Mapping the scientific knowledge of glomalin-related soil protein with implications for carbon sequestration

Shanle Liu, Qizhi Wang, Lu Qian, Binghuang Zhang, Xiangwen Chen, Hualong Hong, Shengjie Wu, Jingchun Liu, Chongling Yan and Haoliang Lu

*Ministry of Education, Key Laboratory of the Coastal and Wetland Ecosystems, Xiamen University, Fujian, China; College of the Environment and Ecology, Fujian Key Laboratory of Coastal Pollution Prevention and Control, Xiamen University, Fujian, China

ABSTRACT
Arbuscular mycorrhizal fungi (AMF) derived refractory organic matter, mainly in glomalin-related soil protein (GRSP), stores globally significant amounts of carbon, attracting wide attention in response to climatic change. However, there is no synthesis review has been done so far to reveal global research progresses on GRSP, especially its ecological role for climate mitigation. Here, we conducted a bibliometric analysis of the papers on GRSP research from 1998 to 2021, based on the Web of Science (WOS) Core Collection. We collected a total of 634 papers and analyzed the characteristics of publication outputs in various countries and institutions. In addition, keyword analysis was conducted to reveal the main research directions and hot trends in the field of GRSP. We confirmed that 1) the number of papers published per year has gradually increased since 2010, and increased sharply in 2019; 2) the United States was the earliest country to conduct GRSP research, while China currently has the largest number of publications; 3) relevant studies focus on the role from AMF and GRSP in soil health and plant health; 4) the contribution of GRSP to soil carbon and its ecological function in mitigating climate change are the hotspot of research. The benefits of GRSP have become a consensus, however, the nature of glomalin and the mechanisms of GRSP-associated benefits still need to explore. As an important soil recalcitrant organic carbon, further studies are need to elucidate the land-ocean biogeochemical processes of GRSP and its contribution to global blue carbon.

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Introduction
Arbuscular mycorrhizal fungi (AMF), one of soil microorganisms widely distributed in various terrestrial ecosystems can form a symbiotic association with roots of more than 80% of terrestrial plants (Gao, Wang, and Wu 2019). AMF can expand the absorption area of host plant roots to improve the absorption of water and mineral nutrients, promoting plant growth (Auge et al. 2003; Auge 2001). AMF produce a recalcitrant glycoprotein with insolubility and heat resistance, glomalin, which is tightly bound within the hyphal wall of AMF and only released or sequestered into the soils after the senescence of hyphal and spores (Wright et al. 1996; Wright and Upadhyaya 1998; Driver, Holben, and Rillig 2005; Wang et al. 2019b). On the one hand, glomalin has a beneficial effect on AMF. For example, glomalin could benefit fungi directly by improving its fungal physical growing space (Rillig and Steinberg 2002). Moreover, Driver, Holben, and Rillig (2005) deduced that glomalin could reduce hyphal palatability to fungal grazers, so as to protect AMF. On the other hand, the ecological functions of AMF, such as soil aggregate formation, carbon fixation or storage and heavy metal adsorption or fixation, are related to the production of glomalin (Bedini et al. 2009; Rillig et al. 2001b). This protein is abundant in soils (Wright and Upadhyaya 1998), and has been found in oceans, agriculture, grassland, forest, desert and non-cultivated soils (Adame et al. 2012; Singh, Singh, and Tripathi 2013). It is worth noting that glomalin was first found by Wright et al. (1996), and considered to be a specific protein produced by AMF (Wright et al. 1996; Wright and Upadhyaya 1998; Rillig et al. 2003). However, the glomalin extracts contain other non-glomalin ingredients (Rillig 2004b). As a result, Rillig (2004b) proposed a new name, glomalin-related soil protein (GRSP), to replace glomalin in soils.

Mass spectrometry analysis suggested that GRSP is an N-linked glycoprotein composed of 3 to 5% N, 36 to 59% C, 4 to 6% hydrogen, 33 to 49% oxygen, 0.8 to 8.8% Fe and 0.03 to 0.1% P (Schindler, Mercer, and Rice 2007; Singh, Singh, and Tripathi 2013). The content of the Fe values indicates why the GRSP extracts present...
red (Wright and Upadhyaya 1998). It is worth noting that GRSP, with abundant carbon (5–13%), is the most important source of soil organic carbon (SOC) (Lovelock et al. 2004; Kumar, Singh, and Ghosh 2018). GRSP, as a “super glue,” can bond small soil particles to microaggregates with diameters of less than 0.25 mm, which promotes the formation of soil aggregates, and the stability of soil aggregates can improve the soil structure (Wright and Upadhyaya 1998; Gispert et al. 2013; Liu et al. 2020). In turn, soil aggregates can protect GRSP, reducing its decomposition (Gao, Wang, and Wu 2019). Additionally, GRSP has a turnover time of 6–42 years and high soil accumulation capacity, which promotes carbon storage in soils (Rillig et al. 2001b; Wang et al. 2019b). Therefore, GRSP plays an important role in the carbon fixation and cycle (Singh et al. 2017).

