Spatio-temporal dynamics of arbuscular mycorrhizal fungi and soil organic carbon in coastal saline soil of China

Huan-Shi Zhang1,2,3,7✉, Ming-Xi Zhou4,7, Xue-Ming Zai5, Fu-Geng Zhao6 & Pei Qin6✉

A comprehensive understanding of the relationship between arbuscular mycorrhizal (AM) fungi and coastal saline soil organic carbon (SOC) is crucial for analysis of the function of coastal wetlands in soil carbon sequestration. In a field experiment, the temporal and spatial dynamics of AM fungi, glomalin-related soil protein (GRSP) – which is described as a N-linked glycoprotein and the putative gene product of AM fungi, SOC, and soil aggregates were investigated in halophyte Kosteletzya virginica rhizosphere soil of coastal saline areas of North Jiangsu, China. Soil samples were collected from a depth of up to 30 cm in two plantation regions from August 2012 to May 2013. Results showed K. virginica formed a strong symbiotic relationship to AM fungi. AM colonization and spore density were the highest in the 10–20 cm soil layer of Jinhai farm in August 2012, because of the presence of numerous fibrous roots in this soil layer. The total GRSP and SOC were the highest in the 0–10 cm soil layer in May 2013 and November 2012, respectively. Correlation coefficient analysis revealed that AM colonization and spore density were positively correlated with total GRSP. Meanwhile, total GRSP was significantly positively correlated with large macroaggregates (>3 mm), SOC, total P, Olsen P, and soil microbial biomass carbon (SMBC), but negatively correlated with microaggregates (<0.25 mm), soil EC, total N, and pH. SOC was positively correlated with spore density, large macroaggregates, small macroaggregates (2–0.25 mm), alkaline N, and SMBC and negatively correlated with microaggregates, EC, pH, and total K. Although it may be a statistical artifact, we found an interesting phenomenon that there was no significant correlation between soil aggregates and AM colonization or spore density. Hence, total GRSP is a vital source of saline soil C pool and an important biological indicator for evaluating coastal saline SOC pool and soil fertility, while AM colonization or spore density may not be.

A lot of research shows hydrological and biogeochemical processes are essential for material and energy exchange among climate-soil-plant systems, and understanding the spatiotemporal features of the water and carbon (C) cycles is of great importance for watershed ecosystem management1,2. Coastal wetlands are one of the most productive ecological systems worldwide and are located in the transition zones of marine and terrestrial ecosystems3. Soil is the core of coastal wetland C budget and the largest organic C pool; as such, slight changes in soil will affect C emissions into the atmosphere4. Furthermore, the structure of soil aggregates, which are the basic unit of soil structure and the site of metabolism and transformation of matter and energy in the soil, confers physical protection against mineralization and decomposition of soil organic C (SOC). Microbial activity is limited within the aggregates, allowing 0.4 to 1.2 Pg C to be sequestered each year in soil aggregates4,5. Therefore, aggregate formation is considered an important soil C sequestration mechanism6. The stability of soil aggregates is also important to maintain soil porosity, gas exchange, erosion resistance, and water holding capacity5. Hammer &
Rillig reported that large amounts of Na⁺ in coastal saline solute induce colloid formation and flocculation, leading to difficulty in the formation of colloidal aggregates. Thus far, limited information is available regarding the relation between soil aggregates and the stability of SOC pool in coastal saline land.

Soil aggregation can be altered directly by management strategies or indirectly by biotic and abiotic factors. Among biotic factors, symbiosis between arbuscular mycorrhizal (AM) fungi, an important group of beneficial microorganisms in soil, and the roots of most land plants contribute to the stability of soil aggregates, including those in high-salinity soils, such as salt marshes. AM fungi naturally occur in salt environments and promote the salinity tolerance of plants by enhancing nutrient acquisition, decreasing Na uptake, and alleviating water stress. The large network of AM external mycelium can entangle soil organic and inorganic particles and thus promote the formation of macroaggregates. Meanwhile, hyphae release an alkaline-soluble glycoprotein compound called glomalin or glomalin-related soil protein (GRSP), which acts as a soil particle binding agent, similar to mucopolysaccharides produced by soil bacteria. GRSP demonstrates 3–10 times higher soil aggregating ability than hot-water extractable carbohydrates, and its concentration in soil is strongly positively correlated with the water-stability of soil aggregates. GRSP is very resistant to decomposition, having a half-life of 7–42 years. It accumulates in soils to account for 4%–5% of soil C, which exceeds the 0.08%–0.2% contribution of soil C from soil microbial biomass. Therefore, GRSP is linked to soil C storage via its effect on soil aggregate stabilization and is a vital component of SOC.

A strong positive relationship exists between GRSP concentration and the amount of water-stable aggregates in non-saline soils. Adame et al. found a negative relationship between GRSP and salinity (which ranged from 0.02 mg·g⁻¹ to 1.38 mg·g⁻¹) in mangrove estuary soils of southeast Australia. Hammer & Rillig suggested that salinity can stimulate AM fungi to secrete GRSP. To our knowledge, no study has directly tested the relationship between AM fungi, soil aggregates, and SOC in coastal saline land.

The main objectives of this study were to evaluate AM fungal status, GRSP, soil organic carbon and soil aggregates concentrations to answer the following questions: (i) are there spatio-temporal differences in SOC, AM fungal colonization and spore density associated with K. virginica in coastal saline soils? And (ii) could AM fungi and GRSP be biological indicators for evaluating coastal saline SOC pools? And (iii) what are the relationships between AM fungi and soil aggregates in coastal saline soils?

**Results**

**Spatial and temporal changes in AM colonization and spore density.** Data on AM colonization and spore density in control plot were not presented because no AM fungi were found. In Qingkou saltern and Jinhai farm plantations, the AM colonization rate and spore density decreased with increasing soil depth (Fig. 1A,B). The AM colonization rate and spore density in the 10–20 cm soil layer were higher than those in the 0–10 cm soil layer of Jinhai farm plantation in August 2012 (Fig. 1B, p > 0.05). In Qingkou saltern plantation, AM colonization rate in 10–20 cm soil layer was lower than that in 20–30 cm soil layer in November 2012 (Fig. 1A). In Qingkou saltern and Jinhai farm, the AM colonization rate decreased from the highest value at August 2012 to the lowest value at February 2013. The spore density in Qingkou saltern showed a similar trend, that is, the highest spore density in the 0–10 cm soil layer was recorded in November 2012.

