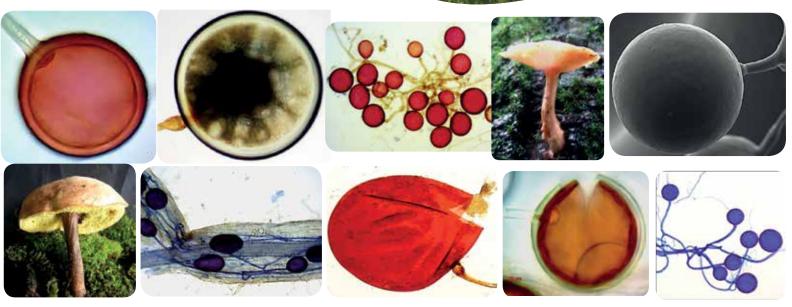
IYCORRHIZA NEW The Quarterly Newsletter of Mycorrhiza Network

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About **TERI**

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The Energy and Resources Institute (TERI) is a dynamic and flexible organization with a global vision and a local focus. TERI's focus is on research in the fields of energy, environment, and sustainable development, and on documentation and information dissemination. The genesis of these activities lies in TERI's firm belief that the efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

TERI's Mycorrhiza Network

TERI's Mycorrhiza Network is primarily responsible for establishing the Mycorrhiza Information Centre (MIC), the Centre for Mycorrhiza **Culture Collection** (CMCC), and publishing Mycorrhiza News. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

Mycorrhiza News

The Mycorrhiza News provides a forum for the dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

For further information, visit www.mycorrhizae.org.in

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RESEARCH FINDING PAPER

Investigation of Community Structure and Arbuscular Mycorrhizal Association in a Dry Mixed Deciduous Forest and a Sal Forest in South–West Bengal

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Abstract

Arbuscular Mycorrhizal Fungi (AMF) are functionally an integral part of the plant community. Both AMF and the plant community are interdependent. In our investigation in the dry mixed deciduous and Sal forests, we explored this relationship through community study and mycorrhizal status of plants. Plant species distribution in both forests differed significantly. Maximum mycorrhizal infection, spore population and spore density was found in the mixed forest species. Intensity of infection in most of the species from mixed forest was very high with arbuscules, vesicles and intraradical spores. In Sal dominated forest, plants were found to be fairly infected, but intensity of colonization and spore densities were poor.

Introduction

Stress conditions, such as hormonal and nutrition imbalance (Razaq, Zhang, Shen, Salahuddin, 2017), ion toxicity, physiological disorders and pathogen attacks, (de Souza, Granada and Sperotto 2016) affect plant growth and its survival. For ensuring survival, 80% vascular plants form symbiotic associations with soil inhabiting fungi known as Arbuscular Mycorrhizal Fungi (AMF). These mycorrhizal fungi form a network of filaments at the plant root-soil interface, thereby, enhancing the growth and survival of numerous plant species through improved uptake of water (Auge 2016) and nutrients (Guissou, Babana, Sanon, and Ba 2016). Plant community and AMF are interdependent and influence each other's community structure (Heijden, Marcel, Sanders, et al. 2003; Lin, McCormack and Guo 2015). Not only do arbuscular mycorrhizae (AM) define the community, but also play an important

role in plant succession (Smith and Read 1997). In the field, besides plant composition, soil factors are also influencers of AM (Johnson, Tilman, and Wedin 1992; Klironomos, McCune, Hart 2000; Bainard, Klironomos, Gordon 2011). Likewise, AM also have a role in plant succession and in defining the community (Smith and Read 1997). This is a vast and diverse area that differs with different ecosystems. In our study, we looked for the relationship between two forest types (dry mixed deciduous and Sal) in the same locality and climate (West Bengal) through plant community structure, soil physicochemical properties, root colonization status and rhizospheric spore density of AMF, as both soil and the plant community influence AM.

