Community structure of fungal pathogens causing spikelet rot disease of naked oat from different ecological regions of China

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Spikelet rot disease (SRD) is an emerging disease of the grain surface of naked oat in China that affects both grain yield and quality. The typical symptom is discoloration from the black structures of the causal fungi. Here, we investigated the fungal communities on the grain surfaces of cultivar Bayou 13 grown in ten ecological oat-producing regions of China, to identify the main pathogens of naked oat SRD. Our results showed that the growth of Alternaria spp. and Davidiella spp. exhibited a competitive relationship and was mainly affected by the elevations of all 10 ecological regions. The dominant pathogens were Davidiella spp. in Shannan Prefecture in Tibet and Haidong Prefecture in Qinghai Province and Alternaria spp. in the other eight regions. The ratios of black pathogens of interest to all pathogens in Shannan Prefecture and Haidong Prefecture were significantly lower than those of the other eight regions, thus indicating that SRD mainly occurred in regions below 2000 m (elevation). We isolated black fungal pathogens from grain surfaces and deduced that they were Alternaria spp. by sequence comparison. The blackened appearance of the grain surfaces was more evident under spray inoculation with a spore suspension of Alternaria than under the control in greenhouse experiments. The recovered pathogen was the same as the pathogen used for inoculation. We thus concluded that Alternaria alone causes naked oat SRD and mainly infects naked oat in regions below 2000 m, which provides a basis for the recognition and management of SRD of naked oat.

Oat (Avena sativa) is one of the most important cereal crops in the world and is cultured in all agricultural regions of the world with a moderate climate. The hexaploid cultivated species A. sativa is represented by both hulled (A. sativa subsp. sativa L.) and naked forms (A. sativa subsp. nudisativa (Husnot.) Rod. et Sold.)2, which are believed to have originated in China. The yield and composition of nutritional and functional active ingredients were higher in naked oat than in hulled oat4,5. Naked oat is the main cultivated oat species in China, accounting for 92% of the total area planted in oat (106.7 hm² of 120.0 hm²)6. Naked oat is mainly distributed in arid and cold regions, including Inner Mongolia, Yunnan Province, Gansu Province, Sichuan Province, Hebei Province, Shanxi Province, Qinghai Province, Jilin Province, and Tibet7. In these regions, rainfall is scarce and mostly concentrated from July to September. The occurrence of continuous rainfall during the harvest season leads to high moisture and warm temperatures being important factors in the spread of plant diseases8. In this period, many fungi on glumes and panicles of weakened plants or moribund tissues of cereal crops result in grain discoloration9. Extensive occurrence of spikelet rot disease (SRD, Supplementary Figure S1) on oat grains in fields is a result of rainfall just before harvest and is a regularly observed phenomenon10. SRD of naked oat is characterized by black fungal discoloration, especially on the surface of the kernels as well as beneath the pericarp of the grain ears11. This discoloration is mainly considered a blemish of the grain but not a factor affecting quality10. This disease has generally received comparatively little attention and is considered a minor disease that does not warrant specific control measures11 when compared with oat diseases such as rusts, blights, smuts, spots, and culm and root rots12-14. Recently, the most general usage of naked oat has been for livestock feed, but oat consumption as a human food has recently increased, perhaps due to the reported health benefits arising from the nutritional value of the grains14. However, fungal infection of grains in the field may result in yield nutritive value reduction and the production of toxins that are harmful to humans and animals15,16. Thus, a high grain quality is of great significance to the safe production of naked oat products.
and on dead or dying plant tissues.10 With sufficient surface moisture, the fungal spores present on the kernels can germinate and grow. With sufficient fungal growth, the husk can become discolored. However, Fusarium spp. were not observed on normal and discolored grains of naked oat in China, while more Alternaria spp. were isolated from discolored grains than from normal grains.27 Currently, there are no systematic reports on SRD on naked oat, but it is considered an increasingly important disease of naked oat in China. Such a lack of knowledge hampers the development of suitable disease risk forecasts and control measures.