**Spatial and temporal distribution of total GRSP.** In Qingkou saltern and Jinhai farm, the total GRSP content decreased with increasing soil depth and ranged from 0.08 mg·g⁻¹ to 0.69 mg·g⁻¹ and 0.32 mg·g⁻¹ to 2.43 mg·g⁻¹, respectively (Fig. 2A,B). The total GRSP content in K. virginica plantation is significantly higher in Qingkou saltern than that in control plot in the same layer (p < 0.05). Irrespective of soil layer, the total GRSP content decreased from August 2012 to the lowest values in February 2013 and then returned to high levels in May 2013.

**Edaphic factors.** Ten edaphic factors were assessed to comprehensively determine the local soil condition. The results were presented by a radar chart. The plantation soil pH, available nitrogen, total nitrogen, and soil microbial biomass carbon (SMBC) are all significantly higher in Qingkou saltern than in control plot from August 2012 to May 2013 (p < 0.05, Fig. 3A–D). The SOC content ranged from 6.34 mg·g⁻¹ to 14.2 mg·g⁻¹ in the plantation. The plantation SOC in the 20–30 cm soil layer is significantly higher than that in the 10–20 cm soil layer in August and November 2012 and significantly lower than that in control plot soil in February 2013 (p < 0.05). The electrical conductivity (EC) in all plantations is significantly lower than that in control plot soils in each soil layer (p < 0.05). The EC increased in the plantation with increasing depths of the soil layer. In August 2012 and May 2013, the Olsen phosphorus of the plantation is significantly higher than that of control plot in each soil layer (p < 0.05). The total phosphorus of the plantation is significantly higher than that of control plot (p = 0.024), except for that in February 2013. However, no significant difference was observed between K content in the plantation and control plot in each soil layer (p > 0.05).
In Jinhai farm, soil pH was not significantly different between the plantation and control plot from August 2012 to May 2013 (Fig. 3E–H). The EC and Olsen potassium in the plantation are significantly lower than those in control plot in each soil layer \((p < 0.05)\). By contrast, the total nitrogen, SMBC, and SOC in the plantation are significantly higher than those in control plot in each soil layer in four measurements \((p < 0.05)\), except for SOC in the 20–30 cm soil layer during November 2012 and February 2013. The SOC content ranged from 2.69 mg·g\(^{-1}\) to 10.71 mg·g\(^{-1}\) in the plantation. The Olsen phosphorus in the plantation is significantly higher than that in control plot in each soil layer during November 2012 and May 2013 \((p < 0.05)\).

**Spatial and temporal distribution of soil aggregates.** In Qingkou saltern, irrespective of plantation or control plot in the four periods, soil aggregates are mainly in the fractions of > 5 mm, 1–0.25 mm, and...
microaggregates (MI) (Fig. 4A–D). In August 2012, all soil aggregate fractions between the plantation and control plot exhibited no significant difference. In November 2012, the soil aggregate contents of 3–5, 2–3, and 1–2 mm fractions in the plantation are significantly higher than those in control plot in the three soil layers (p < 0.05).
However, the content of > 5 mm fraction in the plantation is significantly lower than that in control plot in the 0–10 cm soil layer ($p = 0.026$). The large macroaggregates (LM) and small macroaggregates (SM) contents in the plantation are significantly higher with time than those in control plot in February 2013, but the MI content in the

Figure 4. Aggregates-size distribution as determined by wet sieving for the 0–10 cm, 10–20 cm and 20–30 cm layers in Qingkou saltern (A–D) and Jinhai farm (E–H). P1: 0–10 cm layer in K. virginica plantation. P2: 10–20 cm soil layer in K. virginica plantation. P3: 20–30 cm soil layer in K. virginica plantation. C1: 0–10 cm soil layer in control plot. C2: 10–20 cm soil layer in control plot. C3: 20–30 cm soil layer in control plot. Data are means ± SE of five replicates. Comparisons among means were made with the Least Significant Difference (LSD) test.
planted significantly lower than that in control plot in the three soil layers. In May 2013, the soil aggregate contents in 3–5, 2–3, and 1–2 mm fractions are higher than those in control plot, and the MI is significantly lower than that in control plot in each soil layer.

In Jinhai farm, the results of the parameters tested are similar among the four periods (Fig. 4E–H). In the plantation, soil aggregates are mainly in >5 mm and MI. In control plot, soil aggregates are mainly in MI in the three soil layers during the four periods. In all soil layers and periods, the contents of LM and SM in the plantation are significantly higher in Jinhai farm than those in control plot, and the MI content in control plot is significantly higher than that in Jinhai farm in the plantation (p = 0.031).

**Correlation coefficient analysis.** AM colonization was negatively correlated with pH, EC, total nitrogen, and Olsen potassium (p < 0.01) and positively correlated with Olsen phosphorus (p = 0.038), SMBC, spore density, and total GRSP (p < 0.01, Table 1). Spore density was negatively correlated with pH, EC, and total nitrogen (p < 0.01) and positively correlated with SOC, total potassium (p < 0.05), and total GRSP (p = 0.005). Total GRSP was also positively correlated with LM, SOC, total potassium, Olsen phosphorus, and SMBC but negatively correlated with MI, EC, total nitrogen, and pH (p < 0.01). SOC was positively correlated with LM, SM, available nitrogen (p < 0.01), and SMBC (p = 0.033) and negatively correlated with MI, EC, pH (p < 0.01), and total potassium (p = 0.047). LM content was positively correlated with SM, SOC, available nitrogen, and total GRSP (p < 0.01) and negatively correlated with MI (p = 0.007) and total nitrogen (p = 0.036). SM content was positively correlated with SOC (p = 0.005), available nitrogen, and SMBC (p < 0.05) and negatively correlated with MI, EC, total potassium (p < 0.01), and OK (p = 0.038). MI was negatively correlated with SOC, available nitrogen, and total GRSP (p < 0.01). Finally, no significant correlation was observed between soil aggregates and AM colonization or spore density (p > 0.05).