In soil ecosystems, GRSP also has other functions. Firstly, GRSP produced by AMF can reduce the toxicity of toxic metals to plants (Vodnik et al. 2008), because GRSP can bind with them, stabilizing them and isolating their availability (Rillig 2004b; Wang et al. 2019b; Dhalaria, Kumar, and Kumar et al. 2020). Moreover, GRSP can improve the resistance of plants to toxicity (Folli-Pereira et al. 2012). Recent studies indicated that AMF under some stressed conditions could secrete more GRSP to improve plant stress tolerance by serving as a stress-induced protein (Gao, Wang, and Wu 2019). GRSP under drought stress can improve soil water-stable aggregates, indirectly affecting the soil moisture retention (Wy, Xia, and Zou 2008; Rillig 2004b). Similarly, soil aggregation is highly disturbed under high NaCl levels, but higher production of GRSP could counteract that adverse effect (Hammer, Rillig, and Nielsen 2011). However, not all stresses induce a higher GRSP production, such as osmotic stress (Hammer, Rillig, and Nielsen 2011).

In general, the reviews on GRSP can be classified into the following two categories: 1) Discussion of the application and mechanism of AMF in some fields, such as land use, enhancing plant resistance (including heavy metal and drought), ecological restoration and carbon storage (Riaz, Kamran, and Fang et al. 2021; Janeeshma and Puthur 2020; Kaushal 2019; Wang 2017; Parihar et al. 2020; Wei et al. 2019). And GRSP is one of the reasons why AMF functions; 2) Summary to the function, importance and mechanism of GRSP specifically (Gao, Wang, and Wu 2019; Sousa et al. 2012; Vlcek and Pohanka 2020). However, this category accounts for only 17.95% of all review articles. The mechanisms of activity of AMF and GRSP should be separated, and the number of reviews only on GRSP should be increased. At the same time, the reviews in recent years mostly focus on soil degradation (Singh, Zhu, and Chen et al. 2020), mitigation of heavy metal pollution (Dhalaria, Kumar, and Kumar et al. 2020; Riaz, Kamran, and Fang et al. 2021), and the extraction and naming of GRSP (Irving et al. 2021). Nevertheless, there is a lack of statistical analysis of the published documents of GRSP research.

Bibliometric analysis uses mathematical and statistical techniques to analyze scientific publications, which is a systematically interdisciplinary method (Marcal et al. 2021). Compared with traditional methods, it can help research workers collate some information quickly. It not only can be used to estimate the impact of research areas and identify emerging trends, but also can analyze the characteristics, hotspots, and emerging trends of a given field (Niu, Zhao, and Wu et al. 2021). Bibliometric analysis evaluates literatures through three dimensions: quality, quantity and network (Marcal et al. 2021). This information could help new researchers joining GRSP research to quickly find a range of basic or advanced papers. Besides, through the three aspects, we also know the active journals, countries, institutions and authors of a certain field, and know their partnerships (Niu, Zhao, and Wu et al. 2021; Marcal et al. 2021).

Researchers new to the field could use the messages to get directions for seeking help and collaboration. Among bibliometric analysis tools, CitSpace and VOSviewer are the most commonly used tools owing to their characteristics of simple operation, multifunction and clear and comprehensive visualization (Niu, Zhao, and Wu et al. 2021; Yang, Cheng, and Shen et al. 2017). In this study, a bibliometric analysis was carried out to systematically evaluate the publications of GRSP research using CitSpace and VOSviewer software. We discussed journals, countries, institutions, authors and keywords, tapping into and analyzing research developments and directions of the field. Furthermore, current research hotspots were also investigated.

Materials and methods

Data collection

The data were obtained from the Web of Science (WOS) Core Collection. The WOS Core Collection was chosen because it is considered the most comprehensive database, embodying the most influential and relevant journals (Niu, Zhao, and Wu et al. 2021). We used the following terms: “glomalin” OR “GRSP” OR “Glomalin-related soil protein,” from its inception (1998) to 18 July 2021, locating a total of 677 papers. There were no restrictions on language, document type, data category, or a document’s year (Zhang et al. 2020). It is worth noting that the 677 papers do not contain Wright et al. (1996) and Wright and Upadhyaya (1996), which are not from the WOS Core Collection. We first evaluated the 677 papers’ correlation with GRSP research by reading the title and abstract. For papers
that could not be clearly judged by this way, secondary screening was conducted by checking their full text. Finally, a total of 634 documents for GRSP research were obtained. The study selection and flow charts of the research framework are shown in Figure 1.

**Analysis method**

The data from the 634 papers were further analyzed by bibliometric analysis. VOSviewer software (version 1.6.16) and CiteSpace (version 5.7.R5) were used to analyze the document types and numbers per year, productivity and quality (journals, countries, institutions and authors), partnership network (countries and institutions) and research focii (e.g., keywords co-occurrence and cluster, the strongest citation bursts). In addition, we used Microsoft Excel 2019 to summarize data, and used OriginPro 2021 to plot graphs. In the network of co-occurrence and cluster analysis, the nodes represent the specific key terms such as countries, institutions, or keywords, where the size of the nodes shows their productivity, and the thickness of the line between the nodes indicates the collaboration relationship (Niu, Zhao, and Wu et al. 2021).

