



Review Article

ROLE OF MICROBES ON CARBON SEQUESTRATION

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Abstract- Global warming in the last 100 years is said to be closely associated with increases in the concentration of atmospheric CO₂ almost 46% from pre-industrial era to present condition. Agro ecosystems plays important role in sequestration of carbon (C) to reduce the emission of atmospheric CO₂. Carbon sequestration is usually measured in terms of the total organic carbon stored in the soil. Among the different approaches and strategies, microbial modulation has been considered as one of the strategy to enhance soil C-sequestration. Soil microbial communities are an integral component of many ecosystem processes of which fungal and bacterial dominances, mycorrhizal associations, microalgae and oligotrophs (k- strategies) vs copiotrophs (r-strategies) has been attributed as contributors of soil C-sequestration. In prairie ecosystem high population of fungi and bacteria helps in accretion of carbon in soil. Mycorrhizal fungi have a special protein called glomalin which is secreted by the hyphae and spores of such fungi. Glomalin involves in aggregate stability and C storage in soil. In addition to the fungal dominance and mycorrhizal associations, microalgae have been exploited as potential and promising method for CO₂ capture and storage. The present review highlights the key role that soil microbes can play as an ecosystem service provider in mitigating global carbon-di-oxide emission.

Keywords- Microbes, Carbon, Sequestration

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Introduction

One of the major challenges of the 21st century is to mitigate the effects of global environmental changes brought about by increasing emissions of greenhouse gases (GHGs). Among the various GHGs, carbon dioxide (CO₂) is a key one accounted for 63% of total GHGs emission, whereas methane (CH₄), nitrous oxide (N₂O) and the remaining trace gases account for 24, 10 and 3%, respectively [18,9]. There is thus an imminent need to identify cost-effective strategies for mitigating anthropogenic CO₂ emissions if we are to minimize the impact of climate change and ensure global environmental security. One of the proposed cost effective methods is to increase soil carbon storage through carbon sequestration. There are different carbon pools in the ecosystem. The largest carbon pool is the oceanic carbon pool (38400 pg, 1 pg = 10¹ g = 1 billion ton), the second largest pool is the geologic pool (4130 pg), the third largest pool is the soil carbon pool (2500 PG), the fourth one is the atmospheric pool (760 pg) and the smallest pool is the biotic carbon pool (620 pg). Soil carbon pool mainly comprises of soil organic C (SOC) estimated at 1550 Pg and soil inorganic C (SIC) of approx. 750 Pg, occurred up to 1-m depth [23]. Being the third largest carbon pool and approximately 3.3 times the size of the atmospheric pool and 4.5 times the size of the biotic pool [12,10] therefore soil carbon sink has a significant impact on sequestering atmospheric carbon-di-oxide. The global potential of C sequestration in soils of agro-ecosystems is about 2.1 billion tons C/yr. If the SOC pool in world soils can be increased by 10% (+250 billion tons) over the 21st century, it implies a drawdown of about 110 ppm of atmospheric CO₂ [13]. Carbon sequestration is measured in terms of total organic carbon stored in the soil. Total organic carbon comprises mainly two carbon pools. One is active pool and another is passive pool. Active pool consists of very labile carbon (fraction I), labile carbon (fraction II) and passive pool consists of less labile carbon (fraction III), non-labile carbon (fraction IV). Passive pool is mainly responsible for soil carbon sequestration [23]. There are two major mechanisms of carbon sequestration in soil biochemical

alteration, physicochemical protection:

Biochemical alteration

C stabilization in soil involves alteration of organic matter to chemical forms that are more recalcitrant to microbial attack or more likely to adsorb to soil solids, or both. The alteration typically occurs in two stages.

(a) Decomposition

The first stage of (bio) chemical alteration involves the decomposition of C inputs (chiefly cellulose and lignin biopolymers present in plant litter) into smaller molecules. This process is mediated principally by microorganisms and their extracellular enzymes. Cellulose is readily degraded by both fungi and bacteria. Lignin, however, is more recalcitrant, and its complete degradation is restricted to a selected group of fungi that produce the extracellular lignin peroxidases [4, 27].