**Discussion**

**AM fungus and edaphic factors.** The inoculation of AM fungi can form good symbiotic association with *K. virginica* roots under various salt stress conditions in greenhouse. This study demonstrated that *K. virginica* in the coastal saline soil of North Jiangsu could also form symbiotic relationships to AM fungi. He et al. reported that the maximal value of the AM colonization and spore density occurred at the 0–10 cm soil layer in farming–pastoral zone. In this study, the AM colonization rate and spore density in the 10–20 cm soil layer are higher than those in the 0–10 cm soil layer because of the presence of numerous fibrous roots in the former. For the same reason, *K. virginica* possessed numerous fibrous roots during August 2012, and the highest AM colonization rate was detected during this period. AM fungal hyphae in roots can be related to the absorption and translocation of low-mobility nutrients, such as P, in soil and water from distant areas that are inaccessible to plant roots. In the present study, increased hyphal colonization could help in efficient nutrient and water absorption and transportation from soil to host, leading to increased nutrient demands in August. This result is in accordance with the reports that the maximum abundance of AM fungi occurs during summer and the colonization declines during winter and early spring. However, Füzy et al. reported that AM colonization peaked in late spring to early summer and exhibited a second peak later in autumn. This may be due to different soil and air temperature changes and plant growth characteristics leading to the difference of AM colonization.

Table 1. Correlation matrix of soil aggregates, edaphic factors, AM fungus, and soil total GRSP in Jinhai farm.

| LM  | SM  | MI  | EC  | pH  | SOC  | TN  | TP  | TK  | OP  | AN  | OK  | SMBC | SD  | AC  | TG  |
|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|
| LM  | -   | 1   |     |     |      |     |     |     |     |     |     |      |     |     |     |
| SM  | 0.473* | -   | 1   |     |      |     |     |     |     |     |     |      |     |     |     |
| MI  | -0.986** | -0.615* | -   | 1   |      |     |     |     |     |     |     |      |     |     |     |
| EC  | -0.038 | -0.460** | -0.122 | 1   |      |     |     |     |     |     |     |      |     |     |     |
| pH  | -0.316 | -0.438** | -0.150 | 0.312 | -0.038* | 1   |     |     |     |     |     |      |     |     |     |
| SOC | 0.544** | 0.741** | -0.629** | -0.481** | -0.338* | 1   |     |     |     |     |     |      |     |     |     |
| TN  | -0.334* | -0.223 | 0.342* | -0.310 | 0.610** | -0.188 | 1   |     |     |     |     |      |     |     |     |
| TP  | 0.098 | 0.145 | -0.116 | 0.020 | -0.224 | 0.011 | 0.320 | 1   |     |     |     |      |     |     |     |
| TK  | 0.048 | -0.543** | -0.060 | 0.344* | 0.269 | -0.544** | 0.127 | -0.306 | 1   |     |     |      |     |     |     |
| OP  | 0.189 | 0.208 | -0.209 | 0.055 | -0.393* | 0.257 | -0.496** | 0.721** | -0.473* | 1   |     |      |     |     |     |
| OK  | -0.128 | -0.338* | 0.179 | 0.496* | -0.373* | -0.266 | -0.080 | -0.268 | 0.045 | -0.190 | -0.238 | 1   |     |     |     |
| SMBC | 0.171 | 0.353* | -0.221 | 0.137 | -0.683** | 0.395* | -0.534* | 0.543* | -0.708** | 0.710** | -0.234 | 0.192 | 1   |     |     |
| SD  | 0.217 | -0.294 | -0.139 | -0.558** | -0.616** | 0.402* | -0.458** | 0.052 | 0.372* | 0.016 | -0.264 | 0.063 | 0.183 | 1   |     |     |
| AC  | 0.322 | 0.038 | -0.296 | -0.493* | -0.809** | 0.074 | -0.683* | 0.251 | -0.012 | 0.345* | -0.180 | -0.429** | 0.566* | 0.819* | 1   |
| TG  | 0.566** | 0.323 | -0.568* | -0.585* | -0.791* | 0.383* | -0.606* | 0.490* | -0.290 | 0.625** | -0.059 | 0.145 | 0.729* | 0.519* | 0.772** | 1   |
In this study, the AM colonization and spore density in the two research sites decreased with increasing soil depth (Fig. 1A,B), consistent with the results of He et al. 35,43, Taniguchi et al. 44, Wang et al. 45, and Zhang et al. 46. This phenomenon might be due to the usual distribution of the main roots of K. virginica in 0–20 cm soil. Moreover, the EC and pH increased but the oxygen concentration decreased with increasing soil depth (Fig. 1A–G). The correlation coefficient analysis results also showed significantly negative correlation of AM colonization and spore density with pH and EC (Table 1). These results corroborate reports on reduction in root mycorrhizal rate at high salinity levels 35,36,46–49. This may be due to salinity being able to hamper colonization capacity, spore germination, and growth of AM fungal hyphae 46,50,51. Füzéry et al. 47 suggested that drought may play an important role in governing mycorrhizal activity in saline habitats because AM fungi may help plants acquire water from soil 42; in addition, the formation of additional arbuscules may facilitate the transfer of water and nutrients to plants. Although mycorrhizal colonization is reduced with increasing salt levels, the symbiosis between AM fungi and halophytes may be strengthened in saline environments once the partnership has been established 32.

Benchieri et al. 48 found that the number of AM fungal spores increased with soil salinity level 49. Hildebrandt et al. 53 and Becerra et al. 54 also reported different halophytic plants associated with numerous AM fungus spore populations in saline soils. In addition, Aliaqsharazd et al. 55 reported that salt stress can stimulate AM fungi sporulation. Benchieri et al. 48 suggested that sporulation can be considered a resistance behavior to help AM fungi survive adverse environmental conditions. In contrast to these findings, the present results indicated a significant reduction in spore count as salinity level increased. This phenomenon could be explained by the inhibitory nature of high salinity levels on spore-producing hyphae 49. Zhang et al. 56 suggested that the high spore number found in October coincides with the end of growth season and can also reflect the accumulation of spores throughout the growing season. In the present study, the highest spore density was observed in Qingkou saltern and Jinhai farm during November and August (Fig. 1), respectively. This finding could be due to the effects of the differences in soil type, edaphic factors, and climate characteristics in the two research areas on spore density and hyphal growth; thus, the number of spores might be different in various types of soil 56.