Materials and Methods

Sample collection

The study was conducted in January 2019, in the dry Mixed deciduous Forest of Anandapur, Paschim Medinipir, West Bengal, (22.3347° N and 87.2433° E) and Sal forest of Jhargram, West Bengal, (22.4550° N and 86.9974° E). Community study of the forest was done using five randomly placed quadrats (10 m² each). Frequency, density, and abundance of the selected plant species were measured. From each quadrat, rhizospheric soil and plant root samples were collected up to a depth of 30 cm. In the mixed forest, root and soil samples were collected in airtight polythene bags from Acacia auriculiformis, Azadirachta indica, Diospyros melanoxylon, Mimosa pudica, Shorea robusta, and Syzygium cumini. In the Sal forest, samples were collected from Combretum decandrum, Cyperus rotundus, Demostachya bipinnata, Diospyros melanoxylon, Hemidesmus indicus, Madhuca longifolia,

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Meyna laxiflora, and *Shorea robusta* and composite samples were prepared. These were then transported to the laboratory for various analyses.

Mycorrhiza Studies

Roots were cut into 1 cm segments and treated with 10% potassium hydroxide (KOH) solution, at 15 lb pressure for 10 minutes in an autoclave, decolourized with ammoniacal hydrogen peroxide (H_2O_2) and then stained with 0.05% trypan blue (Phillips and Hayman 1970). Total root colonization was calculated using the equation:

AM Colonization (%) =
$$\left(\frac{\text{Number of infected root segments}}{\text{Total Number of root segments}}\right) \times 100$$

Intensity of colonization was studied using the method followed by Kormanik (Kormanik 1980). The following Three classes were defined as per pattern of colonization:

Class I: Patchy infection in the root segment **Class II:** Parallel infection on both sides of the stele **Class III:** No separate cell without infection Percentage of each intensity class was determined for the root segments. Arbuscular mycorrhizal (AM) spores were isolated by the wet sieving and decanting method (Gerdemann and Nicolson 1963).

Analysis of Physico-chemical Properties

The soil samples were tested for nitrogen, phosphorus, and potassium (NPK) using soil analyses kits, employing methods followed by Jackson (Jackson 1973). The organic carbon content was determined using the method followed by Walkley and Black (Walkley and Black 1934).

Statistical Analysis

Simpson's Diversity index for both the forests was calculated using the equation:

Simpson's Diversity index (D) =
$$\frac{\sum n(n-1)}{N(N-1)}$$

Where, N = total number of individuals in an area;n = number of individuals of a species.The similarity between the two forests was calculatedusing the equation:

Simple Matching Index (S) =
$$\frac{2C(100)}{(a+b)}$$

Where, C= common species; a = number of species in Community 1 (mixed forest); b = number of species in Community 2 (Sal forest).

Results and Discussion

In the quadrat study of the dry mixed deciduous forest (Figure 1), the species with maximum frequency and density was found to be *S. robusta* followed by *A. auriculiformis.* The species that were least in frequency and density were *D. melanoxylon, A. indica* and *S. cumini.* Among the shrubs, *M. pudica* showed maximum shrub abundance while *D. melanoxylon* showed the least abundance. Simpson's Diversity index for the mixed forest was found to be 0.2622.

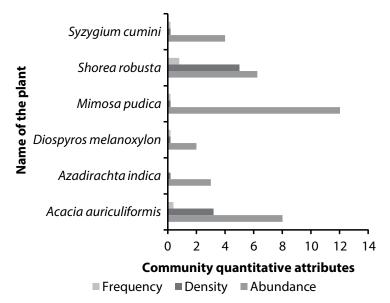
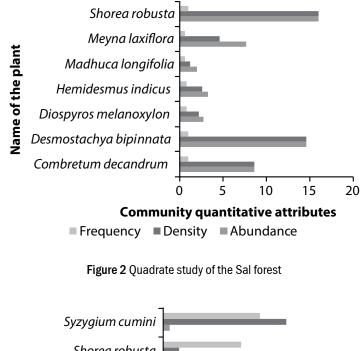


Figure 1 Quadrate study of the dry mixed deciduous forest

Frequency, density, and abundance of the trees in the Sal forest are depicted in Figure 2. Maximum abundance and density was found to be that of *S. robusta*, which was much higher when compared to the other two subdominants—*D. melanoxylon* and *M. longifolia*. Maximum frequency was found to be that of *C. decandrum* and *S. robusta*. *M. longifolia* was found to be the least abundant species with the least density. Among the weeds, maximum abundance and frequency were that of *D. bipinnata*, followed by *C. rotundus*. Simpson's Diversity index for the Sal forest was found to be 0.2296. The similarity between the two forests (mixed and Sal) by the Simple Matching Index was found to be 0.18. S. *robusta* and *D. melanoxylon* were present in both the forests.