**Results**

**Paper type and quantity, research directions, and active journals**

In the study, 634 papers on GRSP were identified. Figure 2(a) shows six types of the 634 papers, namely articles, review articles, proceedings papers, early access, meeting abstracts and editorial materials. Among them, the majority of the documents were article (88.1%), followed by review (5.9%). The proportion of review articles on GRSP is at a medium level compared to other fields, such as microplastics (8.01%) (Zhang et al. 2020), groundwater remediation (3.60%), covalent organic frameworks (10.11%) and metal-organic frameworks (4.5%) (Niu, Zhao, and Wu et al. 2021). Review is a summary and conclusion made by relevant researchers according to the current research status, which can promote the development and progress of related areas. The number of review articles before 2010 accounted for only 20.51%, whereas after 2015 accounted for 53.85%. It reflects that GRSP research has been received more attention to a scientific consensus. Moreover, some recent research and review articles indicate that the potential impact of GRSP for climate mitigation has overlook some points that are important to effective climate change communication, such as carbon sequestration (Agnihotri et al. 2022), soil and plant health (Irving et al. 2021), and connectivity between terrestrial and marine environments (Adame et al. 2012; Wang et al. 2018a).

The proportion of proceedings papers and meeting abstracts (3.6%) appears that the holding of academic conferences may be few. But academic conferences can promote the development and dissemination of research for GRSP. Additionally, the number of papers published in the GRSP area per year has increased steadily and gently, but has enjoyed a sharp increase since 2019 (Figure 2(b)). It implies that the research for GRSP had received great attention since 2019 and has the potential to become a research hotspot. However, we found that there was no obvious change of hot spot by analyzing the research directions of papers published in 2018 and 2019. Therefore, it can only be stated that the research on GRSP is still in the exploratory stage. Furthermore, the first six research fields with the largest numbers of articles were also determined. Among them, the field of soil science was the subject
field with the most papers on GRSP (42.74%), followed by agronomy and agriculture multidisciplinary (20.98%), and then environmental sciences (20.35%). In recent years, the number of published papers in the fields of soil science, agronomy and agriculture multidisciplinary, plant sciences, ecology and microbiology has irregularly increased (Figure 2(b)), and the number of documents in the field of microbiology in these two years is more than that in each previous year. It implies that this field has begun to receive more attention.

Understanding the publication of papers on GRSP in different journals can provide some references for researchers in this area when choosing journals to deliver manuscripts. Studies on GRSP collected by WOS have been published in 210 journals. However,

Table 1. The top 11 journals for GRSP research.

| Rank | Journal                                         | N (%)  | Citations | Citations/N | Country         | IF (2020) |
|------|-------------------------------------------------|--------|-----------|-------------|-----------------|-----------|
| 1    | Soil Biology & Biochemistry                     | 53(8.36%) | 2853     | 53.83       | England         | 7.609/Q1  |
| 2    | Applied Soil Ecology                            | 28(4.42%) | 614      | 21.93       | Netherlands     | 4.046/Q1  |
| 3    | Soil Tillage Research                          | 22(3.47%) | 840      | 38.18       | Netherlands     | 5.374/Q1  |
| 4    | Plant and Soil                                 | 18(2.84%) | 2088     | 116         | Netherlands     | 4.192/Q1  |
| 5    | Science of the Total Environment                | 16(2.52%) | 508      | 31.75       | Netherlands     | 7.963/Q1  |
| 6    | Archives of Agronomy and Soil Science           | 15(2.37%) | 85       | 5.67        | England         | 3.092/Q2  |
| 7    | Catena                                         | 15(2.37%) | 134      | 8.93        | Germany         | 5.198/Q1  |
| 8    | Mycorrhiza                                     | 13(2.05%) | 359      | 27.62       | Germany         | 3.387/Q2  |
| 9    | Geoderma                                       | 11(1.74%) | 274      | 24.91       | Netherlands     | 6.114/Q1  |
| 10   | Scientific Reports                             | 10(1.58%) | 233      | 23.3        | England         | 4.379/Q1  |
| 11   | Soil Science Society of America Journal         | 10(1.42%) | 622      | 62.2        | USA             | 2.3088/Q3 |

Note: N: number; IF: Impact factor (value shows journal quality)
58.57% of all journals had only published one paper. According to the number of documents, the top 11 journals are summarized in Table 1. Citations/Numbers (Citations/N) indicates the average number of citations per paper, and it shows popularity and quality of the journal. The journal with the most published papers was Soil Biology & Biochemistry, which published 58 papers, accounting for 8.36% of the total number, with 7,609 Impact Factor and 53.83 Citations/N. And its Citations/N ranks third in the 11 journals. Meanwhile, the journals ranking first and second are Plant and Soil (116) and Soil Science Society of America Journal (62.2) separately, but Soil Science Society of America Journal is the only Q3 journal of the 11 journals and whose number of papers is the lowest. Notably, the Impact Factor of Science of the Total Environment is 7.963, ranking first, but its other aspects are not encouraging. Moreover, 5 of the journals originate from Netherlands, followed by England, Germany and the United States of America (USA) with values of 3, 2 and 1, respectively. It indicates that the journals from Netherlands have made a great contribution to GRSP research.

**Active countries and institutions**

Understanding the number of documents published in different countries and institutions helps to quickly evaluate the contributions they have made to the GRSP topic. All the papers were from 71 countries, among which China published the highest number with a value of 185, accounting for 29.18%, with 17.67 Citations/N, followed by USA (97, 15.30%, 85.24), Brazil (58, 9.15%, 9.98), India (55, 8.68%, 11.4), Spain (33, 5.21%, 31.70), Chile (29, 4.57%, 33.48). Besides, the number of documents published in the top 6 countries has changed significantly in the field of GRSP. The contribution rates of the top 6 countries from 1998 to 2010 were 6.25% (China), 50% (USA), 1.39% (Brazil), 2.08% (India), 8.33% (Spain), 5.56% (Chile). However, their contribution rates of them in the recent ten years were 35.92% (China), 5.10% (USA), 11.43% (Brazil), 10.61% (India), 4.29% (Spain), 4.29% (Chile). In addition, the total of papers published in the China, Brazil and India account for 47%. This means that research has flourished in China, Brazil and India in the field of GRSP over the past decade.