(b) Condensation/polymerization

Decomposition is followed by condensation or polymerization. In this stage, new compounds which are chemically more recalcitrant are formed from the simpler molecules produced in decomposition. These chemically altered fractions are formed by the condensation reaction of amino compounds (acids and sugars) with quinones or reducing sugars to form melanin-type compounds.

Physicochemical protection

The biochemically altered soil organic matter needs to be protected from further microbial decomposition. Organic matter and other soil particles must rearrange in some order for protection of organic matter. For this rearrangement the new C is sorbed chemically or physically to an existing surface coupled with some sort of physical barrier to prevent further attack by microbial agents [11].

(a) Chemical protection: Chemical protection of humified C involves strong chemical associations with soil minerals by forming organo-mineral complexes. These are formed by various organo-mineral associations such as polyvalent cation bridging, hydrogen bonding, van der Waals forces, and interactions with hydrous oxides and aluminosilicates [2, 3].

(b) Physical protection: Greater protection of chemically protected substrates is obtained when it is physically impeded. Physical protection of organic matter is obtained through the formation of stable aggregates. Primary particles and extremely stable fine-silt-sized aggregates (<20 µm diameter) are bound together into microaggregates (20–250 µm diameter) by plant and microbial debris. Microaggregates along with silt-sized aggregates and primary particles are bound into macroaggregates (>250 µm diameter) by labile organic materials and by fine roots, fungal hyphae, bacteria, and algae. The rate of macroaggregate turnover plays an important role in the stabilization of SOC. If macroaggregate turnover is too rapid, microaggregate formation and stabilization can be inhibited, leading to a reduction in the amount of microaggregate-protected SOC [22, 11].

Soil, water and crop management

It includes (i) conservation tillage for minimising soil disturbance, (ii) using integrated nutrient management strategies of applying manure/compost, biological nitrogen fixation (BNF), biofertilizers along with judicious use of chemical fertilizers, (iii) soil application of biochar, charcoal created by low-temperature pyrolysis of biomass under anaerobic conditions, is also considered as an option to increase the SOC pool [19]. The potential of biochar application for SOC sequestration may be 1 billion tons C/yr or more [15].

Different farming system

It includes (i) crop rotation, which influences soil aggregation and thus increase carbon sequestration. However the effectiveness of crop rotations in sequestering SOC depends on the cropping system and tillage practices. (ii) agroforestry, which have higher potential to sequester carbon than pastures and field crops. (iii) ley farming system enhances SOC concentration with improvement in soil aggregate stability, decrease in bulk density and increase in water infiltration rate, increase nutrient availability. (iv) cover cropping also enhance carbon sequestration by improving soil structure and water stable aggregates [13].

Land use

It includes (i) restorative perennial systems for increased aggregate formation, increased fungal dominated pathways in decomposition, greater inputs of organic matter, reductions in erosion and biomass accumulations, (ii) species with wide adaptation [13].

Microbial Strategies for enhanced carbon sequestration

The microbial contribution to C sequestration is governed by the interactions between the amount of microbial biomass, microbial community structure, microbial by products, and soil properties such as texture, clay mineralogy, pore-size distribution, and aggregate dynamics. Accumulation of microbially derived organic matter (MOM) in soil depends on a balance between production and decomposition of microbial products, that is: (1) the microbial growth efficiency (MGE), the efficiency with which substrates are incorporated into microbial biomass and by products, (2) the degree of protection of microbial biomass in the soil structure, and (3) the rate at which microbial by products are decomposed by other microorganisms [22]. The strategies adopted by microbes for carbon sequestration are:

- (A) Fungal and bacterial dominance for carbon sequestration [24].
- (B) Mycorrhizal Association. [29].
- (C) Microalgae for Efficient CO₂ Capture [17].
- (D) Oligotrophs (k strategists) Vs Copiotrophs (r strategists) [5].

(A) Fungal and bacterial dominance for carbon sequestration: The fungal

and bacterial abundance in soils is associated with C sequestration potential with greater fungal abundance is related to greater C storage [24]. The production of microbial biomass and byproducts will be greater in soils where the microbial community is composed predominantly of fungi because fungi have higher microbial growth efficiency than bacteria. Fungal-dominated communities will, therefore, retain more C in biomass per unit substrate consumed and release less as CO₂. The degradation of MOM will be slower in soils where fungi predominate because fungal products are more chemically resistant to decay and are preferentially protected from decomposition through their interactions with clay minerals and soil aggregates [21]. Agricultural management practices that favour fungal dominance will help enhance soil C sequestration.