In contrast to previous research 41,57, the present work showed that seashore saline soil total nitrogen was negatively correlated with AM colonization and spore density. This finding could be due to the higher amounts of N that accumulated in mycorrhizal plants than in non-mycorrhizal plants 47. With AM fungi colonization, K. virginica mycorrhizal became stronger and intensively absorbed nitrogen from saline soil, leading to a shortage of supply of N in this period. Additionally, the entire soil microbial community became more active with the improvement in soil physico-chemical properties and soil fertility, resulting in significantly intensified ammonification and nitrification and accelerated organic nitrogen mineralization. In the present study, the available P content in plantation soils is higher than that in control plot and was positively correlated with AM colonization. This trend might be explained by the fact that enhanced AM colonization can increase the available P content 58. Magallon-Servin et al. 59 suggested that AM can produce numerous organic acids, which can solubilize mineral P. For example, the metabolic activity of the K. virginica root system enhances the reproduction of AM fungi and other soil microorganisms, thereby accelerating the release of soluble phosphatase into the soil 56.

**AM fungus and GRSP in coastal saline soil.** AM colonization is positively correlated with GRSP content 41,59–62. Consistent with previous works, the present study reported that AM colonization, similar to spore density, was positively correlated with GRSP, illustrating the presence of the majority of GRSP in AM fungal hyphae and spores 64. Similar to the findings of He et al. 35 and Taniguchi et al. 44, the current results showed that the total GRSP content in each plantation decreased with soil depth. In Qingkou saltern, the average total GRSP contents in the 30 cm soil layer are 35.44% and 62.5% lower than those in the 20 and 10 cm soil layers, respectively. In Jinhai farm, the average total GRSP contents in the 30 cm soil layer are 33.98% and 56.75% lower than those in the 20 and 10 cm soil layers, respectively. These findings may be due to the distribution depth of the mature K. virginica root. The usual distribution depth of the mature K. virginica roots is 20 cm, which supports the result of Taniguchi et al. 44 and confirms that the reduction of root biomass may cause the decrease in AM fungi colonization and GRSP content with soil depth. The microbial activity was strong in highly fertile soil in the 0–20 cm layer, especially at 10 cm, and favored microbial reproduction expansion and GRSP accumulation. Thus, GRSP content was concentrated in the 0–20 cm soil layer, especially in 0–10 cm.

GRSP is a very stable biomolecule with a half-life of 6–42 years in soil 52,63, but concentrations can fluctuate throughout the growing season 65. Moreover, inoculation of AM fungi stimulated the synthesis of easily extractable GRSP (EE-GRSP), a fraction of soil GRSP 11, which is newly produced by the hyphae and spores of AM fungi 62. In the present study, the highest concentrations of total GRSP in saline soil were observed in May or August, and the lowest was detected in February. These results are consistent with those reported in previous studies 43,65, which indicated that the highest total GRSP content was observed in May; Zhang et al. 48 reported that both total GRSP and EE-GRSP contents were the highest in August and lowest in October. This finding may be due to the corresponding mycorrhizal infection in different sampling periods. The local area has more suitable temperature and water and light conditions in May and August, and the plants are in a vigorous growth period. In addition, the formation of additional arbuscules may facilitate the transfer of water and nutrients to plants. Hence, the total GRSP content fluctuated seasonally because AM fungi and K. virginica were affected by seasonal changes, demonstrating time heterogeneity.

Different kinds of soil texture exhibit varied influences on total GRSP content. In agricultural, natural grassland, desert, and mangrove forest ecosystems, the total GRSP contents are 1–21 mg·g⁻¹, 4.5–5.0 mg·g⁻¹, 2.49–4.11 mg·g⁻¹, and 0.46–1.38 mg·g⁻¹, respectively 21,25,41,49. In the present study, the total GRSP content in the K. virginica plantation in Jinhai farm is 0.85 mg·g⁻¹ to 2.43 mg·g⁻¹, which is lower than that in natural grassland soil but higher than that in the mangrove forest ecosystem. This finding indicates that K. virginica could establish good symbiotic association with AMP 35,36 and contribute to the restoration of saline soil. The total GRSP content is higher in Jinhai farm than in Qingkou saltern (Fig. 2) because of the heavy clay soil texture in the latter and the
sandy loam in the former. AM fungi obtained more suitable soil conditions for growth in the Jinhai farm soil, thus demonstrating higher AM colonization and spore density in August, 2012 (Fig. 1B). Furthermore, GRSP concentration may be influenced by soil mineral and fertility. In the current study, total GRSP showed a significantly positive relationship to SOC, total phosphorus, Olsen phosphorus, and SMBC, consistent with the findings of many researches. This result could be due to the requirement of mycorrhizal fungal growth and metabolism. Rillig reported that total GRSP is an important part of soil N; in addition, many studies indicated that GRSP content is positively correlated with available nitrogen. In the present study, total GRSP was significantly correlated with available nitrogen and negatively correlated with soil total nitrogen. This can be explained by former results that there is a higher portion of available nitrogen in the plantation plots.

The amount of GRSP was negatively correlated with soil EC and pH. Our results are strongly supported by other authors, who reported the significant negative effects of soil salinity on GRSP. Lovelock et al. found that GRSP levels in soil and in vitro cultures were negatively correlated with hyphal length, suggesting that the production of this compound may be a stress response. Hammer and Rillig reported that the GRSP content increased up to a threshold level of 150 mM NaCl but decreased with further increase in the salt concentration. Garcia et al. reports that maximum salinity stress (2.0 dS m⁻¹) increased 6% and 18% GRSP production than 1.0 dS m⁻¹, respectively. Hence, GRSP may be involved in the inducible stress responses of AM fungi to salinity.