Marked difference was observed in the diversity, spore population and density (per 100 g soil) of AMF species, in the two forests (Figures 3 and 4).

The diversity of AMF species, spore population and density per 100 g soil in the mixed forest were found to be higher than that of the Sal forest. Spores ranging between sizes 90 µm and 180 µm were dominant in the Sal forest. In the mixed forest, spores ranging between 90 µm and 180 µm were dominant in the rhizospheres of A. indica, and S. cumini, and those between 50 µm and 90 µm were found to be dominant in the rhizospheres of D. melanoxylon, M. pudica and S. robusta. In A. auriculiformis spores of both the size ranges were almost codominant. Maximum number of spores of sizes 90–180 µm and 180–300 µm were observed with A. indica. When compared to the mixed forest, large (180-300 µm) spores were more in number in the Sal forest. The number of medium-sized (90-180 µm) spores was more in the mixed forest (more than 200, except the Sal rhizosphere).



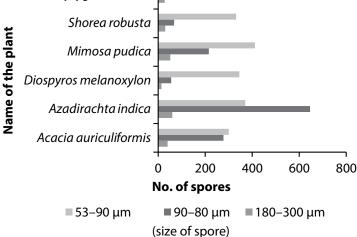


Figure 3 Spore counting in the dry mixed deciduous forest

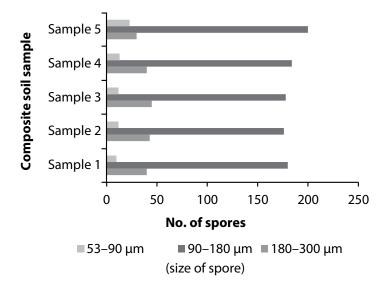


Figure 4 Spore counting in the Sal rhizospheric soil samples

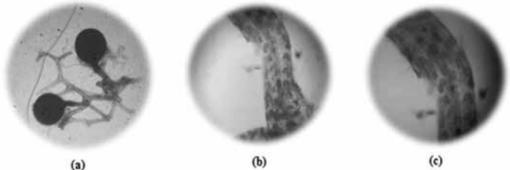
AM root colonization and intensity of colonization showed similar trends in the two forests (Tables 1 and 2). Both *S. robusta* and *D. melanoxylon* showed AMF colonization, with higher percentage found in the mixed forest. However, the intensity of AMF colonization exhibited by the Sal forest was highest. It is interesting to note, in both forest types, sporulation did not correlate with colonization. In general, three species in the genus Acaulospora, three in Glomus, one in Sclerocystis, Gigaspora margarita, G. heterogama, G. gigantea, and Scutellospora nigra were found. Glomus sporocarp was profuse in the rhizosphere of A. indica and S. nigra was found only in rhizosphere of Acacia and Syzygium (Figure 5).

S. No.	Plant	Vesicle (%)	Arbuscule (%)	Mycelium (only) (%)	Colonization (%)	Intensi	ty class (%	6)
						Ι	II	III
1	Acacia auriculiformis	88	84	6	96	10	80	6
2	Azadirachta indica	76	60	4	92	-	12	80
3	Diospyros melanoxylon	92	76	4	100	-	10	90
4	Mimosa pudica	64	47	4	80	-	80	-
5	Shorea robusta	92	88	6	100	20	60	20
6	Syzygium cumini	96	76	4	100	16	60	24

Table 1 Mycorrhizal colonization and intensity percentages in the dry mixed deciduous forest