Network maps can provide cooperation information between countries (Figure 3(a)). The cooperation among various countries was very close, with China having the most frequent exchanges with India, Czech Republic, USA, Germany, Australia and England. Additionally, the USA, excepting China, has exchanges with Brazil, Spain, Germany, Australia, England, Mexico and Canada (Figure 3(a)). Citations/Numbers (Citations/N) indicates that the average number of citations per paper, which represents the quality and dissemination of the papers to a certain extent in this field. Figure 3(a) shows that papers published in China, Brazil and India have lower value of citation/N compared with other three countries. It is worth noting that the impact factors (IF) of journals published can also reflect the quality of papers to a certain extent in this field. However, published journals’ average IF of all documents published by China is in the vicinity of 4.49, compared with 4.39 in USA, 2.94 in Brazil 3.56 in India, 5.40 in Spain and 4.50 in Chile. Therefore, the impact of publications on GRSP research in Brazil and India is relatively low.

The United States of America, as a pioneer in GRSP research, has rich research content in the field of GRSP, including the measurement of GRSP in various ecosystem (Lovelock et al. 2004), the role of GRSP in agriculture (Wright, Green, and Cavigelli 2007), in soil and plant health (Hruso et al. 2018; Garcia, Dattamudi, and Chanda et al. 2019), in carbon sequestration (Parihar et al. 2020) and in mitigating pollution (Chern, Tsai, and Ogunseitan 2007) and the quantification of GRSP and the purification of glomalin (Rosier, Hoye, and Rillig 2006; Janos, Garamszegi, and Beltran 2008). As now the world’s largest GRSP research center, China has effectively expanded the research direction of GRSP. The special feature of GRSP research in China is the research on wetlands (Pei, Ye, and Yuan et al. 2020; Wang et al. 2020). Different countries not only have different emphases and advantages in GRSP research, but also have unique geographical features. For example, GRSP research in Brazil and India focuses on agriculture (Bertagnoli et al. 2020), and in particular, India uses carbon change to monitor farmland quality (Singh, Rai, and Singh 2016). Spain has some studies on carbon pool in the field of GRSP (Singh et al. 2017; Gispert, Phang, and Carrasco-Barea 2020). And GRSP research in Chile is unique in volcanoes, wheat and aluminum (Seguel et al. 2017). Therefore, it is necessary to strengthen international exchanges and cooperation, which can reveal the ecological role of GRSP in the ecosystem.

Moreover, a total of 757 institutions were found to contribute to GRSP research. The top 6 productive institutions are summarized in Figure 3(b), among which the Chinese Academy of Sciences has published the highest number of 64 papers, accounting for 10.09%, with 22.64 Citations/N, followed by Yangtze University (39, 6.15%, 21.46), Agricultural Research Service of United States Department of Agriculture (36, 5.68%, 92.89), University of Montana (32, 5.05%, 129.53), University Of La Frontera (29, 4.57%, 33.48) and Spanish National Research Council (21, 3.31%, 38.71). Although Brazil and India rank high in the number of published papers, there are no representative institutions which have published more than 10 papers. It shows that GRSP research in India and Brazil is scattered. Furthermore, the Chinese Academy of Sciences and Yangtze University are the
most productive organization with a total of 64 and 39 publications respectively, whereas Agricultural Research Service of United States Department of Agriculture and University of Montana are the most influential organizations with 92.89 and 129.53 Citations/N within the studied period.

The network of the top 25 productive institutions is showed in Figure 3(b), among which the node size represents the number of published articles, and the connection between the two institutions represents their cooperation (Niu, Zhao, and Wu et al. 2021). Among these 25 institutions, the number of Chinese institutions is the largest, accounting for 44%, including the Chinese Academy of Sciences, Yangtze University, Zhejiang Academy of Agricultural Sciences, Huazhong Agriculture University, Henan University of Science and Technology, Nanjing University, Northeast Forestry University, Xiamen University, Hebei University, Northwest A&F University and Nanjing Agriculture University. Moreover, the Chinese Academy of Sciences has cooperated with most of them, excepting Nanjing Agriculture University and Huazhong Agriculture University. Referring to the top 6 institutions, there have been frequent exchanges between the Chinese Academy of Sciences and Yangtze University, between the Agricultural Research Service of United States Department of Agriculture and University of Montana, and between the University of La Frontera and the Spanish National Research Council (Figure 3(b)). This implies that institutional cooperation still needs to be strengthened among different countries, and even different regions.