### GRSP and soil aggregates in coastal saline soil

The distribution patterns of soil aggregates differed in the two sites. In Qingkou saltern, both in plantation and control plot, soil aggregates in all soil layers are mainly in > 5 mm, 1–0.25 mm, and MI. In Jinhai farm, soil aggregates are mainly in > 5 mm and MI in the plantation, and 95% are MI in control plot. This finding could be directly due to difference in soil texture in the studied areas; that is, Qingkou saltern has heavy clay with high soil bulk density and low nutrition, whereas Jinhai farm has sandy loam and up to 65.50% sand (Table 2). Soil in these two sites showed very poor granulation structure. Such results suggest that soil aggregation decreased as levels of sand and carbonate increased, likely due to concurrent decreases in levels of clay in the soil. In addition, the contents of macroaggregates (≥ 0.25 mm) effectively increased in the plantation soil compared with that in CK soil. Macroaggregates were negatively correlated with microaggregates (< 0.25 mm), suggesting that the former was gradually formed from the latter during planting K. virginica periods. This finding may be related to amount of fibrous roots can wind up dispersion soil particles and form large aggregates. Furthermore, the stability of soil aggregates is probably affected more by the direct and indirect actions of the plant–fungal system, rather than by plant root metabolism. In present study, SM content was positively correlated with SMBC, and LM content was positively correlated with total GRSP; as such, microorganisms are one of the most active and important biological factors.

Wright et al. indicated that glomalin is an insoluble glue-like substance only released by AM fungi into the soil environment during hyphal turnover and after the death of the fungus; this compound may contribute to binding within microaggregates and macroaggregates. Wright and Anderson found that aggregate stability and GRSP were linearly correlated (r = 0.73, n = 54, p < 0.001) across all treatments from different cropping systems. In relation to this finding, the present results indicated the positive correlation between LM and TG, with coefficient of 0.566 (p < 0.01), and the negative correlation between MI and total GRSP, with coefficient of 0.568 (p < 0.01). Hence, soil GRSP presents strong cementing ability, inducing the formation of aggregates with increased structural stability. In addition, external hyphae can promote the formation of soil aggregates by physical tangles and changing soil dry–wet circulation. Bearden and Petersen suggested that mycorrhizae primarily influence the stability of macroaggregates. By contrast, no significant correlation was found between soil aggregate and AM colonization or spore density (p > 0.05) in the present study. This may be due to the formation of soil aggregates takes a long time, but the fibrous roots used to check AM colonization have a shorter time to grow from the main and lateral roots of K. virginica. Meanwhile, it may also be affected by the detection methods and environmental factors, and there is a large gap between the test results and the actual situation of AM colonization or spore density. Rillig et al. reported that the direct effect of GRSP on aggregate stability is higher

|                  | Qingkou saltern | Jinhai farm |
|------------------|-----------------|-------------|
| pH               | 8.22            | 8.43        |
| Electrical conductivity (dS·m⁻¹) | 4.13            | 3.21        |
| Soil salinity(‰) | 15.35           | 10.16       |
| Soil organic carbon (%) | 4.52            | 4.97        |
| Total nitrogen (g·kg⁻¹) | 0.16            | 0.49        |
| Total phosphorus (g·kg⁻¹) | 0.45            | 0.80        |
| Total potassium (g·kg⁻¹) | 30.22           | 18.21       |
| Alkaline nitrogen (mg·kg⁻¹) | 10.72           | 39.42       |
| Olsen phosphorus (mg·kg⁻¹) | 5.52            | 2.19        |
| Olsen potassium (mg·kg⁻¹) | 552.07          | 212.50      |
| Sand (%)         | 15.50           | 65.50       |
| Silt (%)         | 22.50           | 13.50       |
| Clay (%)         | 61.50           | 20.50       |

Table 2. Physical and chemical properties of soils at two sites.
than the total (direct and indirect) effect of hyphae on soil aggregate stability in oak-hickory (Quercus-Carya) forests of eastern North America; this phenomenon also can partly explain the weak correlation between soil aggregate and AM fungi. Barto et al. suggested that abiotic factors (mowing, grazing, and fertilization) can be more important for determining soil aggregation than biotic factors (root length and mass, AM colonization, extraradical AMF hyphal length), especially in highly aggregated soils. Hence, contrary to He et al.'s research conclusion that spore density, colonization of hyphae, the contents of glomalin can be used as parameters to monitor the development of organic carbon dynamic and nutrition cycle in sand soil, AM colonization or spore density may not be used to evaluate coastal saline SOC pool and soil fertility. Furthermore, we cannot ignore another possibility that the lack of correlation between soil aggregate and AM fungi was a statistical artifact. The mechanism underlying this phenomenon has not been fully understood yet. The results also showed that SM content was negatively correlated with soil EC, similar to the report of Lax et al. This probably due to large amount of Na ions complicate the formation of colloidal aggregates in coastal saline soil.

**AM fungus and SOC in coastal saline soil.** Coastal soil ecosystems dominated by plants play a critical role in the global sequestration of C, and the SOC pool acts as a crucial regulator of C fluxes between biosphere and atmosphere. Our previous studies revealed that plant biomass improved by AM fungi inoculation might be beneficial to coastal soil ecosystems in sequestering large amounts of C. We also found that AM fungi inoculation could strongly promote plant dry biomass and nutrient uptake by *K. virginica*, regardless of salinity level. In the present work, after planting *K. virginica* for 3 years, the SOC in the plantation is significantly higher than that in control plot in each soil layer in several periods (p < 0.05). This finding could be due to the ability of *K. virginica* to sequester SOC within numerous living biomass aboveground and belowground, litter, and dead wood. Moreover, the mycorrhizal roots of *K. virginica* can provide photosynthetic C, which, in turn, is delivered to soil via fungal hyphae. This finding is confirmed by the positive correlation between spore density and SOC (p < 0.05) (Table 1). Rillig stated that the C in GRSP largely contributes to the total soil C pool. In tropical soils, the amount of C in GRSP is estimated to be 37%, which represents 3% of soil C pools. The total GRSP-to-SOC ratio of sand and soil is between 34.88% and 66.85%. In the present study, total GRSP was positively correlated with SOC (p < 0.05), with the highest total GRSP-to-SOC ratio of up to 53.29% in Jinhai farm. Hence, total GRSP is a vital source of saline soil C pool, and important biological indicator for evaluating coastal saline SOC pool and soil fertility.