Contribution of Fungi and bacteria to Soil Organic Matter under different Agricultural practices

Li *et al.* 2015 [16], conducted an experiment for eight years in a mollisol to elucidate the contribution of fungi and bacteria to soil organic matter under different agricultural practices. They did six field treatments including two no tilled soils supporting perennial plants (Alfalfa, T₁ and natural fallow, T₂), and four tilled soils under rotation between maize and soyabean with Cropping without mineral and organic addition (T₃), Crop with mineral fertilization (T₄), Crop rotation with mineral fertilization and fixed amount of straw incorporation (T₅), Crop with mineral fertilization with all aboveground biomass incorporation (T₆). Parameters assessed for contribution of fungi and bacteria towards soil organic carbon are microbial biomass and necromass. Necromass is estimated by comparing amino sugars (ASs) contained in living and dead cell walls and phosphor lipid fatty acids (PLFAs) contained in living cell membranes. The soil organic carbon (SOC) ranged from 6.9 to 12 g C/kg soil being highest in T₆ and lowest in T₃. The SOC content fell in the order of T₆>T₂>T₅>T₁>T₄>T₃, which is a similar order of the amount of organic carbon input. Total microbial biomass carbon follows the order T₂ >T₅>T₆>T₁>T₄>T₃. Bacterial biomass carbon contributes more towards total organic carbon than fungal biomass carbon in all the agricultural practices. The biomass carbon in T₂ and T₅ is more than T₁ and T₆ respectively, despite the largest plant diversity in treatment 1 and highest amount of organic carbon input in treatment 6. This indicates the effect of the quality of organic carbon inputs on microbial community. In T₂ leguminous plants was present which leads to higher nitrogen availability. In T₅ additional baked soyabean and maize was applied, but in T₆ all above ground biomass of soyabean and maize was applied. In case of necromass fungal necromass contribute more towards total necromass than bacterial necromass. Highest bacterial necromass was found in T₂ and highest fungal and total necromass was found in T₅. Correlation of PLFA towards SOC is less than the correlation of total necromass towards SOC. This indicates that living biomass less correlated to SOC than the dead biomass. Fungal necromass highly correlated towards SOC than bacterial SOC which indicates that fungal contribution towards SOC is more than bacteria. Contribution towards SOC is more from necromass than from the living microbial biomass. Contribution of fungal necromass ranged from 12.3 to 18 g C/kg SOC and bacterial necromass is ranged from 5.3 to 11.8 g C/kg SOC. While contribution of PLFA ranged from 0.7 to 1.4 g C/kg SOC for fungi and 3.9 to 5.6 g C/kg SOC for bacteria, which reflects the contribution of living microbial biomass.

The bacterial PLFA is more in SOC than fungal PLFA, this indicate that living bacterial population is more than fungi. But in case of necromass the fungal-derived necromass was dominant over the bacterial derived necromass in SOC, suggesting that fungal necromass was protected in the soils. From this study, it is clear that living fungi and fungal necromass are important for the formation and stabilization of soil aggregates, resulting in more fungal-derived organic matter protected physically.

Fungal and Bacterial contributions for enhanced C-sequestration in different Ecosystems

Bailey *et al.*, [1] 2002 assessed the contribution of fungi and bacteria towards total organic carbon through microbial biomass and activity of soils from five different ecosystems.

The total organic carbon content found highest in Douglas fir followed by Tall grass Prairie (restored). Although Tall grass Prairie has a higher F:B ratio than Douglas fir, it has lower total organic carbon content because Douglas fir soil has mycorrhizal biomass which accounted for 50% of total biomass of the soil and which exceed the biomass of fine roots.

They found a high correlation between fungal activity and total organic carbon (50%) under different ecosystems and increase fungal activities [Fig-7] more than F:B ratios were associated with increase soil C. But correlation of bacterial activity is very less towards total organic carbon (17%) in comparison to fungal activity. It is seen that higher amount of F:B activity and total organic carbon was observed in restored Prairie than neighboring farmland as well as greater amount of F:B activity and total organic carbon in no-tilled soil in comparison to conventionally tilled soil. It reflect the hypothesis that passive land use practices promote fungal activity and thereby increase the amount of stored carbon, that is carbon sequestration.

