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Viewpoints

Myristate and the ecology of AM fungi: significance, opportunities, applications and challenges

Summary

A recent study by Sugiura and coworkers reported the nonsymbiotic growth and spore production of an arbuscular mycorrhizal (AM) fungus, *Rhizophagus irregularis*, when the fungus received an external supply of certain fatty acids, myristates (C:14). This discovery follows the insight that AM fungi receive fatty acids from their hosts when in symbiosis. If this result holds up and can be repeated under nonsterile conditions and with a broader range of fungi, it has numerous consequences for our understanding of AM fungal ecology, from the level of the fungus, at the plant community level, and to functional consequences in ecosystems. In addition, myristate may open up several avenues from a more applied perspective, including improved fungal culture and supplementation of AM fungi or inoculum in the field. We here map these potential opportunities, and additionally offer thoughts on potential risks of this potentially new technology. Lastly, we discuss the specific research challenges that need to be overcome to come to an understanding of the potential role of myristate in AM ecology.

Background: arbuscular mycorrhizal fungi complete lifecycle nonsymbiotically in the presence of myristate

Recent years have seen a step increase in our understanding of the biology of the nutrient and carbon exchange between arbuscular mycorrhizal (AM) fungi and their host plant (Jiang *et al.*, 2017; Keymer *et al.*, 2017; Luginbuehl *et al.*, 2017). In addition to hexoses, plants transfer lipids to their fungal partners, the latter apparently lacking genes for their own biosynthesis of long-chain fatty acids. These results mean that AM fungi require an external supply of lipids. Recently, Sugiura *et al.* (2019), in a study published as a preprint on *bioRxiv*, provided evidence that AM fungi can complete their life cycle in the absence of a host when supplied with certain fatty acids, myristates (C:14, a common organic acid in plant root exudates; Li *et al.*, 2017), in a variety of formulations (also see an earlier study on fatty acid effects on AM fungal growth by the same group; Kameoka *et al.*, 2019). The significance of this finding for understanding the biology of these fungi and for their independent culture are immediately apparent. But what might this mean for the ecology of AM fungi, and what opportunities (and challenges) are there for ecological applications?

Before addressing these questions, it needs to be acknowledged that the study by Sugiura *et al.* (2019) was a pioneering study. As such, it needs to still be independently verified as it was carried out with only one isolate, *Rhizophagus irregularis*, under controlled laboratory conditions. Spores produced symbiotically and on myristate-cultured AM fungi differed also in traits, most notably size. There might also be other differences in hyphal physiological functions. Much of the following discussion hinges on further corroboration of these findings outside the Petri dish, addressing questions such as: can the action of myristate be confirmed under sterile and nonsterile conditions? How does myristate interact with soil minerals and how does that change its persistence? Does this also apply to other AM fungi? How reflective of symbiotic growth is fungal physiology and ecology that unfolds in the asymbiotic stage? Yet, this finding is fascinating, if it holds, and with these caveats in mind, we here explore the significance and opportunities this might offer for ecological research on AM fungi, and for potential applications (Fig. 1).

Significance and opportunities

Ecological questions and implications

There are several potential implications predominantly for the ecology of the AM fungi themselves, but also for plant communities, and for ecosystem roles of AM fungi. These all center on the use of external, nonsymbiotically obtained myristate as a carbon source by the fungal individual. Effects of myristate at the level of the individual fungus or fungal community could have consequences at other levels of the ecological hierarchy, the plant community and the ecosystem. We discuss these effects in the following in this order.

Fungal perspective An exciting opportunity is to examine and study AM fungal mycelium and spore traits in the absence of a host plant, opening the path towards a host plant-independent ecology of AM fungi (with the caveat that asymbiotic spores differed from those produced by symbiotic mycelium). Studying direct fungal responses to environmental drivers would be a step change in mechanistically dissecting symbiotic responses. This could entail arena competition experiments in the absence of a host, studies examining community assembly, biotic interactions (e.g. grazing, association with prokaryotes), diversity–ecosystem function relationships, and fungal stoichiometric flexibility (e.g. carbon and nitrogen efficiency) – simply many basic ecological aspects that have so far been challenging or impossible to answer because the host exerts an effect as well. There are a range of questions that arise