In addition, as insoluble glue, GRSP can stabilize soil aggregates and significantly reduce organic matter degradation by protecting labile compounds within soil aggregates. In agreement with these findings, the present study showed that LM was positively correlated with SOC and total GRSP (p < 0.01). Meanwhile, the negative correlation of MI content with SOC and total GRSP (p < 0.01) illustrates the importance of soil aggregate stabilization in inhibiting the degradation of organic matter and promoting soil C sequestration. In our previous study, the introduced microbe (*Glomus mosseae*, and a phosphate-solubilizing fungus *Mortierella sp.*) can collaborate with indigenous microorganisms to promote the humification of organic materials. Plant–AM fungi mutualism can improve the reestablishment of vegetation in bare saline-alkaline soil and drives the vegetation restoration to a community dominated by the original species. In the present work, the significant positive relationship among AM colonization, total GRSP, SMBC, and SOC suggests that AM fungi play a crucial role in stimulating the growth of indigenous microorganisms, enhancing the stability of saline SOC pool and soil fertility, and promoting the reestablishment of vegetation when *K. virginica* was introduced into the coastal saline soil of North Jiangsu and other sites in China.

The SOC contents in the 20–30 cm soil layer of the two plantations intensively fluctuated following spatio-temporal dynamics and are even lower than that in control plot. These results suggest that SOC in the 20–30 cm soil layer of *K. virginica* plantation demonstrated instability and spatio-temporal heterogeneity in coastal saline soil. However, further research is required to understand the relative mechanisms.

**Conclusion**
A strong symbiotic relationship was found between *K. virginica* and AM fungi after their introduction into the coastal saline soil of North Jiangsu for 3 years. This study demonstrated highly variable temporal and spatial patterns in the dynamics of AM fungi, total GRSP, and SOC, which were affected negatively by soil salinity. The significantly positive relation among AM fungi, total GRSP, and SOC were also confirmed. The results also revealed that soil aggregate stabilization, especially soil large macroaggregates (>3 mm), is crucial to maintain the stability of total GRSP and saline SOC pool. Although it may be a statistical artifact, a new phenomenon that no significant correlation exists between soil aggregate and AM fungi was observed, which contradicts previous findings. Hence, total GRSP is a vital source of saline soil C pool and an important biological indicator for evaluating coastal saline SOC pool and soil fertility, while AM colonization or spore density may not be. Future research must investigate the relation between AM fungi and saline soil aggregate to improve understanding of their roles in coastal ecosystems.

**Materials and Methods**

**Study site.** North Jiangsu experiences a typical temperate and monsoonal climate with four clearly distinct seasons. The study sites selected are Qingkou saltern of Lianyungang City (34°45′N, 119°11′E) and Jinhai farm of Yancheng City (32°59′N, 120°46′E), which are located in North Jiangsu and possess the highest annual average temperatures of 25.8 °C and 28.1 °C, respectively, in July and the lowest annual average temperatures of 0.7 °C and 2.0 °C, respectively, in January. The annual average precipitations of Lianyungang and Yancheng are 896.7 and 1020.5 mm, respectively, with 50% of the precipitation occurring from June to September. The soil types of Qingkou saltern and Jinhai farm are coastal meadow saline soil, and the soil textures are heavy clay and sandy loam, respectively (Table 2).
Field and experimental sampling. At each study site, the 1200 m² area without vegetation for high salt content was divided into six parts. The experimental design was full factorial, three parts were randomly selected as K. virginica plantation, and three K. virginica seeds were planted in one hole according to 0.5 m × 0.5 m design on May 4, 2009. 10 d after germination, germinants were thinned from 3 to 1 in each hole and each part had 800 plants. The residual three parts were selected as control plot without K. virginica. After seedling thinning, K. virginica was allowed to grow unmanaged from 2009 onwards. And five sites in each part were randomly selected for soil sampling, which started on August 15, 2012 and every three months thereafter until May 25, 2013. Soil samples (n = 5) were collected from section at depths of 0–10, 10–20, and 20–30 cm. Prior to sample collection, the upper layer of soil (approximately 5 mm) was scraped off to remove litter. The collected soil samples were stored in sealed polyethylene bags placed in an insulated container and transported to a laboratory. The soil samples (50 g) for soil microbial biomass C analyses were dried at room temperature, passed through a 4 mm sieve, and stored in sealed plastic bags at 4 °C until analysis. The remaining samples were divided into two parts after air drying. One part (100 g) was milled to obtain particles with size < 2 mm for determination of physico-chemical properties, and the other part (350 g) was used for water stability structure analysis.

Analyses of total GRSP. Total GRSP was extracted from 1 g of soil by using the method described by Wright and Upadhyaya7. Extraction was conducted with 8 ml of 50 mM Na citrate (pH 8.0) at autoclave cycles of 121 °C for 60 min until the supernatant showed no red brown color typical of GRSP. Based on our previous experiment, two to three cycles are recommended. The fractions were determined through Bradford assay using bovine serum albumin as standard.

Analyses of AM fungus spore and colonization. Soil samples (25 g each) were used to determine spore density of AM fungus. Spore number was determined by wet sieving in a 40 μm mesh and decanting, followed by sucrose density centrifugation. The suspension was carefully decanted and added with 40% sucrose solution. AM fungus spores were counted under a stereoscopic microscope at 40 ×. Sporocarps were dissected with forceps, and the released spores were counted. Spore density was expressed as number of spores per 10 g of dry soil.

Fresh roots were cut into 0.5–1.0 cm segments and washed until free of soil. The roots were then stained with 0.5% (w/v) acid fuchsinsolution according to the method described by Phillips & Hayman83 and Zhao & He84. AM colonization was quantified by glass slide method, where 50 randomly selected 1 cm root segment units were microscopically examined90. Total colonization was expressed as the percentage of root segments colonized for a root sample.