Figure 3. The network map of (a) the top 36 productive countries, and (b) the top 25 productive institutions for GRSP research.
**Active authors and cited papers**

CiteSpace and Google maps have been used to visualize the distribution of authors for GRSP research (Figure 4). It shows that the distribution of the research workers is consistent with the statistical results of the top 15 active countries, which mainly distribute in the North America and Sino-American (the United States, Canada and Mexico), South America (Brazil and Chile), Europe (Spain, Czech Republic, Germany, England, Italy, France and Argentina), Oceania (Australia) and Asia (China and India), except Central Asia. So, the active regions with a high number of documents can be summarized as Western Europe, China, India, USA, Brazil and Chile. A total of 2,257 authors were involved in the study of GRSP. However, only 36 authors have published more than 5 papers, accounting for 1.60%. Though many researchers are involved in the relevant work, there are very few high-yield authors. According to statistics, it was found that there were 1,845 authors with only one paper, accounting for 81.75% of the authors. This indicates that only a small number of researchers has been focusing on the topic of GRSP.

The top 10 cited papers because of the value of citations are shown in Table 2, among which Rillig participated in 7 documents (ranking 1,3,4,6,7,8,10) and is the first author in 5 documents (ranking 1,3,6,7,8), Wright participated in 4 papers (ranking 2,7,8,9) and is the first author of the second ranked paper. It appears that Rillig’s research team has frequent cooperation with Wright’s research team. It is worth noting that 8 among the top 10 cited papers are from the USA. Although China is ahead of the USA in the number of publications, it falls behind the USA in the number of citations. Furthermore, there is no one on the list in the recent 10 years, which indicates that the 10 papers are classic and groundbreaking. On the other side, it also reflects that the research in this field started late and is still in its infancy.

Table 3 shows the top 10 authors because of the number of papers, among which the top 3 researchers in GRSP field are Rillig, Wright and Wu, Qiang-Sheng. Wright et al. (1996) first isolated and named “glomalin,” which opened a new chapter for mycorrhizal research. And the paper, A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi, stated that the soil microbes at the molecular level play a positive role on the stability of soil structure, and pointed out the value to agriculture (Wright and Upadhyaya 1998).

**Keywords**

**Co-occurrence keywords analysis**

The keywords from the 2,780 GRSP studies were summarized and counted. Fifty-five (55) keywords that have appeared at least 20 occurrences are shown in Figure 5(a), and the size of the nodes in this figure represents this frequency. Obviously, the keywords with higher frequency are arbuscular mycorrhizal fungi (AMF), glomalin, aggregate stability, hyphae,
Table 2. The top 10 cited papers in GRSP research.

| Rank | Title                                                                 | Author(s) (Publication year)               | Organization (Country)     | Citation | Average per Year |
|------|-----------------------------------------------------------------------|--------------------------------------------|-----------------------------|----------|------------------|
| 1    | Mycorrhizas and soil structure                                       | Rillig and Mummey (2006)                  | Univ Montana (USA)          | 756      | 47.25            |
| 2    | A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi | Wright and Upadhyaya (1998)                | USAD ARS (USA)              | 657      | 27.38            |
| 3    | Arbuscular mycorrhizae, glomalin, and soil aggregation               | Rillig (2004b)                            | Univ Montana (USA)          | 466      | 25.89            |
| 4    | Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments | Wilson et al. (2009)                      | Oklahoma State Univ (USA)   | 383      | 29.46            |
| 5    | Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation | Khan (2005)                              | Univ Western Sydney (Australia) | 346      | 20.35            |
| 6    | Arbuscular mycorrhizae and terrestrial ecosystem processes           | Rillig (2004a)                            | Univ Montana (USA)          | 346      | 19.22            |
| 7    | Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils | Rillig, Wright, and Nichols et al. (2001b) | Univ Montana (USA)          | 327      | 15.57            |
| 8    | The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species | Rillig, Wright, and Eviner (2002)         | Univ Montana (USA)          | 292      | 14.6             |
| 9    | The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements | Gonzalez-Chavez et al. (2004)              | Nat Resources Inst, Colegio Postgrad, Mexico City | 262      | 14.56            |
| 10   | Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi | Driver, Holben, and Rillig (2005)         | Univ Montana (USA)          | 212      | 12.47            |

Table 3. The top 10 active authors for GRSP research.

| Rank | Author       | N  | Citations | Citations/N | Country |
|------|--------------|----|-----------|-------------|---------|
| 1    | Rillig, MC   | 41 | 5079      | 123.88      | USA     |
| 2    | Wu, Qiang-Sheng | 36 | 651       | 18.08       | China   |
| 3    | Wright, SF   | 28 | 3103      | 110.82      | USA     |
| 4    | Borie, Fernando | 25 | 931       | 37.24       | Chile   |
| 5    | Cornejo, Pablo | 24 | 779       | 32.46       | Chile   |
| 6    | Zou, Ying-ning | 18 | 660       | 36.67       | China   |
| 7    | Srivastava, AK | 14 | 323       | 23.07       | India   |
| 8    | Roldan, A.   | 12 | 657       | 54.75       | Spain   |
| 9    | Nichols, Ka  | 10 | 887       | 88.7        | USA     |
| 10   | Seguel, Alex | 9  | 243       | 27          | Chile   |

protein, carbon, organic-matter and nitrogen, which can indicate the focus of GRSP research field to a certain degree. Additionally, these 56 keywords were divided into 5 clusters, among which the clusters clearly showing research directions are cluster 1 and cluster 2. Research in cluster 1 focuses on the benefits of AMF and GRSP on soil quality, such as soil stability or soil aggregate stability, soil structure, soil organic matter, soil organic carbon and soil nitrogen. Moreover, research in cluster 1 also includes the influencing factors of AMF and GRSP, such as land management (tillage or no-tillage), elevated carbon dioxide and microbial biomass. We can find many keywords of carbon, such as "organic matter," "organic carbon," "elevated CO2," and "carbon sequestration." Finally, cluster 2 concerns the plant effects from the colonization of AMF on plants and glomalin produced by AMF, including promoting plant growth and improving plant tolerance.