The species abundance and composition of the microbiota are important factors in determining the quality of the grain.15 Throughout the growing season, weather conditions such as temperature and humidity influence the distribution of the infecting fungal species and lead to geographical variation in the species distribution.26–31. As the main cultivar of naked oat in China, Bayou 13 is planted in different ecological regions, including Tibet, Shanxi Province, Gansu Province, Inner Mongolia, Qinghai Province and Hebei Province. Therefore, in this study, we investigated fungal pathogens found on the surfaces of grains produced in these regions associated with typical SRD symptoms, identified the associated black pathogens, and carried out greenhouse infection experiments to determine the main pathogen and pathogen distribution for this disease.

### Results

**Metagenomic sequence characteristics of fungal pathogens on grains from different ecological regions.** Metagenomic sequencing of the fungal communities on the grain surfaces of Bayou 13 obtained from 10 ecological regions during the harvest season yielded 1,458,785 valid sequences, with 99.29% (1,448,477) being high-quality sequences. The proportion of high-quality sequences was more than 97% for all regions. The samples from Shannan Prefecture produced the fewest valid and high-quality sequences (114,171 and 113,867, respectively). The samples from Dingxi City in Gansu yielded the most valid and high-quality sequences (200,080 and 199,194, respectively) (Table 1). The sequences had lengths ranging from 173 to 283 bp, and 113,867, respectively). The samples from Shannan Prefecture produced the fewest valid and high-quality sequences (114,171 and 113,867, respectively). The samples from Dingxi City in Gansu yielded the most valid and high-quality sequences (200,080 and 199,194, respectively) (Table 1). The sequences had lengths ranging from 173 to 283 bp, with a total length of 1,458,785. The number of sequences was > 1000.

### Table 1. Valid and high-quality sequences obtained from metagenomic sequencing of fungal pathogens on the surfaces of Bayou 13 grains from 10 ecological regions.

| Ecological region                             | Valid sequences | High-quality sequences | High-quality sequences/valid sequences (%) |
|----------------------------------------------|-----------------|------------------------|-------------------------------------------|
| Kelan County, Shanxi Province                | 158,447         | 157,375                | 99.32                                     |
| Dingxi City, Gansu Province                  | 200,080         | 199,194                | 99.56                                     |
| Datong City, Shanxi Province                 | 125,628         | 125,155                | 99.62                                     |
| Chifeng City, Inner Mongolia                 | 185,197         | 182,857                | 98.74                                     |
| Jining City, Inner Mongolia                  | 133,994         | 133,281                | 99.47                                     |
| Hinggan League, Inner Mongolia               | 120,721         | 119,073                | 98.63                                     |
| Zhangjiakou City, Hebei Province             | 146,646         | 146,049                | 99.59                                     |
| Baicheng City, Jilin Province                | 153,187         | 151,395                | 98.83                                     |
| Shannan Prefecture, Tibet                    | 114,171         | 113,867                | 99.73                                     |
| Haidong Prefecture, Qinghai Province         | 120,714         | 120,231                | 99.60                                     |
| Total                                        | 1,458,785       | 1,448,477              | 99.29                                     |

### Species diversity and richness of fungal pathogens on grains from different ecological regions.

In total, 1880 OTUs were produced from the sequencing of fungal pathogens on grains from 10 regions. When the calculated diversity indices were compared, the Chao1 and ACE indices were similar to the OTUs. The highest Chao1 and ACE index values were observed in samples from Dingxi City, and the lowest Chao1 and ACE index values were observed in samples from Datong City. The highest values for the Shannon indices were observed in samples from Baicheng City, and the lowest values were observed in samples from Shannan Prefecture, Tibet (Table 2).

In total, 69 genera were detected on grain surface samples, with the dominant pathogens belonging to the genera Alternaria (5.4–64.0%), and Davidiella (1.4–64.6%). Alternaria was the dominant species in the Kelan (33.6%), Dingxi (36.7%), Datong (56.3%), Chifeng (37.7%), Jining (31.1%), Hinggan League (64.0%), Zhangjiakou (40.5%), and Baicheng (28.8%) regions. Davidiella was the dominant species in Shannan Prefecture (64.4%) and Haidong Prefecture (53.9%). Judging from the proportions, Alternaria was much more common than Davidiella in all regions (Fig. 1).