Analyses of soil aggregates. Soil aggregates were fractionated using a wet-sieving procedure85,86. After capillary wetting of 100 g of air-dried soil to field capacity, the samples were immersed in water on a nest of 5, 3, 2, 1, and 0.25 mm sieves and shaken vertically at 3 cm height for 50 times during a 2 min period. This wet-sieving procedure resulted in fractions of > 3 mm LM, 2–0.25 mm SM, and < 0.25 mm MI. Soil aggregates retained on each sieve were backwashed into pre-weighed containers, oven dried at 50 °C for 2–3 days, and weighed.

Analyses of general soil properties. Soil pH was analyzed in a 1:5 soil-to-water ratio. SOC was determined by dichromate oxidation85. EC of soil was measured with a conductivity meter (Model DDS-11A; Leizi, Shanghai, China). SMBC was analyzed by fumigation–extraction method99. Available nitrogen was measured using alkaline hydrolysis diffusion method90. Olsen potassium in soil was determined by ammonium acetate through flame photometry. Olsen phosphorus was determined by chlorostannus-reduced molybdophosphoric blue color method after extraction with 0.5 M sodium bicarbonate for 30 min90. total nitrogen, total phosphorus, and total potassium concentrations of soils were determined using the method of Olsen et al90.

Statistical analysis. Data were subjected to ANOVA using IBM SPSS Statistics (version 19.0; IBM Corp., Armonk, NY, USA). Differences were considered significant at p < 0.05. The means of main effects were compared using least significant difference test after a significant ANOVA test result. Pearson linear correlations among the parameters were evaluated with SPSS 19.0.