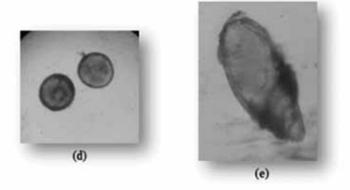
S. No.	Plant	Vesicle (%)	Arbuscule (%)	Mycelium (only) (%)	Colonization (%)	Intensity class (%)		
3. NU.	Flant				COIOIIIZAUOII (%)	Ι	II	III
1	Shorea robusta	66	70	12	84	12	12	50
2	Madhuca longifolia	66	72	8	80	8	60	12
3	Diospyros melanoxylon	90	78	8	90	6	70	14
4	Combretum decandrum	80	78	6	90	10	40	40
5	Desmostachya bipinnata	78	76	10	88	16	60	12
6	Meyna laxiflora	72	72	6	82	30	40	2
7	Hemidesmus indicus	84	78	4	88	40	40	8

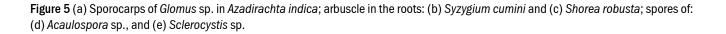
Table 2 Mycorrhizal colonization and intensity percentages in Sal forest



(a)







It can be concluded that there were differences in soil characteristics between the two forests (Table 3). pH, moisture, P, N, K, and organic carbon had an influence on the AM symbiosis. Improved nitrogen content in the rhizosphere has been known to influence the AM community (Lonhienne, Yeoh, Kasinadhuni, et al. 2015). Controlled burning and high-potash concentration in Sal forests are mainly attributed for the difference in various physicochemical parameters as well as the AMF population (Neary, Klopatek, DeBano, et al. 1999). In Sal forests, controlled burning is done on a regular Lin, McCormack and Guo 2015), in our study we found the plant influence is notable on the AMF community as well. In fact it has been found to be more influential than soil conditions (Krüger, Petr, Martina, et al. 2017).

Acknowledgements

The authors are thankful to Rasthriya Uchchatara Siksa Abhiyan (RUSA) for financial assistance and Mr G. C. Bera, Principal, Midnapore College, for supporting the research.

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that some species were favored by particular species of	Gerdemann J. W. and Nicolson T. H. 1963. Spores of
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by the AM flora found in the annuals in different	sieving and decanting. Transactions of the British Mycological
forests (Torrecillas, Alguacil, and Roldán 2012); and	Society. 46 (2): 235–244
in turn have an effect on the productivity and soil	Guissou T., Babana A. T., Sanon K. B., and Ba A.M. 2016.

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Table 3 Soil properties of the dry mixed deciduous and Sal forests

Forest	рН	Moisture (%)	Available P (P ₂ O ₅ kg/hectare)	Total N (g/ 100 g)	K content (K ₂ 0 kg/ hectare)	Organic carbon content (%)	Number of AM species
Dry mixed deciduous	5.8	4.8	203	0.18	490	0.036	11
Sal	5.5	2.3	172	0.11	519	0.020	6

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basis and this leads to hardening of the soil. Many studies have found an overall decrease in mycorrhizal colonization after occurrence of deliberate fire episodes (Dhillion, Anderson, Liberta, 1988). The removal of organic matter from the soil surface and the topsoil (by forest fires) brings about changes in the soil structure. These changes include decreased soil pore size (which could lead to increased surface water runoff and erosion) and reduced soil water retention (Neary, Klopatek, DeBano, et al. 1999).

Generally, soil nutrients and moisture could influence the difference (Johnson, Tilman, and Wedin 1992) in mycotrophy between the forests under consideration; plant community too has a role to play (Liu, Plenchette, and Hamel 2007). Also under different conditions, plants tend to associate with different AMF (Bainard, Klironomos, Gordon, 2011). In this case, plant species from the same community differed in colonization pattern, AM association and intensity of colonization. It could also be observed structure (by promoting diversity) (Kirolomonus 2000; Koide and Dickie 2002). Though researchers have a strong opinion that AM influences the plant communities (Heijden, Marcel, Sanders, et al. 2003;