Mycorrhizal association for C-sequestration

Mycorrhizal association significantly increases the total C assimilation by plants. A commonly known pathway by which AM fungi sequester C in soil is the transfer of photosynthates from the host plants to the AM fungal intraradical hyphae and subsequently to extraradical hyphae before release to the soil matrix [14]. Could result in a 4–20 % drain of carbon from the host plant to their hyphae and indirectly influence C-sequestration in soils [8]. Directly influence soil C sequestration through the growth and turnover of extraradical hyphae in rhizosphere and bulk soil. The overall contribution of AM fungi to soil C sequestration dependent on the volume of hyphal biomass produced, the turnover time of accumulated hyphal biomass and the role played by these fungi in the stabilisation of soil aggregate formation [32]. Hyphae produces one glycoprotein called glomalin which increases aggregate stability. Greater stability of aggregates leads to larger amounts of protected organic carbon and thereby larger C-sequestration [29].

Glomalin directly enhances the aggregate stability

Wright and Upadhyaya [29], in 1998 did experiment on thirty-seven samples from four geographic areas of the U.S. and one area of Scotland to test the effect of glomalin in aggregate stability. They took aggregate of size 1-2 mm for calculating the percentage of stability. The Glomalin on mycorrhizal hyphae in soil aggregates was assessed by immunofluorescence and it was observed on some surfaces of aggregates from all soils examined, but was most evident on aggregates with high glomalin concentrations. They found a high correlation of aggregate stability with all measures of glomalin. The highest correlation of aggregate stability was seen with immunoreactive easily extractable glomalin (IREEG) (84%), followed by immunoreactive total glomalin (IRTG) (79%) and lowest from easily extractable glomalin (69%). They also found a good correlation of all measures of glomalin with % carbon content.

These results indicate that glomalin is highly correlated with aggregate stability. Easily extractable glomalin and the immunoreactive fractions of glomalin in aggregates were more closely correlated with aggregate stability than total glomalin. Alternatively, the most stable aggregates had higher EEG and IREEG values than the less stable aggregates. This indicates that higher amount of glomalin leads to higher aggregate stability and hence higher amount of protected organic carbon.

Glomalin, soil aggregate stability and SOC as affected by land use variation

Fokom *et al.* 2012 [6], assessed land use impact on glomalin related soil proteins (GRSPs), soil quality and aggregation in the humid forest zone in southern Cameroon with soil samples collected from forest, short fallow and field crop production soil. They founded a decrease in GRSPs, soil organic carbon, organic matter and waterstable aggregates from the forest to fallow and the field crop production systems.

The easily extractable glomalin was 30% and 39% less under fallow and field crop production respectively, relative to the forest soils. Similarly, glomalin was 17%

and 46% less under fallow and field crop production respectively, relative to the forest soils. The waterstable aggregates was 20% less under fallow, and 33% less under field crop production relative to the forest system. This study has shown that glomalin concentrations were strongly affected by soil disturbance as soil samples from the undisturbed ecosystems (forest and fallow) have higher glomalin than those from the disturbed ecosystem (agricultural field). This is due to disruption of hyphal network by agricultural practices lead to a reduction of glomalin production which leads to reduction of amount of waterstable aggregates and thereby less amount soil organic carbon. From this study it is clear that conversion of natural forest into agricultural lands affects soil mycorrhizal population and hence affect organic matter content of soil. Here, they have found a positive correlation of glomalin with organic matter. So, it is evident that mycorrhizal fungi could regulate organic matter by the production of glomalin in soil. Wilson *et al.* [28], 2009 studied role of abundance of Mycorrhizal fungi on soil aggregate stability and carbon sequestration in long term basis on Tall grass Prairie from 1986-2009. They did two treatments, one is with fungicide application and another is without fungicide application.