Hierarchical cluster analysis at the genus level revealed that 13 genera, including Alternaria, Pyrenophora, and Gibberella, belonged to one cluster, and 13 others, including Davidiella, Botrytis, and Bipolaris, belonged to...
another cluster. When region-based clustering was performed, it was found that Haidong Prefecture formed a cluster of its own, and all other regions formed another cluster (Fig. 2).

Based on the richness of each species in the various samples, the correlations among species were calculated using the richness information at the genus level for the top 50 species. A correlation network consisting of significantly correlated species was constructed for the prediction of interactions among species. From Fig. 3, it can be seen that, with the exception of one negative correlation (i.e., competitive relationship), all other correlations between Davidiella and Alternaria were positive (i.e., collaborative relationships).

By eliminating these six indices, we found one significant single linear relationship between the percentage of Alternaria spp. \(y\) and elevation \(x\): \(y = 53.272 - 0.013x, R^2 = 0.568, (F_{1,9} = 10.54, P < 0.05)\), and there were significant differences in elevation \((t = -3.246, P < 0.05)\); another relationship was found between the percentage of Davidiella spp. \(y\) and elevation \(x\): \(y = -5.59 + 0.018x, R^2 = 0.805, (F_{1,9} = 33.064, P < 0.05)\), and there were significant differences in elevation \((t = 5.75, P < 0.05)\).

**Figure 1.** Genus-level taxonomic composition of fungal pathogens on grain surfaces of Bayou 13 from 10 ecological regions. SXKL, TSN, QHHD, SXDT, GSDX, IMCF, IMJN, IMHL, HBZJK, and JLBC indicate Kelan County in Shanxi Province; Shannan Prefecture in Tibet; Haidong Prefecture in Qinghai Province; Datong City in Shanxi Province; Dingxi City in Gansu Province; Chifeng City, Jining City, and Hinggan League in Inner Mongolia; Zhangjiakou City in Hebei Province; and Baicheng City in Jilin Province, respectively.

| Ecological region                        | OTUs | Chao1 estimator | ACE estimator | Shannon index |
|------------------------------------------|------|-----------------|---------------|---------------|
| Kelan County, Shanxi Province            | 172  | 162.821         | 165.875       | 2.770         |
| Dingxi City, Gansu Province              | 188  | 201.954         | 201.729       | 2.121         |
| Datong City, Shanxi Province             | 156  | 138.167         | 150.132       | 1.928         |
| Chifeng City, Inner Mongolia             | 210  | 197.431         | 201.655       | 2.777         |
| Jining City, Inner Mongolia              | 180  | 176.888         | 181.924       | 2.273         |
| Hinggan League, Inner Mongolia           | 162  | 161.372         | 158.034       | 1.790         |
| Zhangjiakou City, Hebei Province         | 174  | 185.212         | 171.447       | 2.432         |
| Baicheng City, Jilin Province            | 182  | 189.288         | 174.731       | 3.131         |
| Shannan Prefecture, Tibet                | 152  | 151.611         | 165.347       | 1.779         |
| Haidong Prefecture, Qinghai Province     | 155  | 146.633         | 159.059       | 2.425         |

Table 2. Diversity indices of fungal pathogens on grains of Bayou 13 from 10 ecological regions.

**Culture of fungal pathogens from grains from different ecological regions.** Based on the information obtained on the fungal pathogen community (Supplementary Table S2, Supplementary Figure S2), the infestation ratio of all pathogens on the grains from Haidong Prefecture in Qinghai Province was significantly higher than that from Shannan Prefecture in Tibet and significantly lower than that in the other regions \((F = 64.96, df = 29.9, P < 0.05)\) (Fig. 4). Moreover, the infestation ratios of black pathogens from Shannan Prefecture in Tibet and Haidong Prefectures were significantly lower than those from the other regions \((F = 49.557, df = 29.9, P < 0.05)\) (Fig. 4).
Verification of SRD-causing fungal pathogens on grain surfaces. The black pathogen strains purified on the grain surfaces from the 10 ecological regions were identified as *Alternaria* spp. through sequencing analysis and morphological identification (Fig. 5). After the granules and glumes of the three groups had been cultured on PDA medium for 5 days, greenhouse experiments were performed, and the infestation ratios of granules (*F* = 142.855, df = 8.6, *P* < 0.05) and glumes (*F* = 49.258, df = 8.6, *P* < 0.05) in the control group were significantly higher than those in the normal growth group (untreated) and lower than those in the experimental group (Fig. 6). The black pathogen strains were again isolated from the granules and glumes of the experimental group. The isolates were observed to be consistent with the original isolates, thus fulfilling Koch’s postulates.