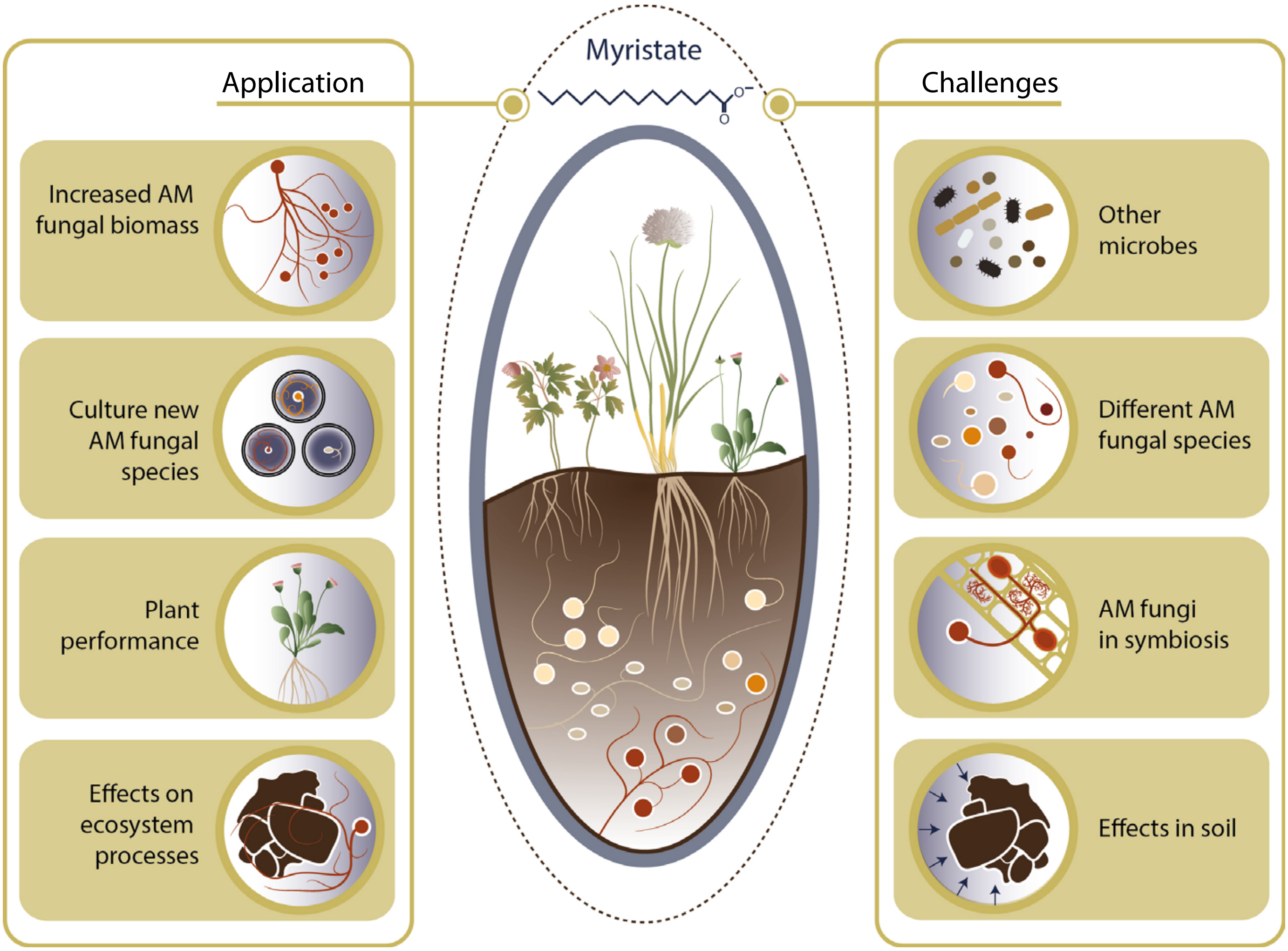

Fig 1 Potential applications of myristate (in agriculture, restoration and forestry) and challenges that await researchers. Potential benefits of applying myristate could include an increased arbuscular mycorrhizal (AM) fungal biomass in soil, the culture of new AM fungal species, enhanced plant performance, and beneficial effects on ecosystems process (including soil aggregation). Challenges include the following: other microbes may utilize myristate added to soil before AM fungi can access it; AM fungi may differentially benefit from myristate addition (and not necessarily the most beneficial genotypes may profit); it is unclear if AM fungi in a symbiosis with plants can access myristate (as this has so far only been shown for nonsymbiotic fungi); and myristate may have either nontarget effects on soil (including toxic effects) or may be rendered ineffective by the soil environment (e.g. made unavailable due to sorption to soil surfaces).

from the fact that AM fungi may be able to tap into nonsymbiotic carbon sources (Table 1).