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News HIGHLIGHTS



Soil fungi and rare-species advantage

Scientists have identified the mechanism regulating species coexistence in a subtropical forest. Trees at the Gutianshan National Nature Reserve in Zhejiang province, China, have been found to be immune to pathogenic fungal infection even when growing close to infected same-species neighbours. Ectomycorrhizal trees were found to have slower pathogenic fungal accumulation rates and a weaker rare-species advantage while it was found to be the other way around in trees associated with arbuscular mycorrhizal fungi.¹

Bid goodbye to fertilizers

According to a new study published in *Global Change Biology*, wheat's uptake of key nutrients could be boosted and new 'climate smart' varieties of crops could be produced through the addition of fungi. Researchers have demonstrated that this partnership could be utilized to develop new farming systems, especially ones which are less reliant on fertilizers.²

A symbiotic boost for tomato

Increase in the expression of a particular gene, belonging to a family of genes responsible for removing sodium from cells, due to the colonization of a symbiotic fungus has been shown to increase salt tolerance in tomato plants.³

Slowing down climate change

Plant-ectomycorrhizal fungal symbioses play a key role in sequestering carbon in soils and hence restoring these ecosystems could be one of the strategies of slowing climate change, says a study published in *Nature Communications.*⁴

In the Myristica swamps

An ancient ecosystem dominated by evergreen trees is known to be a haven for species endemic to the Western Ghats. A study published in the journal Phytotaxa talks about the molecular analysis of a recently discovered fungus (from the swamp), that revealed it to be a new species of the genus *Laccaria*.⁵

Seaweeds and mycorrhizae

A graduare student from Dalhousie University, Canada, has been working on how seaweed biostimulants affect arbuscular mycorrhizal fungi. "In my project I looked at how the biostimulant directly affects the growth of mycorrhizal fungi and how it affects the plant–microbe communication on a phenotypic— measuring how much the mycorrhizae is colonizing the plant—and on a molecular level", explains Sarah.⁶

Genomic gymnastics

A study published in the *Proceedings of the National Academy of Sciences* provides the first detailed look at how *Sorghum bicolor* (L.) Moench exercises exquisite control over its genome to survive in challenging surroundings. The plant was found to switch on and off some of the genes responsible for various life processes (for example, symbiotic associations and photosynthesis) whenever necessary.⁷

Supplying the appropriate microbe

A study carried out at the coniferous forests of Tangmarg, Mammar and Pahalgam explains the use of microorganisms (such as ectomycorrhizal fungi) at early stages of seedling development. Ectomycorrhizal fungi increase seedling vigour when resources are limited and enhance the competitive ability of the seedlings during establishment.⁸

¹ Details available at https://phys.org/news/

² Details available at https://www.sciencedaily.com

³ Details available at https://phys.org/news/

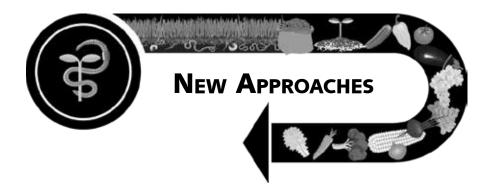
⁴ Details available at https://www.sciencedaily.com

⁵ Details available at https://www.cnbctv18.com/

⁶ Details available at https://www.dal.ca/news/

⁷ Details available at https://www.sciencedaily.com

⁸ Details available at https://www.thekashmirmonitor.net/



New Approaches and Techniques Counting Arbuscular Mycorrhizal Fungal Spores Using a Semi-automated System

Scientists have already established that the presence and quantity of Arbuscular Mycorrhizal Fungal (AMF) spores in a certain area can be determined through manual counting by observing samples under a microscope. This counting process has been found to be prone to errors because of the presence of mineral and organic particles, which not only make it a tedious task but also one that requires a great amount of attention and expertise. A semi-automated counting model, for counting AMF spores in microscope images, based on Artificial Neural Network (ANN) has been introduced. In this hybrid method, the images are processed through Circle Hough Transform (CHT) method (an online method) and classified automatically according to the pattern recognized by a trained ANN (a conventional method). The method not only counts the elements that appear in the images, but also classifies them as 'spores' and 'non spores'. The proposed System for Detection, Identification, and Counting (SDIC), algorithm relies on 'shape' information to obtain sub-images of AMF spores and elements similar to them. While spore extraction, image acquisition, and identification and classification by experts are the manual steps of the system, preprocessing steps and ANN classification are the automated phases. After extracting the spores using Wet Sieving Process (WSP), the images are obtained through microscopy (and high-resolution cameras).