Discussion
Through the investigation of the community structure of fungal pathogens on the surface of Bayou 13 oat grains from 10 ecological regions, our results showed that the fungal pathogens mainly belonged to 69 genera of Ascomycota and Basidiomycota, with the dominant pathogens belonging to *Alternaria* spp. and *Davidiella* spp. The same results were found in *Lolium perenne* and *Trifolium repens*. These two fungal genera are widely distributed in different proportions in plants, aquatic ecosystems and soils. Ascomycetes are more easily amplified than Basidiomycota with the ITS2 subsite, while *Davidiella* was the dominant genus amplified with the ITS2 region. Furthermore, we observed that these two genera exhibited a competitive relationship. A negative interaction...
Figure 3. Network analyses at the genus level according to the abundance of fungal pathogens on the surface of Bayou 13 grains from 10 ecological regions. Network analyses of Spearman correlation coefficients reveal the cooccurrence patterns among genera. Different colors represent different genera. Edges (lines) between nodes are colored red for positive correlations between genera and green for negative correlations between genera.

Figure 4. The comparison of infections by fungal pathogens after 5 days of culture on PDA on the surface of Bayou 13 grains from 10 ecological regions, China. The white and black columns represent the means for all pathogens and black pathogens, respectively, and the bars represent the SE. Different uppercase letters and lowercase letters above the columns indicate significant differences within the ten regions (least significant difference test, \( P < 0.05 \)) for all pathogens and black pathogens, respectively. SXKL, TSN, QHHD, SXDT, GSDX, IMCF, IMJN, IMHL, HBZJK and JLBC indicate Kelan County in Shanxi Province; Shannan Prefecture in Tibet; Haidong Prefecture in Qinghai Province; Datong City in Shanxi Province; Dingxi City in Gansu Province; Chifeng City, Jining City, and Hinggan League in Inner Mongolia; Zhangjiakou City in Hebei Province; and Baicheng City in Jilin Province, respectively.
Figure 5. Molecular identification and BLAST comparison of *Alternaria* spp. on black spots of grains from three plates for each of the 10 ecological regions. A plate containing 10 mildewed grains of Bayou 13 cultured on PDA medium for 5 days.

Figure 6. The comparison of infections by *Alternaria* spp. after 5 days of culture on PDA in the experimental group (2 weeks spraying with spore suspension of *Alternaria*), control group (2 weeks spraying with water containing 0.1% Tween-20), and normal growth group (untreated) for either glumes or grains of Bayou 13 from Kelan County in Shanxi Province, China. The gray and white columns represent the means for granules and glumes, respectively, and the bars represent the SE. Different uppercase and lowercase letters above the columns indicate significant differences within the three groups (least significant difference test, *P* < 0.05) for granules and glumes, respectively.
between *Fusarium* spp. and *Alternaria* spp. was found on the surface of wheat and barley grains of reduced quality\(^{16,22}\). Fungal distribution is mainly affected by fungal interactions such as spatial competition\(^{18}\). This may explain why, except for Shannan Prefecture and Haidong Prefecture, the proportion of Ascomycota in the other eight regions was significantly higher than that of Basidiomycota, and *Alternaria* spp. were the dominant fungi in the eight regions. In addition to being proven with plate culture, fungal cooccurrence has been investigated by metagenomic approaches and network analysis\(^{39,40}\). We also found that *Alternaria* and *Davidiella* spp. were negatively and positively correlated with elevation, respectively. Elevational gradients exert a strong influence on the relationships among crops and their microbiota in alpine regions\(^{41,42}\). This result thus indicated that elevation mainly affected the relative occurrence of *Alternaria* and *Davidiella* spp., presenting an inverse relationship with blackened surfaces of Chinese naked oat grains.

