

## Characteristics of Pollen Assemblages in Grazing Livestock Dung and Their Environmental Significance in the Alpine Meadow Region of the Eastern Qinghai-Tibet Plateau (Postprint)

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**Date:** 2021-03-03T00:00:00+00:00

### Abstract

The Tibetan Plateau constitutes a critical alpine meadow distribution area and pastoral region in China, where the ecological impacts of livestock grazing on regional vegetation have garnered extensive scholarly attention. This study collected 49 grazing livestock dung samples from the eastern Tibetan Plateau alpine meadow region (30 yak dung, 11 horse dung, and 8 sheep dung), and conducted pollen analysis based on regional vegetation surveys. Results indicate that pollen assemblages across the three dung types are dominated by Cyperaceae, Poaceae, Gentianaceae, and Ranunculaceae pollen, effectively reflecting regional vegetation community characteristics. The presence of minor cereal Poaceae pollen in herbivore dung suggests local residents cultivate small quantities of oats for livestock feed. The three livestock species exhibit distinct feeding preferences: yaks consume marginally more plant species than sheep and horses; yaks and horses show lower feeding rates on weed Poaceae and *Thalictrum* compared to sheep, but significantly higher rates on Gentianaceae, Asteraceae, and *Artemisia*. Through selective consumption of palatable dominant Cyperaceae and Poaceae species, grazing livestock directly influence alpine meadow vegetation community structure and function. Comparative analysis between dung pollen and topsoil pollen assemblages reveals significantly higher arboreal and Aster pollen contents in topsoil samples, whereas Poaceae and Gentianaceae pollen contents are elevated in dung samples. These differences demonstrate that dung pollen better reflects regional vegetation community characteristics, with selective grazing creating distinctions between dung and topsoil pollen assemblages. This research provides novel methodological frameworks for evaluating grazing livestock feeding habits and pastoral impacts on Tibetan Plateau vegetation, while furnishing critical modern-process evidence for future pollen

analysis of preserved dung samples from archaeological sites to reconstruct paleovegetation and ancient human production activities.

## Full Text

### Characteristics and Environmental Implications of Dung Pollen Assemblages of Grazing Livestock in Alpine Meadow in the Eastern Qinghai-Tibetan Plateau

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## Abstract

The Qinghai-Tibetan Plateau is an important area of alpine meadow pasture and livestock husbandry in China, and the impact of grazing activity on regional vegetation and ecology has attracted widespread scholarly attention. To investigate this impact, we collected 49 dung samples from grazing livestock (30 yak dung, 11 horse dung, and 8 sheep dung) in typical alpine meadows of the eastern Qinghai-Tibetan Plateau. Our study area extended from 34°30'08" N, 101°30'57" E - 101°42'04" E, with elevations ranging between 3456-3815 m. Vegetation communities at sampling sites were investigated concurrently. Principal component analysis was employed to explore characteristics of different dung samples and pollen taxa, and dung pollen assemblages were compared with regional topsoil pollen assemblages to examine selective grazing characteristics.

Results indicate that pollen assemblages in all three livestock dung types were dominated by Cyperaceae, Poaceae, Gentianaceae, and Ranunculaceae, generally reflecting regional plant community characteristics. Cereal Poaceae pollen in herbivore dung reflects local residents' practice of cultivating oats for livestock feed. The three grazing livestock species exhibit distinct feeding habits: yaks and horses show lower feeding rates on weed Poaceae and *Thalictrum* than sheep, but significantly higher rates on Gentianaceae, Compositae, and *Artemisia*. Grazing livestock directly affect alpine meadow vegetation community structure and function through selective feeding on palatable dominant species such as Cyperaceae and Poaceae.

Comparison of dung and topsoil pollen assemblages reveals similar contents of Cyperaceae, Polygonaceae, Ranunculaceae, *Taraxacum*, and *Artemisia*. However, Poaceae and Gentianaceae contents are higher in dung samples, while *Aster* content is higher in topsoil samples, indicating selective feeding characteristics. Additionally, arboreal pollen (*Betula*, *Pinus*, *Picea*, and *Hippophae*) contents are higher in topsoil samples, suggesting that dung pollen assemblages better reflect local vegetation community characteristics. This study provides a novel approach for assessing livestock grazing habits and grazing impacts on regional vegetation in the Qinghai-Tibetan Plateau, and offers important modern-process reference data for future pollen analysis of preserved dung samples from archaeological sites to reconstruct paleovegetation and ancient human production activities.

