviews Too, Ecology for all Seasons*. Herpetological Review 48(3): 695 696.
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INVITED MEETING SYMPOSIA PARTICIPATION

- **Pinou, T**. (2017). Man vs Wild: Lessons on the Earth and Human Impacts. 2017 Connecticut Science Educators Annual Conference, Hamden, CT, USA, 18 November.
- **Pinou, T.** and Lazo-Wasem (2008). Epibionts from *Leidochelys olivacea*, in Jalisco, Mexico. The 28th Annual Symposium on Sea Turtle Biology and Conservation, Loreto, Baja California Sur, Mexico, 18-26 January.
- **Pinou, T.** and Lazo-Wasem (2008). Marine Turtle Epibionts Field Methods, Procedures, and Data Protocol. The 28th Annual Symposium on Sea Turtle Biology and Conservation, Loreto, Baja California Sur, Mexico, 18-26 January.
- **Pinou, T.** and Lazo-Wasem (2008). Status of the barnacle *Stomatolepas* from Jalisco, Mexico. The 28th Annual Symposium on Sea Turtle Biology and Conservation, Loreto, Baja California Sur, Mexico, 18-26 January.

ABSTRACTS OF PAPERS PRESENTED AT PROFESSIONAL MEETINGS.

- LaBanca, F., and **Pinou, T**. 2021. Impact of situated learning in informal settings on science motivation and content knowledge. Paper presented at the American Educational Research Association Annual Meeting: Science Teaching and Learning SIG, Out-of-School Science Roundtable, Washington, DC.
- **Pinou** T., Robinson N.J., Zardus J.D., Rojas L., Lazo-Wasem E. A. 2020. Microdermatoglyphic patterns of sea turtle carapaces do not offer phylogenetic insight nor explain characteristic epibiotic associations. 40th Annual International Symposium On Sea Turtle Biology and Conservation. Cartagena, Columbia. Poster presentation.
- *Schrull C., Pinou T., Connally N. 2020. Predicting Diamondback Terrapin Road Crossing Mortality Hotspots in Connecticut. 2020 Northeast Natural History Conference. Stamford, CT, USA. Oral Presentation.
- Robinson N. J., *Deguzman K., Bonacci-Sullivan L., DiGiovanni R., **Pinou T**. 2019. Satellite tracking of juvenile green, loggerhead, and Kemp's Ridley turtles from rehabilitation centers in southern New England and Long Island, USA. 2019 Long Island Sound Research Conference. Port Jefferson, NY, USA. Poster session and lightning talk.
- Robinson N. J., *Deguzman K., Bonacci-Sullivan L., DiGiovanni R.A., **Pinou T**. 2019. Satellite Tracking of Juvenile Green, Loggerhead, and Kemp's Ridley Turtles from Rehabilitation Centers in Southern New England and Long Island, USA. 39th Annual International Symposium On Sea Turtle Biology and Conservation. Charleston, South Carolina, USA. Poster presentation.
- Majewska R., Tessier, S., Scheinberg, L., Robinson, N., Lazo-Wasem, E., Rojas, L., **Pinou, T.,** Zyskowski, K., Van de Vijver, B., Hamilton, P., and Poulin, M. (2018). Zoological museum collections as a valuable source of information for epizoic diatom studies. 31st Congress of the Phycological Society of Southern Africa. Poster Presentation.
- Majewska R., Tessier, S., Scheinberg, L., Robinson, N., Lazo-Wasem, E., Rojas, L., **Pinou, T.,** Zyskowski, K., Van de Vijver, B., Hamilton, P., and Poulin, M. (2018). A forgotten treasure: how zoological museum collections can contribute to epizoic diatom research. International Diatom Symposium. Berlin. Oral Presentation.
- Majewska R., Robinson N.J., Lazo-Wasem E.A., Nel R., Paladino F.V., Rojas L., Zardus J.D., **Pinou T.** (2017). Diatoms on sea turtles: a summary of recent discoveries. 37th Annual Symposium on Sea Turtle Biology and Conservation. Las Vegas, U.S.A. Oral presentation.
- Robinson, N.J., Majewska, R., Nel, R., Paladino, F.V., Rojas, L., Zardus, J., **Pinou, T**. (2016). Epibiotic diatoms are universally present on all sea turtle species. 36th Annual Symposium on Sea Turtle Biology and Conservation. Lima, Peru. Poster presentation.
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