On the other hand, the infestation ratios on grains from Shannan Prefecture and Haidong Prefecture were significantly lower than those from the other eight regions. According to the Chinese Three Gradient Terrains\(^{43}\), Shannan Prefecture and Haidong Prefecture belong to the first terrain, Kelan, Dingxi, Datong, Jining, and Zhangjiakou belong to the second group, and Chifeng, Hinggan League, and Baicheng belong to the third group. Clearly, visible differences in black pigmentation were also found among the surface fungi of naked oat grains between the first terrain and the other terrains. This result thus indicated that SRD of naked oat characterized by black fungal discoloration mainly occurred in regions below 2000 m (elevation). Our previous reports showed that for grains of naked oat planted in Kelan, the infection level of *Alternaria* spp. on the black surface (38.7%) was five times as much as that on the normal surface (7.6%), while the infection level of *Davidiella* spp. on the black surface (3.9%) was similar to that on the normal surface (2.3%)\(^{47}\). *Alternaria* are dematiaceous fungi characterized by dark colonies ranging from gray to olive/brown\(^{44}\). Taking these results together, the black pathogen(s) causing SRD in eight of the regions examined may be *Alternaria* spp.

Further experimental results showed that the black pathogens cultured on PDA were *Alternaria* spp., which was consistent with the sequencing results; additionally, the greenhouse experiment results confirmed that *Alternaria* spp. was the cause of SRD of naked oat. Naked oat SRD differs from rice SRD\(^{57,18}\) in that it is associated with one pathogen. Oat leaf spot disease is only caused by *Alternaria alternata*\(^{45-47}\). *Alternaria* is one of the main mycotoxigenic fungal genera found in cereals worldwide\(^{48}\), causing diseases in over 400 host plants and postharvest spoilage of several crops\(^{49,50}\). The prevalence of this fungus in cereals indicates a high disease incidence, with more than 90% of the grains affected in the field\(^{51}\) and more than 50% of the grains affected in the greenhouse experiment in our study. Reports of cereal diseases characterized by leaf blackening and blight associated with *Alternaria* are continually published\(^{46}\), and cereal grains are constantly affected by *Alternaria* spp. and their toxins\(^{51}\). Although *Alternaria* toxins are often neglected in grains, possibly due to the lack of severe economic losses directly caused by the genus, recent studies have shown that their frequency and ability to produce a wide range of toxins is significant\(^{51,18}\). With governments attaching great importance to the sustainable development of the environment and agriculture and people's concern for health, research and application of biological control are increasing. Microbial agents, botanical agents and resistance inducers are receiving increasing attention regarding their roles in the biocontrol of plant diseases caused by *Alternaria*\(^{52}\). Oat grains can be processed into many kinds of foods\(^{18}\). Thus, the finding that SRD of naked oat is caused by only *Alternaria* spp. presents an obvious advantage for the biological control of this disease.

**Conclusions**

SRD has become an emerging naked oat disease in China, as losses caused by the disease have been regularly increasing over recent years. The pathogens of SRD have been investigated in many regions of Tibet, Shanxi Province, Gansu Province, Inner Mongolia, Qinghai Province, and Hebei Province, China. Using metagenomic analysis, molecular techniques and field validation, one causal agent was identified as *Alternaria* spp., and this disease mainly occurred at altitudes below 2000 m. Our results can help improve the recognition, diagnosis, and management of this important disease. It is emphasized in this study that this disease is caused by a single pathogenic taxon, and further research into the application of antagonistic microbes against *Alternaria* spp. to prevent the occurrence of pre- and postharvest SRD of naked oat are required to ensure the production of certified oat materials.