Plant community At the plant community level, it could be relevant to ask if plant species have different myristate concentrations in their root exudates; for example, are there differences in myristate in nonmycorrhizal and mycorrhizal plants, and are there differences within mycorrhizal plants that contribute to explaining host preference patterns? Certainly, plant community composition could respond strongly to local myristate availability if this subsidizes AM fungi that are important for certain host plants in a given plant community (Powell & Rillig, 2018). Thus, myristate production could be a potentially important additional trait to include in plant trait-based ecology in the future.

Ecosystem roles Our understanding of ecosystem roles and functions of AM fungi could also be affected if AM fungi can tap into carbon sources other than directly from the host plant (e.g. decaying plant material, litter, other microbes). AM fungi may be making contributions to ecosystem processes that are independent of direct access to host plant carbon, for example through their positive effects on soil aggregation and carbon storage. In addition, AM fungal respiration and stoichiometry are likely to be partially independent of host plant carbon supply and nutrient demands, with variation within and among AM fungal species likely to be functionally important within ecosystems (Powell & Rillig, 2018; Riley *et al.*, 2019); myristate may provide the means to obtain direct measures of this variation. An interesting question is if net soil carbon budgets will change when AM fungi switch for symbiotically obtained to nonsymbiotically obtained carbon; a question that needs to be addressed with isotopic labeling. In the context of global change it will also be important to establish whether drivers of global change (such as warming, precipitation change) can affect myristate production and thus AM fungi abundance; this would

Table 1 Potential implications of the use of external myristate as a carbon source for the ecology of arbuscular mycorrhizal (AM) fungi.

Question/opportunity	Explanation	Approach
Can AM fungi use myristate to survive extreme situations (e.g. in the absence of a host)?	AM fungi might be able to compensate for loss of supply in host carbon, or might supplement this symbiotic supply during ecological crunches	Confront AM fungal mycelia with extreme stress situations (host death, drought) in the presence and absence of myristate in the growth substrate
Do less symbiotic AM fungi have a preference for use of external carbon, such as myristate?	AM fungi exist on a gradient of interactions with host plants, but what factors control this outcome are not always clear. Do some fungi exhibit preference for host carbon vs myristate?	Test for differences in myristate responsiveness among AM fungal isolates
Are there nonsymbiotic AM fungi in nature?	Have some AM fungi adopted a nonsymbiotic lifestyle, depending exclusively on external carbon, such as myristate?	High-throughput attempt to bring AM fungi into culture on myristate-containing media in the absence of a host
Is myristate use an ancient character shared by other early diverging fungi (e.g. Mortierella)? How is this trait phylogenetically distributed?	If the trait is shared with other fungi, competition for myristates is likely, but this could be a legacy trait in AM fungi that have specialized on symbiosis with plants. If the trait is not shared, this would imply niche partitioning at deep phylogenetic depth	Test myristate use in pure culture and in soil for a range of soil fungi and analyze phylogenetic distribution
Do AM fungi display chemotaxis towards myristate?	Do AM fungi respond to a myristate gradient and is there a concentration at which they stop growing towards the source; myristic acid could serve as a semiochemical attractant to induce colonization and infection of *Rhizophagus solanacearum* (Li *et al.*, 2017)	*In vitro* culture studies offering a gradient of myristate in the medium
How important are mixes of fatty acids for AM fungi? Do different AM fungi species perform better with different fatty acids supplies?	This could be a mechanism of resource partitioning, which could help explain high diversity in AM fungi communities, despite the (alleged) high niche overlap	Screen different isolates of AM fungi for growth responses to myristate and other fatty acids, as well as to fatty acid diversity and composition
Do we underestimate the importance of AM fungi in the rhizosphere by only considering root colonization?	Nonmycorrhizal plants may still stimulate AM fungal growth when exuding myristate	Shift focus in experiments to include nonsymbiotic growth of AM fungi in the rhizosphere of host and nonhost plants
Can we better estimate carbon cost and nutrient economy of the AM fungal mycelium?	It is difficult to discern inherent aspects of AM fungal mycelium construction and metabolism in the presence of roots and other soil biota	Build up AM fungal mycelium without a host and under axenic conditions, then measure biomass CNP and respiration under variable carbon (myristate concentration) and nutrient supply rates
How is myristate use correlated with other AM fungal traits such as root and soil colonization?	The presence of myristate could induce changes in root/soil colonization by the fungus if dependence on symbiotic root carbon transfer is reduced	Assess root and soil colonization with or without myristate supply. Test correlation between myristate use and soil/root colonization for different AM fungi