The overlay visualization map of these 55 keywords is shown in Figure 5(b), and the yellower the color of the node in the figure is, the newer the keyword. We set the overlay colors range from 2013 to 2017, so that the 55 keywords can have obvious color discrimination. It is worth noting that the color of nodes is almost blue when setting the overlay colors range from 1998 to 2010, more nodes in yellow when setting the overlay colors range from 2010 to 2015 and more nodes in blue when setting the overlay colors range from 2016 to 2021. Figure 5(b) shows that there was no significant change in the research direction of GRSP during this period, although the annual number of publications from 2018 to 2020 increased.

Turning down the requirements on the number of occurrences of keywords, more nodes will appear. As shown in Figure 6(a), 61 additional keywords that have appeared at least 10 times are included, forming new clusters. However, cluster 1, cluster 2 and cluster 3 are just the particulars of the cluster 1 in Figure 5(a). In particular, the keywords related to the mechanism between carbon and GRSP form cluster 1. Moreover, research in cluster 2 focuses on the content of matter in soil, involving the quantification and dynamics of nitrogen and organic carbon. Finally, cluster 3 concerns the effects of AMF and GRSP on soil aggregate stability, and then promoting carbon stability. We need...
to know that though the three clusters all include carbon, research directions and emphases are different. Meanwhile, every cluster shows new nodes, which demonstrates that every research direction for GRSP has had development and progress. The overlay colors were set to range from 2010 to 2021, but there are no obvious yellow nodes. This may also indicate that there is no newly obvious direction for GRSP research in recent three years.

From the overall perspective of co-occurrence keyword analysis, although a large number of keywords appears at the outset, it does not mean that the research on GRSP has completely stagnated. Since the number of annual publications has increased in recent years, GRSP research is still vigorous. It is just that the research direction on GRSP is more detailed, and the concerning research area still focuses on the relationship between soil health and plant health-AMF.
Therefore, the overall number of occurrences of keywords is relatively small, especially as the keywords of the new research directions are inevitably ignored. This brings certain difficulties to keyword analysis. When the minimum number of occurrences of keywords is reduced to 5, the number of keywords increases to 223. In addition, keywords such as ocean and agriculture begin to appear on the periphery of the map. And the keywords related to pollution, soil pollution and heavy metals appear more frequently, and their colors become lighter. In order to explore possible new research directions in recent three years, we continued to reduce the limitation on the minimum number of occurrences of keywords, and the overlay colors are always set to range from 2010 to 2021. Several obvious yellow nodes do not appear until the minimum number of occurrences of keywords reduced to 3, such as climate change, pollution, term land-use, soil respiration and so on. However, these are just some new attempts in GRSP research and cannot form new clusters, which indicates that GRSP research is still under exploration. In the past three years, GRSP research has not only continued to develop on the original basis, but also explores new research areas.

Figure 6. (a) Network and (b) overlay visualization map of co-occurrence keywords for GRSP research from 1998 to 2021.
Burst keywords analysis
The top 19 keywords with the strongest citation burst were shown in Figure 7, and the red bars mean the keywords were cited frequently. These keywords have all appeared since 1999, and the emphasis on carbon dioxide lasted for the longest time. We can see that the keywords related to carbon has always been a research hotspot, but its form has changed: “Carbon dioxide” was the hot spot from 1999 to 2012, followed by “soil carbon” (from 2003 to 2006), “carbon” (from 2014 to 2015), “organic carbon” (from 2017 to 2021). In addition to keywords related to carbon, the popularity of keywords related to space has also had some changes. “Soil” was the earliest research focus, and then it transitioned to plants and then to forests, which means that the research space begins to be integrated, not just confined to a plant. Not only that, some studies concern farmland (Wang, Wang, and He et al. 2015), grassland (Lutgen et al. 2003) and wetland (Wang et al. 2020). It is worth noting that two experimental plants have been favored by researchers, which are citrus and maize. Similar attention has been paid to, AMF in GRSP research, and it can be seen from Figure 7 the keywords that are hyphae, microbial biomass (bioma in the figure), fungi and mycorrhiza. Finally, organic-carbon, maize and enzyme activity have become new focuses of the study.

Discussion
Carbon cycle and carbon sequestration
In order to address global climate change, Paris Agreement proposes that all countries should achieve the peak of greenhouse gas emissions as soon as possible and net emissions of greenhouse gas of the world should achieve zero in the second half of the 21st century. In order to achieve targeted emissions reductions, scientists in various countries are exploring potential carbon pools. Exactly, GRSP has the potential value as a kind of carbon pool and has potential role to carbon cycle and carbon sequestration (Wright, Rillig, and Nichols 2000; Rillig et al. 2001b). Therefore, the GRSP research on carbon has attracted increasingly attention.