They have reported that there is significant effect of fungicide in mycorrhizal hyphal suppression. Mycorrhizal hyphal length was significantly higher in fungicide untreated plots. Consequently, easily extractable glomalin (EEG), immunoreactive easily extractable glomalin (IREEG), immunoreactive total glomalin (IRTG) and total glomalin (TG) were higher in the fungicide untreated plots which results in high aggregate stability and high total organic carbon. Percent root length colonized by mycorrhizal fungi were reduced by 63%, following fungicide applications. Likewise, glomalin fractions, EEG and TG were reduced by 18%, IREEG fraction by 53% and the IRTG fraction by 76%, following fungicide applications. Contribution of macroaggregates (> 250 µm) towards total aggregates was significantly higher in fungicide untreated plots as compared with fungicide-treated plots. Both classes of macro aggregates, 250–2000 and > 2000 µm in diameter, were reduced in the suppression plots, in contrast, a concomitant increase in both classes of micro aggregates (20–53 and 53–250 µm in diameter). Mycorrhizal hyphal abundance was highly correlated with the proportion of soil macro aggregates (aggregates > 250 µm in diameter). In contrast, the relationship between hyphae and proportion of micro aggregates was negative, indicating that mycorrhizal hyphae and associated glomalin likely contributed to the increase in the proportion of macro aggregates at the expense of the micro aggregates, due to promotion of the binding of micro aggregates into macro aggregates. For every meter loss of mycorrhizal hyphal abundance, there was a concomitant cost in soil aggregation for which no other processes compensated. The role of mycorrhizal hyphae with macro aggregate stability suggests that productivity of mycorrhizal fungi influence soil C indirectly by stabilizing soil.

Carbon sequestration by microalgae

CO₂ fixation via microalgae is a potential and promising method for CO₂ capture and storage [31]. CO₂ fixation and storage via microalgae are photosynthetic, which can transform water and CO₂ to organic compounds without extra energy addition or consumption and without secondary pollution. Compared to other carbon capture and storage methods, CO₂ fixation via microalgae has many benefits, such as a high photosynthesis rate (e.g. 6.9×10^4 cells/ml/h [25], a rapid growth rate (0.7–3.2 day⁻¹ [20] good environmental adaptability and low cost of operation. The rate of CO₂ fixation via microalgae and biomass production depends on the cultivation conditions (e.g., temperature, light, pH, and nutrient availability), species of microalgae, CO₂ concentration. Microalgal biomass contains approximately 50% carbon by dry weight [31].

Algal diversity in the sequestration of C in paddy soil and non paddy soil

Yuan *et al.* 2012 [30], experimented role of algal diversity in sequestration of carbon in paddy and non-paddy upland soil. Soils were incubated for 80 days in a continuously labelled. Rubisco enzyme activity was measured as a measure of algal activity in the six different soils. It is seen that a higher algal diversity and activity present in paddy soil in comparison to non paddy soil.

The rate of synthesis of SOC and total SOC also higher in paddy soil than non paddy soil. This is due to submerged condition of paddy soil favour the growth and development of algal population leading to a high algal diversity. Moreover, algae produce a highly stable form of carbon which are largely protected in paddy soil from mineralization by complexation with active iron oxides. But in upland non paddy soil, amount of active iron are lower, therefore the capacity for organic matter complexation is reduced in these soils. Therefore, increase in algal diversity, Rubisco activity, and production of highly stable form of carbon make in paddy soil for maximizing the sequestration of carbon. This work has shown that algal diversity offers significant potential for the microbial assimilation of atmospheric carbon-dioxide.

Carbon dioxide fixed by industrially important microalgae

Sydney *et al.* 2010 [26], experimented carbon dioxide fixation rate of four industrially important microalgae *Dunaliella tertiolecta*, *Chlorella vulgaris*, *Spirulina platensis* and *Botryococcus braunii*. Carbon dioxide fixed was mainly used for microalgal biomass production. Biomass production is main destination of C in microalgal cultivation for industrial applications for making carbon papers. The determination of carbon dioxide fixation by each microalga was done based on the CO₂ consumption profile. *Botryococcus braunii* presented the highest CO₂ fixation (496.98 mg CO₂/ L/Day) rate followed by *Spirulina platensis* (318.16 mg CO₂/ L/Day), *Dunaliella tertiolecta* (272.40 mg CO₂/ L/Day), *Chlorella vulgaris* (251.64 mg CO₂/ L/Day). Per cent carbon in the biomass and CO₂ fixated per ton of biomass was also highest in case of *Botryococcus braunii*. This is because these algae can concentrate more amount of hydrocarbon into their biomass which is 30-40% of their dry weight in comparison to other algae.