open an avenue to test for a novel mechanism explaining responses of soil biota to global change. Myristate application might also facilitate factorial experiments investigating the indirect effects (via impacts on AM fungi) of a variety of environmental drivers on plants and soil properties by providing a means of manipulating AM fungal biomass in soil independently of the host.

Opportunities for application of myristate to AM fungi

The most immediately obvious applied consequence of myristate use by AM fungi is that this may help get a wide range of AM fungi into culture in artificial media (or in soil). Clearly, AM fungal ecology (Powell & Rillig, 2018) and application in agriculture, forestry and restoration have been limited by having only relatively few strains reliably in culture. In particular, it is possible that the use of a host plant (typically just a few host plants are used) acts as a filter for certain fungi. This means that certain species may be successfully cultured using myristate for which certain aspects of host plant biochemistry or other environmental factors may now represent a barrier, even though myristate-mediated culture may reveal additional filters to cultivation. A positive consequence of substantially increasing culturability of AM fungi could be increased availability of local genoytypes for subsequent use in inoculation approaches, thus reducing risks associated with the spread of foreign inocula (Schwartz *et al.*, 2006). Furthermore, if introduced as an inoculum under field or glasshouse conditions, germination and subsequent infection rates of AM fungal spores are often quite low. Myristate could be used to stimulate spore germination before application, thus producing an 'activated' inoculum with a potentially higher colonization rate in the field.

An equally exciting possibility is to enhance the build-up of AM fungal biomass *in situ*, reducing the need for inoculation. This could be achieved by adding myristates, in suitable formulation, directly to the soil, where it could then potentially be taken up by AM fungal hyphae and used for biomass production. This could certainly be directly applicable in agriculture, where often AM fungal abundance is suppressed by a range of management practices (e.g. Rillig *et al.*, 2016). This way, several of AM-fungus mediated host-independent ecosystem functions, such as soil aggregation, could also be directly stimulated, and thus also carbon sequestration and long-term availability of nutrients. Also, adding myristate at the same time as fertilizer on agricultural fields could reduce stress on the crop plant, by potentially preventing AM fungi from becoming a large carbon drain, while increasing plant access to water and nutrients. Additionally, myristate addition could also help bridge fallow-time related stress on AM fungi. A completely different application in an agricultural context could relate to breeding the crop plant for myristate production to generate AM fungi-'friendly' plants and varieties.

Very similar arguments could be made for AM fungal applications in a restoration context. Soil application of myristate, if successful, could enhance restoration success, particularly during ecological crunches in the absence of a host. This could be particularly important in more challenging environments (e.g. drylands or strongly degraded habitats) where recovery processes are particularly slow (Whisenant, 2001; Cortina *et al.*, 2011).

Challenges ahead, and potential risks

A big research challenge evident from the discussion earlier relates to if and how myristate would work in nonsterile field soil (Fig. 1). Can it be accessed by AM fungi from the environment, for example when added to a soil, or will it be quickly metabolized by other microbes? Can the mode of delivery and the formulation make a difference, and are there materials with a high content of myristate that could be cheaper alternatives?

With any new technology come potential risks and unforeseen consequences. If myristate will be used in agriculture, restoration or forestry, there could be risks that are worth considering and examining from the outset.

Scientific risks are in interpreting the results of experiments carried out with myristate: how relevant are they to the real world, and how much is artifact? Certainly, results obtained from the nonsymbiotic growth of AM fungi in the laboratory will need to be interpreted with caution.

Biological and ecological risks are also important to consider, most urgently regarding nontarget effects on other organism groups or processes in soil. It is also extremely likely that not all AM fungi will equally benefit from a potential soil application, and are these community changes desirable or not for processes of interest (promoting plant growth, soil aggregation, etc.)? An additional uncertainty is potential toxicity of the substance to nontarget organisms, including humans; this would need to be carefully tested using ecotoxicological approaches. A somewhat less obvious risk concerns potential evolutionary changes in the AM fungi themselves; could they become less mutualistic after prolonged application of myristate? Would such evolutionary outcomes (loss of mutualistic traits) differ depending on the species/genotype in question, that is their position on the mutualist-parasite continuum (Johnson *et al.*, 1997)? Other unforeseen consequences could include the inadvertent promotion of invasive mycorrhizal plant species, or the promotion of nonnative AM fungal species.