We can see the emergence of various forms of carbon from the co-occurrence keywords analysis, such as “organic matter,” “organic carbon,” “elevated CO₂” and “carbon sequestration.” Figure 6(a) suggests that the GRSP research related carbon includes the mechanism of carbon sequestration (Agnihotri et al. 2022), extraction and measurement of carbon (Kumar, Singh, and Ghosh 2018) and benefits to soil carbon stability (Sousa et al. 2012). In addition, burst keywords analysis shows that the keywords related to carbon has always been a research hotspot from 1999 to 2021, including
“carbon dioxide,” “soil carbon,” “carbon” and “organic carbon.” Rise in carbon dioxide changes soil structure (Rillig et al. 1999) published on nature in 1999 pointed out that GRSP can promote the formation of soil aggregates, have potential carbon sequestration value, and play a great role in promoting the research on the ecological function of GRSP. Thus, GRSP research opened up a new research direction: carbon sequestration. The relationship between CO₂ and GRSP is mainly that soil organic carbon is the main source and sink of atmospheric carbon (Sandeep et al. 2016). Global warming may increase the CO₂ released into the atmosphere through the decomposition of soil organic matter. However, GRSP plays an important role in promoting the stability soil organic carbon (Rillig et al. 2007). Also, AMF can promote carbon production and storage via stimulating plant photosynthesis, which thus increases carbon allocation and promotes the carbon cycle (Wang et al. 2016). Naturally, GRSP concentration in soils has a large increase as response to CO₂ exposure (Rillig and Allen 1999; Zhang et al. 2015). Besides, the biomass and diversity of AM fungi increased in response to elevated CO₂ (Rillig et al. 2001a; Treseder et al. 2003). Therefore, GRSP may alleviate the greenhouse effect.

There are many studies on organic carbon, which mainly focus on farmland (Sekaran, Sagar, and Kumar 2021; Singh et al. 2017), grassland (Banegas, Dos Santos, and Molina et al. 2020; Xu et al. 2017), forest (Xu et al. 2017; Singh et al. 2017) and wetlands including mangroves and salt marshes (Das, Ganguly, and Chakraborty et al. 2020; Gisbert, Phang, and Carrasco-Barea 2020; Pei, Ye, and Yuan et al. 2020; Wang et al. 2018b). These effects are caused by GRSP directly acting on soil, or by AMF indirectly acting on soil by releasing GRSP. Moreover, a large number of research results show that there is a significant positive correlation between GRSP content and soil organic carbon content in soil (Gisbert et al. 2018; Singh et al. 2017). This is not only because GRSP promotes carbon sequestration, but also because GRSP is a part of soil organic carbon (Kumar, Singh, and Ghosh 2018). Besides, GRSP existing in mangroves, can be transported by pore water and accumulating in the sediment profile, offsetting the impact of mangrove carbon loss (Wang et al. 2018b). The content of organic carbon in soil is affected by many factors, and land use was studied more. Farming methods and land management methods are the main factors affecting the GRSP content of farmland (Sekaran, Sagar, and Kumar 2021; Sharma et al. 2019; Sandeep et al. 2016). Direct inoculation of AMF will also increase the carbon sequestration capacity of crops such as grains and beans (Subramanian et al. 2019). Some researchers use the content of organic carbon and GRSP in soil as indicators to monitor the quality of farmland or explore the impact of different land management methods (Carter 2002; Kumar, Singh, and Ghosh 2018; Singh et al. 2017).

The heterogeneity of soil systems depends on soil aggregate and pore spaces from the perspective of structure (Wang et al. 2019a). Studies emphasized a close relationship among CO₂, GRSP, soil aggregate water stability and SOC were found (Rillig et al. 2001a). Different land use patterns and global climatic change, such as enhanced CO₂ concentration, drought and altered precipitation pattern, will cause the change of soil aggregates, resulting in the dynamic change of carbon (Sandeep and Manjaih 2016; Su et al. 2020; Zhou, Zhang, and Lambers et al. 2022). Importantly, the faster deposition of GRSP in soil will accelerate the accumulation of SOC, which will be helpful to understand the role of GRSP in soil carbon sequestration under the scenario of future global environmental change (Zhang et al. 2015). Therefore, it is important to establish the global distribution database of GRSP to understand the contribution of GRSP to SOC.

Progress and direction analysis of GRSP research

Through the cluster analysis of keywords, the GRSP research was mainly divided into two research directions: AMF-GRSP-soil and AMF-GRSP-plants. Hundreds of studies have ascribed numerous soil and plant health to GRSP (Irving et al. 2021), and we can see them from Table 4. However, some mechanisms of action in the ecological roles of GRSP are still unclear, such as alleviating heavy metal pollution (Riaz, Kamran, and Fang et al. 2021) and improving plant resistance (Lenoir, Fontaine, and Sahraoui 2016). Some studies addressed that both AMF and GRSP can also be used to alleviate soil degradation (including soil desertification, pollution), while others indicated promote the reconstruction of degraded ecosystems (Singh, Zhu, and Chen et al. 2020; Yu et al. 2017; Bi, Wang, and Wang 2018). From the published papers in recent years, the practice of AMF and GRSP in restoring soil health and plant health has great future prospects (Matos et al. 2022; Solis-Ramos, Coto-Lopez, and Andrade-Torres 2021; Ma et al. 2016).