References
1. Zhao, F. et al. Spatiotemporal features of the hydro-biogeochemical cycles in a typical loess gully watershed. *Ecol. Indic.* 91, 542–554 (2018).
2. Sun, P. et al. Remote sensing and modeling fusion for investigating the ecosystem water-carbon coupling processes. *Sci. Total Environ.* 697, 134064 (2019).
3. Oslam, M. J. et al. Climate and plant controls on soil organic matter in coastal wetlands. *Global Change Biol.* 24(11), 5361–5371 (2018).
4. Blanco-Canqui, H. & Lal, R. Mechanisms of carbon sequestration in soil aggregates. *Crit. Rev. Plant Sci.* 23, 481–504 (2004).
5. Jiménez, J. & Lal, R. Mechanisms of C sequestration in soils of Latin America. *Crit. Rev. Plant Sci.* 25, 337–363 (2006).
6. Tang, H. et al. Carbon sequestration of cropland and sandy soils in China: potential, driving factors, and mechanisms. *Growth. Gases 95(5), 872–885 (2019).
7. Six, J. et al. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* 70, 555–569 (2006).
8. Hammer, E. & Rillig, M. The influence of different stresses on glomalin levels in an arbuscular mycorrhizal fungus—salinity increases glomalin content. *Plos One* 6, e28426 (2011).
9. Zhang, Z. et al. Effects of arbuscular mycorrhizal fungi on inoculated seedling growth and rhizosphere soil aggregates. *Soil Till. Res.* 194, 104340 (2019).
10. Li, X. et al. Contribution of arbuscular mycorrhizal fungi of sedges to soil aggregation along an altitudinal alpine grassland gradient on the Tibetan Plateau. *Environ. Microbiol.* 17, 2841–2857 (2015).
11. Wu, Q. et al. Systematicness of glomalin in roots and mycorrhizosphere of a split-root trifoliate orange. *Plant Soil Envir.* **62**, 508–514 (2016).
12. Caravaca, F. et al. Plant type mediates rhizospheric microbial activities and soil aggregation in a semiarid Mediterranean salt marsh. *Geoderma* **124**, 375–382 (2005).
13. Aliasgharzad, N. et al. Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. *Mycorrhiza* **11**, 119–122 (2001).
14. Cui, X. et al. Reclamation negatively influences arbuscular mycorrhizal fungal community structure and diversity in coastal saline-alkaline land in Eastern China as revealed by illumina sequencing. *Appl. Soil. Ecol.* **98**, 140–149 (2016).
15. Yamato, M. et al. Community of arbuscular mycorrhizal fungi in coastal vegetation on Okinawa Island and effect of the isolated fungi on growth of sorghum under salt-treated conditions. *Mycorrhiza* **18**, 241–249 (2008).
16. Khalloufi, M. et al. The interaction between foliar GlA3 application and arbuscular mycorrhizal fungi inoculation improves growth in salinated tomato (*Solanum lycopersicum*) plants by modifying the hormonal balance. *J. Plant Physiol.* **214**, 134–144 (2017).
17. Lenon, I. et al. Arbuscular mycorrhizal fungal responses to abiotic stresses: A review. *Phytochemistry* **123**, 4–15 (2016).
18. Miller, R. & Jastrow, J. Mycorrhizal fungi influence soil structure. In: Kapulnik, Y., Douds, D.D. (Eds.), *Arbuscular Mycorrhizas: Molecular Biology and Physiology*. Kluer Academic, Dordrecht, 3–18 (2000).
19. Bearden, B. & Petersen, L. Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of vertisols. *Plant Soil* **218**, 173–183 (2000).
20. Rillig, M. Arbuscular mycorrhiza: glomalin and soil quality. *Can. J. Soil Sci.* **84**, 355–363 (2004).
21. Wright, S. & Upadhyaya, A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* **198**, 97–107 (1998).
22. Rillig, M. et al. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil* **233**, 167–177 (2001).
23. Xiao, L. et al. Increased soil aggregate stability is strongly correlated with root and soil properties along a gradient of secondary succession on the Loess Plateau. *Ecol. Eng.* **143**, 105671 (2020).
24. Rillig, M. et al. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant Soil* **238**, 325–333 (2002).
25. Adamse, M. et al. Sedimentation within and among mangrove forests along a gradient of geomorphological settings. *Environ. Coast. Shelf. Sci.* **86**, 21–30 (2010).
26. Guo, Y. et al. Gene expression of halophyte *Kosteletzya virginica* seedlings under salt stress at early stage, 2009. *Genetica* **137**, 189–199 (2009).
27. Zhou, M. X. et al. Effect of NaCl on proline and glycinebetaine metabolism in *Kosteletzya pentacarpos* exposed to Cd and Zn toxicities. *Plant Soil* **441**, 525–542 (2019).
28. Zhou, M. X. et al. Salinity modifies the interactive effects of cadmium and zinc on ethylene and polyamine synthesis in the halophyte plant species *Kosteletzya pentacarpos*. *Chemosphere* **209**, 892–900 (2018).
29. Zhou, M. X. et al. Salinity modifies heavy metals and arsenic absorption by the halophyte plant species *Kosteletzya pentacarpos* and pollutant leaching from a polycanminated substrate. *Ecotaxon. Environ. Saf.* **182**, 109460 (2019).
30. Zhou, M. X. et al. NaCl impact on *Kosteletzya pentacarpos* seedlings simultaneously exposed to cadmium and zinc toxicities. *Environ. Sci. Poll. Res.* **25**, 17444–17456 (2018).
31. Zhou, M. X. et al. The cytokinin trans-zeatin riboside increased resistance to heavy metals in the halophyte plant species *Kosteletzya pentacarpos* in the absence but not in the presence of NaCl. *Chemosphere* **233**, 954–965 (2019).
32. Qin, P. et al. Ecological engineering through the biosecure introduction of *Kosteletzya virginica* (seashore mallow) to saline lands in China: A review of 20 years of activity. *Ecol. Envir. Sci.* **74**, 174–186 (2014).
33. Ruan, C. et al. *Kosteletzya virginica*, an agroecogineering halophytic species for alternative agricultural production in China’s east coast: ecological adaptation and benefits, seed yield, oil content, fatty acid and biodiesel properties. *Ecol. Eng.* **32**, 320–328 (2008).
34. Pan, L. & Qin, P. Preparation, composition, structure and properties of the *Kosteletzya virginica* bastfiber. *Fiber. Polym.* **12**, 911–918 (2011).
35. Zhang, H. et al. Evidence that arbuscular mycorrhizal and phosphate-solubilizing fungi alleviate NaCl stress in the halophyte *Kosteletzya virginica*: nutrient uptake and ion distribution within root tissues. *Mycorrhiza* **24**, 383–395 (2014).
36. Zhang, H. et al. Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella sp.*) and their effects on *Kosteletzya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. *Biol. Fert. Soils* **47**, 543–554 (2011).
37. He, X. et al. Spatial distribution of arbuscular mycorrhizal fungi and glomalin of *Hippophae rhamnoides* I. infarming-pastoral zone from the two northern provinces of China. *Acta. Ecol. Sinica.* **31**, 1653–1661 (2011).
38. Lalitha, M. et al. Role of vesicular-arbuscular mycorrhize in mobilization of soil phosphorus. In: Meena V., Mishra P., Bish J., Pattanayak A. (eds) *Agriculturally Important Microbes for Sustainable Agriculture*, 317–331 (2017).
39. Lugo, M. et al. Arbuscular mycorrhizal fungi in a mountain grassland II: seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia* **95**, 407–415 (2003).
40. Clark, N. et al. Arbuscular mycorrhizal fungal abundance in the Mojave Desert: seasonal dynamics and impacts of elevated CO₂. *J. Arid Environ.* **73**, 834–843 (2009).
41. Zhang, Y. et al. Dynamics of arbuscular mycorrhizal fungi and glomalin under *Psammochloa villosa* along a typical dune in desert, North China. *Symbiosis* **73**, 145–153 (2017).
42. Wu, Q. et al. Drought, but not salinity, determines the apparent effectiveness of halophytes colonized by arbuscular mycorrhizal fungi. *J. Plant Physiol.* **165**, 1181–1192 (2008).
43. He, X. et al. Dynamics of arbuscular mycorrhizal fungi and glomalin in the rhizosphere of Arctium lappa L. in Mu Us sandland, China. *Soil Biol. Biochem.* **42**, 1313–1319 (2010).
44. Taniguchi, T. et al. Colonization and community structure of root-associated microorganisms of *Sabina vulgaris* with soil depth in a semiarid desert ecosystem with shallow groundwater. *Mycorrhiza* **22**, 419–428 (2012).
45. Wang, Q. et al. Spatial-temporal dynamics of arbuscular mycorrhizal fungi with glomalin-related soil protein and soil enzymes in different managed semiarid steppes. *Mycorrhiza* **24**, 525–538 (2014).
46. Giri, B. et al. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. *Microb. Ecol.* **54**, 753–760 (2007).
47. Garg, N. & Chandel, S. Effect of mycorrhizal inoculation on growth, nitrogen fixation, and nutrient uptake in *Cicer arietinum* (L.) under salt stress. *Turk. J. Agric. For.* **35**, 205–214 (2011).
48. Krishnamorthy, R. et al. Changes of arbuscular mycorrhizal traits and community structure with respect to soil salinity in a coastal reclamation land. *Soil Biol. Biochem.* **72**, 1–10 (2014).
49. Benchirif, K. et al. Impact of soil salinity on arbuscular mycorrhizal fungi biodiversity and microflora biomass associated with *Tamarix articulata* Vahl rhizosphere in arid and semi-arid Algerian areas. *Sci. Total Environ.* **533**, 488–494 (2015).
50. Delvian & Rambey R. Effect of salinity on spore germination, hyphal length & rd root colonization of the Arbuscular Mycorrhizal Fungi. In *IOP Conference Series: Earth and Environmental Science* 260, 012124 (2019).
51. Ramasamy, K. et al. Changes of arbuscular mycorrhizal traits and community structure with respect to soil salinity in a coastal reclamation land. *Soil Biol. Biochem.* **72**, 1–10 (2014).
Acknowledgements
This research was supported by the National Key R and D Program of China (2017YFC0506005) and the Natural Science Foundation of Jiangsu Province (Grants No BK20141064 and BK20151098). We would like to thank Chengcheng Gang of Nanjing University for their helpful comments on the manuscript and the charting method.

Author contributions
Huan-Shi Zhang and Pei Qin planned and designed the research. Ming-Xi Zhou and Huan-Shi Zhang performed experiments, analyzed data, and wrote the manuscript. Xue-Ming Zai and Fugeng Zhao performed data analysis and paper editing. All authors read and approved the final manuscript.
Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to H.-S.Z. or P.Q.
Reprints and permissions information is available at www.nature.com/reprints.
Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020