As with the regular use of AM fungal inoculants in agriculture, myristate applications risk becoming maintenance repairs that preclude more transformational changes in how we sustainably manage (agro-)ecosystems (Henke, 2008). Such technological fixes often expressly avoid the difficult questions of how to change socially, economically and politically entrenched practices of industrial agriculture, those practices that decimated AM fungal communities in the first place. In order to institute transformational repair, myristate applications would have to be supplemented with agricultural practices known to ameliorate damaged agro-ecologies (e.g. reduced fallow periods, greater crop diversity via intercropping or enhanced crop rotation). It should be clear that interventions with myristates (or other substances) can only be used for limited periods of time to manage transitions or for the purposes of speeding up recovery. They can be no substitute for sustainably managing soil biodiversity. In this way, myristate applications (used with AM inoculants) contain great potential for reforestation or afforestation efforts, or for the restoration of sensitive flora in challenging environments.

Conclusions

It is clear that myristate, if its effects are confirmed, could offer plenty of opportunities that await the mycorrhizal ecologist, a field that is traditionally often viewed as limited by methods and experimental approaches. Mycorrhizal ecologists could add a new, exciting tool for experimentation in the laboratory and, most importantly, in the field. It will be interesting to observe how this topic develops, and also if new applications for arbuscular mycorrhizas in agriculture, forestry and restoration emerge.

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Author contributions

MCR wrote the first draft of this article. All authors contributed ideas and helped write the final version. AL designed the figure.

ORCID

Joana Bergmann https://orcid.org/0000-0002-2008-4198
V. Bala Chaudhary https://orcid.org/0000-0002-7232-1757
Coline Deveautour https://orcid.org/0000-0001-6887-0414
Jeff R. Powell https://orcid.org/0000-0003-1091-2452
Matthias C. Rillig https://orcid.org/0000-0003-3541-7853
Julien Roy https://orcid.org/0000-0003-2964-1314

Matthias C. Rillig[1,2*], Carlos A. Aguilar-Trigueros[1,2], Ian C. Anderson[3], Janis Antonovics[4], Max-Bernhard Ballhausen[1,2], Joana Bergmann[1,2], Milos Bielcik[1,2], V. Bala Chaudhary[5], Coline Deveautour[3,6,7], Leonie Grünfeld[1,2], Stefan Hempel[1,2], Milica Lakovic[1,2], Daniel R. Lammel[1,2], Anika Lehmann[1,2], Johannes Lehmann[8,9], Eva F. Leifheit[1,2], Yun Liang[1,2], Erqin Li[1,2], Yudi M. Lozano[1,2], Annette Manntschke[1,2], India Mansour[1,2], Peter Oviatt[10], Liliana Pinek[1,2], Jeff R. Powell[3], Julien Roy[1,2], Masahiro Ryo[1,2], Moisés A. Sosa-Hernández[1,2], Stavros D. Veresoglou[1,2], Dongwei Wang[1,2], Gaowen Yang[1,2] and Haiyang Zhang[3]

[1]Institut für Biologie, Freie Universität Berlin, Altensteinstr. 6, D-14195, Berlin, Germany;
[2]Berlin-Brandenburg Institute of Advanced Biodiversity Research, D-14195, Berlin, Germany;
[3]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia;
[4]Department of Biology, University of Virginia, Charlottesville, VA 22904, USA;
[5]Department of Environmental Science and Studies, DePaul University, Chicago, IL 60614, USA;
[6]National University of Ireland, University Road, Galway, H91 TK33, Ireland;
[7]Environment, Soils and Land-Use Department, Teagasc, Johnstown Castle, Y35 Y521, Co. Wexford, Ireland;
[8]School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, USA;
[9]Atkinson Center for a Sustainable Future, Cornell University, Ithaca, NY 14853, USA;
[10]Program in History, Anthropology and Science and Technology Studies, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
(*Author for correspondence: tel +49 30 838 53165; email matthias.rillig@fu-berlin.de)

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