Moreover, “soil” and “plant” appear in the top 19 keywords with the strongest citation burst, and “soil” appeared earlier than “plant.” GRSP is extracted from soil, which includes land soil (forest, farmland, grassland and desert) and deposit sediment (lakes, rivers, wetlands and oceans). It is worth mentioning that the GRSP research in wetland ecosystem began in 2010 (Adame et al. 2010). However, there are less than 20 documents related to wetlands, most of which are published by Chinese researchers. Moreover, the documents for GRSP research about ocean are less. In keyword analysis, “marine” appears only when we set the minimum number of occurrences of keywords to 5. The content of GRSP in wetland sediments is next to that in tropical forests, ranking second (Wang et al.
There are two sources of GRSP in coastal wetlands: 1) secreted by AMF that form a symbiotic relationship with wetland vegetation; 2) the external land source transported by river, wind and groundwater (Adame et al. 2012; Wang et al. 2021). Among them, GRSP imported from external sources accounts for 80% (Pei, Ye, and Yuan et al. 2020). At regional scales, GRSP has been widely found in coastal salt-marsh (Gispert, Phang, and Carrasco-Barea 2020) and seagrass ecosystem (Kaal et al. 2020). However, the specific contribution of GRSP to blue carbon needs to be further studied.

Glomalin is a kind of MAb32B11 antigen (Wright et al. 1996), and mass spectrometry analysis showed that glomalin is the R. irregularis 60 kDa heat shock protein (RIHSP60) (Gadkar and Rillig 2006). However, it is hard to purify glomalin. GRSP is just an operationally defined protein, relies on nonspecific protein quantification of high-temperature citrate extracts (HTCEs) (Holatko et al. 2021). Though using Mab32B11 for enzyme linked immunosorbent assay (ELISA) to quantify glomalin is more accurate, most laboratories have not equipped (Irving et al. 2021). Moreover, Irving et al. (2021) had discussed the defects and future development of Mab32B11 for ELISA. However, it is not clear for the role and significance of glomalin. If glomalin can be purified, it may reclaim a new research direction: the structure, properties and functions of glomalin and so on. In recent years, some progress has been made in purifying glomalin: 1) Mothay and Ramesh (2021) plotted the structure of “glomalin” according to the similarity between glomalin and Hsp60 amino acid sequence; 2) Quartz crystal microbalance (QCM) biosensor can be constructed as a tool to test glomalin (Pohanka 2021). X-ray absorption, Py-Fims and Proteomics have shown that GRSP is a mixture of proteins, humus, lipids and inorganic substances (Gillespie et al. 2011). Hence, accurate quantification of GRSP can help us better evaluate the contribution of GRSP from the perspective of global environmental change to carbon cycle and carbon sequestration, especially global blue carbon sequestration. The role of GRSP in soil and plant health and the special protein properties of glomalin make it particularly important to understand the composition of GRSP and the molecular structure of glomalin. In addition, a unified standard also needs to be established for the definition, molecular structure and extraction methods of glomalin and GRSP.

### Summary

GRSP has been widely studied by researchers at home and abroad since 1998. With the increasing papers on GRSP, we can use bibliometric analysis to help researchers mathematically and statistically understand research development, research trends and topical issues. According to statistics, the subject fields involved in GRSP research most are soil science, agronomy and agriculture multidisciplinary, environmental sciences. Besides, 81.75% of the authors published only one paper, and scholars from China and USA are very active, having many research achievements and high influence. The earliest research on GRSP originated in USA in 1998, which made great progress in the following ten years. There are many relevant research outputs in Europe and America, and a solid internal cooperative relationship has been established. However, in most other countries, GRSP research has developed since 2010. Among them, China is the fastest developing and most active country. In the future, international research cooperation should be strengthened to discuss some frontier issues related to GRSP, even across regions.
In GRSP research, carbon sequestration has always been the focus of attention. Researchers have done some work on the mechanism and influencing factors of carbon sequestration. Although some scholars speculated that it could alleviate the greenhouse effect, there is a lack of quantitative research on it. In addition, there are still many questions in GRSP research that need to be discussed in depth: (1) At present, GRSP has been found in many ecosystems such as farmland, forest, grassland, mangrove and ocean. However, as an important carbon source from land to ocean, its large-scale distribution in coastal wetland ecosystems and its role in the carbon cycle between sea and land deserves further study; (2) Because the problem of specific extraction of glomalin has not been solved, there are some disputes in naming, including glomalin, GRSP and HTECs (high-temperature citrate extracts). Therefore, the composition of GRSP is still the problem needing to be overcome in the future. Furthermore, whether glomalin is pure is still controversial, and the proportion of glomalin in GRSP is not clear. Finding a way to extract “cleaner glomalin” is also important; (3) Many mechanisms of GRSP are still unclear, and there is no obvious distinction of function among AMF and GRSP; (4) The research on the pollution mitigation function of GRSP mostly focuses on heavy metals and polycyclic aromatic hydrocarbons, but its role with petroleum, pesticides and radioactive pollutants remains to be found. In addition to theoretical research, it is also important to combine research results with practice as soon as possible. How to apply AMF or GRSP more simply and efficiently to improve soil structure, increase crop yield or control heavy metal pollution is also a challenge to be faced. Considering GRSP as a recalcitrant soil organic carbon, the land-sea transport of GRSP and its burial in the coastal ocean has an important contribution to climate change mitigation. Explaining the biogeochemical processes of GRSP on land-ocean scale will provide a deeper understanding of the role of GRSP benefits in the global carbon sequestration.

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