An image containing the objects to be identified must be preprocessed (segmented) through an edge detection filter and a binarization filter. The patterns of possible spores located by CHT in the original image are cut into sub-images (in RGB) and some of the sub-images are resized by standardizing the data that will feed the ANN. The Network, as a classifier, is used to distinguish spores from non-spores (because CHT would have detected all the curved structures in the material). ANN mainly deals with function approximation problems, data clustering, and pattern classification. ANN once trained can generalize the information to an unknown set of examples (in this case, spores), emulating the human brain. The use of such technologies (when compared to the conventional ones) that employ images to identify and quantify microscopic objects could be effective as automation of the counting process improves the working conditions as well as the results of the study (De Melo, Lopes, Andrade, et al. 2019).

Reference

De Melo C. A. O., Lopes J. G., Andrade A. O., Trindade R. M. P., and Magalhàes R. S. 2019. Semi-automated counting model for arbuscular mycorrhizal fungi spores using the Circle Hough Transform and an artificial neural network. *Annals of the Brazilian Academy of Sciences* **91**(4)

RECENT REFERENCES

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

- Agronomy
- AoB Plants
- Archives of Agronomy and Soil Science
- Canadian Journal of Forest Research
- Ecology
- Ecosystems
- Environmental and Experimental Botany
- Frontiers in Microbiology
- Frontiers in Plant Science
- International Journal of Molecular Sciences
- Microorganisms

- Mycorrhiza
- Mycoscience
- Plant Physiology and Biochemistry
- Plants (Basel, Switzerland)
- PLoS One
- Proceedings of the National Academy of Sciences
- Scientific Reports
- Soil and Sediment Contamination
- The Journal of Applied Ecology

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
Abdellatif L [*] , Lokuruge P, and Hamel C. 2019	Axenic growth of the arbuscular mycorrhizal fungus Rhizophagus irregularisand growth stimulation by co-culture with plant growth-promotingrhizobacteriaMycorrhiza 29 (6): 591–598[*Swift Current Research and Development Centre, Agriculture andAgri-Food Canada, Swift Current, Saskatchewan, Canada]
Ågren G I [*] , Hyvönen R, and Baskaran P. 2019	Ectomycorrhzia, friend or foe? Ecosystems 22 (7): 1561–1572 [*Department of Ecology, Swedish University of Agricultural Science, Uppsala, Sweden]
Averill C [*] , Bharnagar J M, Dietze M C, Pearse W D, and Kivlin S N. 2019	Global imprint of mycorrhizal fungi on whole-plant nutrient economics <i>Proceedings of the National Academy of Sciences</i> 116 (46): 23163–23168 [*Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996]
Bahadur A [*] , Batool A, Nasir F, Jiang S, Mingsen Q, Zhang Q, Pan J, Liu Y, and Feng H. 2019	Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants International Journal of Molecular Sciences 20 (17): 4199 [*MOE Key Laboratory of Cell Activities and Stress Adaptation, School of Life Sciences, Lanzhou University, Lanzhou 730000, China]
Begum N [*] , Qin C, Ahanger M A, Raza S, Khan M I, Ashraf M, Ahmed N, and Zhang L. 2019	Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance <i>Frontiers in Plant Science</i> 10 : 1068 [*College of Life Sciences, Northwest A&F University, Yangling, China]
Bernaola L^* and Stout M J. 2019	Effects of arbuscular mycorrhizal fungi on rice-herbivore interactions are soil-dependent Scientific Reports 9: 14037 [*Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, 70803, Louisiana, USA]

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Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)		
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