**Materials and methods**

**Plant material.** Based on the Chinese oat and buckwheat industry, Bayou 13 plants have been grown for over five years in 10 different oat-producing regions (Datong City and Kelan County in Shanxi Province, Dingxi City in Gansu Province, Zhangjiakou City in Hebei Province, Baicheng City in Jilin Province, Shannan Prefecture in Tibet, Haidong Prefecture in Qinghai Province, and Chifeng City, Jining City, and Hinggan League in Inner Mongolia). The elevations, geographical coordinates, and meteorological data of the 10 regions are summarized in Supplementary Table S1. In Datong City, Dingxi City, Baicheng City, Chifeng City, and Hinggan League, Bayou 13 plants were sown in early April and harvested in early August. In Kelan County, Zhangjiakou City, Shannan Prefecture, Haidong Prefecture, and Jining City, Bayou 13 plants were sown in mid-late May and harvested in mid-September. In 2017, the seeds were sown in drills 3–5 cm deep, spaced ca. 25 cm apart and at an average rate of 450 seeds m\(^{-2}\) in these regions. Irrigation and fertilization were scheduled according to the oat requirements, soil storage capacity, and climate. The cropping system followed the guidelines of the National Oat and Buckwheat Industrial Technology System\(^6\). For each of the ten sites, we randomly selected three sampling plots; the plot dimensions were 10 m × 5.0 m, and each plot contained 20 rows. At the ripening stage of the Bayou 13 plants, 500 g of grains per plot for each region was collected by the Center for Agricultural Genetic Resources Research unit and stored in a polyethylene bag at –80 °C.
**Collection of fungal pathogens on the grain surfaces and DNA extraction.** The surfaces of oat grain samples were washed three times with phosphate-buffered saline (PBS), and the resultant pathogen-containing solutions were centrifuged at 15,000 x g for 30 s. After the supernatant was discarded, total pathogen genomic DNA samples were extracted using Fast DNA SPIN extraction kits (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions and stored at −20 °C prior to further analysis. The quantity and quality of extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

**16S rDNA amplicon sequencing.** PCR amplification of the fungal ITS1 region was performed using the forward primer ITS5 (5′-GGA AGT AAA AGT CGT AAC AAGG-3′) and the reverse primer ITS2 (5′-GCTGCG TTCTTATCGATGC-3′). Sample-specific 7-bp barcodes were incorporated into the primers for multiplex sequencing. PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and paired-end 2 x 300 bp sequencing was performed using the Illumina MiSeq platform with a MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China).

**Sequence analysis**. The Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) bioinformatic pipeline was employed to process the sequencing data, as previously described[55]. Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. The low-quality sequences were filtered with previously reported criteria[55,56]. Paired-end reads were assembled using FLASH[57]. After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity by UCLUST[58]. A representative sequence was selected from each OTU using default parameters. OTUs were taxonomically classified by BLAST, and the representative sequence set was searched against the Unite Database[59] using the best hits[60]. An OTU table was also generated to record the abundance and taxonomy of each OTU in each sample. OTUs containing less than 0.001% of total sequences across all samples were discarded. To minimize the difference in sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under 90% of the minimum sequencing depth for further analysis.

**Bioinformatics and statistical analysis.** Sequence data analyses were mainly performed using QIIME and R packages (v3.2.0)[61]. OTU-level alpha diversity indices, such as the Chao1 richness estimator, abundance-based coverage estimator (ACE) metric, and Shannon diversity index, were calculated using the OTU table in QIIME. Taxon abundances at the genus level were statistically compared among samples by Metastats[62] and visualized as violin plots. Cooccurrence analysis was performed by calculating Spearman’s rank correlations between predominant taxa. Correlations with |RHO|>0.6 and P<0.01 were visualized as a cooccurrence network using Cytoscape[63].

**Culture of fungal pathogens from surfaces of oat grains from different ecological regions.** From the samples of each region, 30 oat grains from the initial 500 g of grains were randomly selected. A conventional tissue separation method was used for isolating and culturing samples[64]. After the germ had been removed from each grain using a sterilized blade, the grains were disinfected in 75% ethanol for 30 s, washed three times with sterile water, placed on sterile filter paper to absorb the excess water on the grain surfaces, and evenly arranged in height. For each group, six pots were placed in a 3 m × 1.5 m plot as one repeat and arranged in two rows and three columns.