Microalgae for Carbon capture in increasing CO₂ concentrations

Gonclaves *et al.* 2016 [7], studied capacity of microalgae to grow and capture CO₂ under increasing concentrations of CO₂. They determined carbon capture capacity in terms of specific growth rate, maximum biomass concentration and average biomass productivity. All the parameters had a lowest value in the atmospheric concentration of air for both the algae. For specific growth rate values, had increased from air until CO₂ concentration reached 5% for both the algae and decreased gradually as CO₂ concentration increases upto 10%. This decrease is due to some changes in the photosynthetic characteristics of the algae. It has already been reported that, when grown under high CO₂ concentrations (5% v/v or more), some microalgae display lower affinity to CO₂, higher photosynthetic sensitivity to O₂ and lower activity of carbonic anhydrase, the enzyme responsible for photosynthetic utilization of inorganic carbon. Maximum biomass concentration ranged from 0.472 (gdw/ L) to 1.11 (gdw/ L) for *Chlorella vulgaris* and 0.483 gdw/ L to 0.903 gdw/ L for *Pseudokirchneriella subcapitata*. Highest maximum biomass concentration was achieved for *Chlorella vulgaris* at 7% concentration and for *Pseudokirchneriella subcapitata* at 9% concentration with a decrease above. For average biomass productivity have shown a similar behaviour. These results suggest that the selected microorganisms can grow well under CO₂ concentrations ranging from those commonly present in the atmosphere up to 10% (v/v). However, the best results were achieved for CO₂ concentrations of 7% (v/v) for *Chlorella vulgaris* and 7 for *Pseudokirchneriella subcapitata*. These findings can be useful for the selection of microalgal species able to grow in this range of CO₂ concentrations. These results have shown that the selected microorganisms can be effective in CO₂ capture, especially when cultured with enriched air streams.

Oligotroph and copiotroph for carbon sequestration

Based on the C mineralization potential and growth rates, soil bacteria classified into two ecological functional categories of copiotrophs and oligotrophs. Copiotroph or r-strategists are adapted to maximize their intrinsic rate of growth when resources are abundant while oligotroph or k-strategists are adapted to compete and survive when populations are near carrying capacity and resources are limited. Soils dominated by oligotrophs may have low C turnover and, consequently, low CO₂ emissions and thus higher C sequestration. When soluble nutrients are abundant, microbial mining of SOM should decrease, leading to a

greater sequestration of nutrients in SOM oligotrophs (k-strategists). Soil dominated with copiotrophs will have more soil carbon because they consume more labile forms over recalcitrant organic C, which makes up the bulk of the soil organic pool. Shortage of soluble nutrients could favor microbial species able to mine nutrients in soil organic matter (oligotrophs or k-strategists) over species that feed on fresh-C only and immobilize nutrients from the soil solution (Copiotroph, r-strategists) [5]. But evidence for such life styles under relevant environmental conditions is lacking.

Conclusion

Microorganisms contribute greatly to ecosystem C budgets through their roles as detritivores, plant symbionts, or pathogens, thereby modifying nutrient availability and influencing C turnover and retention in soil. Microbes decompose biomass which results in C loss from the soil due to microbial respiration, while a small proportion of the original C is retained in the soil through the formation of stable organic matter. When C inputs from photosynthesis exceed C losses through soil respiration, SOC levels increase over time resulting in net soil C sequestration. It has been proposed that terrestrial ecosystems can potentially be manipulated through land use and land management practices for the build-up of distinct microbial communities that favour C sequestration. It has been estimated that through judicious management, the world agriculture and degraded soils could sequester an additional 0.4–1.2 Gt C/year, which is equivalent to 5–15% of global fossil fuel emissions [12]. Although microbial communities play a vital and undisputable role in soil C storage but microbial control over processes that facilitate soil C storage remains a topic of debate. Numerous questions remain to be addressed for understanding microbial control over C cycling in terrestrial ecosystems.

Application of review: Comprehensive understanding of terrestrial microbial communities and specific processes that determine the rate and fate of C dynamics will increase the likelihood of successful manipulation of the terrestrial ecosystem for increasing stable C inventories. Challenges in manipulating microbial community for enhanced C sequestration arise from the enormous diversity and unculturability of soil microbial communities, which has precluded their comprehensive characterization and limited understanding on their ecological functions.

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