**Identification of SRD-causing fungal pathogens on the surfaces of naked oat grains.** According to the single spore isolation method[65], single spores derived from all colonies around each grain from each region were selected from the culture medium. The purified strain was inoculated three to five times on new corn meal agar (CMA) medium (corn [Kunming, China], 20 g l−1; glucose [Solarbio, Beijing, China], 20 g l−1; agar, 15 g l−1). For each ecological region, three CMA plates with 10 grains each were prepared, i.e., three parallel experiments were conducted. The loaded CMA plates were incubated in an upright position at 25 °C without light for 5 days[66]. The number of black grains was counted, and the morphology of the pathogens on the surfaces of naked oat grains was preliminarily observed using an Olympus BX53 microscope (Olympus, Japan).

**Pathogenicity tests of Alternaria spp. on oat grains in the greenhouse.** A pot cultivation trial using cultivar Bayou 13 plants collected from Kelan County was conducted in an environmentally controlled glass greenhouse at 25 °C and under a 12:12 LD photoperiod from early November 2018 to late May 2019. This experiment included three groups with three replicates each: the normal growth group (untreated), control group (sprayed with water containing 0.1% Tween-20), and experimental group (sprayed with an Alternaria spore suspension). Approximately 20 normal seeds were sown in a porcelain pot 40 cm in diameter and 30 cm in height. For each group, six pots were placed in a 3 m × 1.5 m plot as one repeat and arranged in two rows with interpot distances of 40 cm; in total, 18 pots and three hundred and sixty seeds were used for each group.
Each group was at least five meters apart. The preserved Alternaria was cultured on PDA at 25 °C under a 12:12 LD photoperiod for 7 days. Subsequently, suspensions of conidia were prepared using sterile water containing 0.1% Tween-20, and the conidial suspensions were passed through two layers of a sterile cheesecloth to remove hyphal fragments. Spore concentration was calculated using a hemocytometer and adjusted to $1 \times 10^6$ spores/ml. During the maturation period, the experimental group was uniformly sprayed with the spore suspension, the control group was sprayed with sterile water containing 0.1% Tween-20, and the normal growth group was left untreated. Using a hand sprayer, 50 ml of spore suspension or 0.1% Tween-20 solution for each batch of six pots was directly sprayed on the rachises; each rachis was then put in a transparent plastic bag to prevent drying. Spraying was performed once every morning, noon, and night for 2 weeks during the maturity stage. From each plot, 50 granules and 50 glumes were randomly selected, surface disinfected using 75% ethanol for 30 s, washed three times with sterile water, evenly arranged on CMA medium, and incubated at 25 °C. After 5 days, the number of black granules and glumes was recorded. The pathogens were isolated from the black areas of grains and glumes. The colony and conidial characteristics of the recovered pathogen were observed using an Olympus BX53 microscope (Olympus, Japan) and compared with those of the pathogen used for inoculation.

**Statistical analysis.** The infestation ratio was calculated using the following equation:

\[
\text{Infestation ratio} = \left( \frac{\text{total number of infested grains or glumes}}{\text{total number of investigated grains or glumes}} \right) \times 100
\]

Data collected from the infestation ratio of grains, granules, or glumes were used for one-way analysis of variance (Fisher’s protected least significant difference), and normalized data were transformed using an arcsine square root function before analysis. Among ecological indices such as elevation, sunshine hours, temperature, rainfall, latitude and longitude from Supplementary Table S1, two models of key indices and two genera, Alternaria and Davidiella spp. were generated using stepwise backward selection. All analyses were conducted in SPSS for Windows Version 16.0 (SPSS Inc., Chicago, Illinois, United States of America).

Received: 13 September 2020; Accepted: 18 December 2020
Published online: 13 January 2021

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**Acknowledgements**
We wish to thank the China Agriculture Research System (CARS-07-A-02) for its financial assistance. We would like to thank professor Siyu Hou, Shanxi agricultural university, China, for drawing these images by sequence alignment analysis and R software.

**Author contributions**
L.L. led the relevant project and designed the study. L.Z., Z.L., and J.Z. prepared the oat samples. M.M. collected the test data. L.L. and M.M. performed the data analyses. L.L. drafted the manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**
**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-020-80273-6.

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