**Key words:** dung pollen assemblage; principal component analysis; grazing livestock; paleo-vegetation; Qinghai-Tibetan Plateau

## Introduction

The Qinghai-Tibetan Plateau, with an average elevation exceeding 4000 m, is the world's highest natural geographic unit—known as the “Third Pole”—and its cold, unique environment and fragile ecosystem are highly sensitive to global climate change [1-3]. The plateau also represents China's largest alpine grassland, accounting for approximately 30% of the nation's total grassland area and serving as a major livestock husbandry region [4-5]. In recent years, increasing livestock scale and intensity have severely impacted the plateau's fragile vegetation. Studies indicate that overgrazing significantly affects vegetation cover [6-7], community structure [8-9], total biomass, and species diversity [10-11], leading to grassland degradation [12-13].

Livestock grazing behavior represents the most direct influence on regional vegetation. Through selective feeding, livestock reduce preferred dominant plant species while increasing the proportion of unpalatable weeds and toxic plants, directly affecting vegetation community structure and composition [14-15]. Therefore, assessing and analyzing livestock feeding habits is crucial for grassland resource protection and sustainable utilization. Current research on ungulate feeding habits primarily relies on plant community quadrat surveys and observation [16-17], stomach content analysis, and fecal microhistological analysis [18-20]. While quadrat surveys can intuitively reflect grazing habits, they are subject to numerous factors such as discrepancies between animal grazing ranges and quadrat placement, small sample sizes, and difficulty identifying grazing traces [21]. Moreover, because ungulates digest different plant growth stages and parts at varying rates, and each plant epidermal fragment has different identifiability [22], stomach content and microhistological analyses often miss or misidentify species, introducing uncertainty and variability.

Pollen constitutes an important part of plant reproductive organs, and ungulates ingest large quantities during feeding that pass through the digestive sys-

tem and are excreted in feces. Pollen exines contain sporopollenin (C<sub>96</sub>H<sub>22</sub>O<sub>24</sub>) [23], making them highly resistant to gastric acid and digestive decomposition, allowing intact preservation in animal feces [24-25]. Pollen is also abundant and quantifiable [26], enabling quantitative analysis of ungulate feeding habits through pollen taxa and percentages in herbivore dung. Furthermore, ingested pollen directly relates to surrounding vegetation, allowing direct inference of vegetation conditions from dung pollen assemblages—valuable for paleoenvironmental reconstruction and environmental archaeology. Pollen analysis of preserved animal feces in strata can reveal feeding habits and surrounding vegetation characteristics [27-28], infer human animal management and fodder structure, and aid in analyzing ancient subsistence patterns. Scholars have analyzed coprolites from ground sloths [29], goats [30-31], and camels [32-33] to reconstruct dietary characteristics and paleoenvironmental contexts, finding more pollen types in herbivore feces than in sediment layers, demonstrating the importance of coprolite palynology in paleoenvironmental reconstruction [34]. Recently, Zhang et al. [35] conducted pollen analysis on preserved cattle dung from the Early Bronze Age Xiaohe Cemetery in Xinjiang, inferring an oasis environment where cattle fed primarily on *Phragmites australis*, *Eragrostis*, *Aster*, and *Artemisia*. Li et al. [36] analyzed coprolite pollen from the Neolithic Tianluoshan site in the lower Yangtze River, reconstructing ancient vegetation composition and human activity contexts.

Thus, studying dung pollen assemblages of grazing livestock, combined with regional vegetation surveys, can quantitatively analyze feeding habits, assess grazing impacts on vegetation community structure, and provide important reference for environmental archaeology using fossil dung pollen assemblages. However, domestic research on dung pollen remains scarce, with only Wu et al. [37] investigating yak dung pollen assemblages in northern Xinjiang, finding fewer pollen taxa than in topsoil samples but good indication of modern vegetation composition. As the Qinghai-Tibetan Plateau represents an important livestock husbandry and ecological protection region lacking such research, this study analyzes pollen assemblages from three livestock types (yak, sheep, and horse) in typical eastern plateau alpine meadows to characterize dung pollen features, compare differences among livestock types, and discuss selective grazing characteristics through comparison with topsoil pollen assemblages. This research also provides important reference for future pollen analysis of animal dung in archaeological strata to reconstruct paleovegetation, environmental contexts, and interpret ancient human production modes and subsistence patterns.

## Study Area

The study area lies in the eastern Qinghai-Tibetan Plateau, with sampling sites distributed across Zeku County and Henan County in the Huangnan Tibetan Autonomous Prefecture of Qinghai Province, between 34°30'08"–35°06'28" N and 101°30'57"–101°42'04" E. The regional climate is plateau continental, characterized by low temperatures, short frost-free periods, and precipitation concen-

trated in May–September, with evaporation far exceeding precipitation [38]. Zeku County has an average annual temperature of  $-1.41^{\circ}\text{C}$ , annual precipitation of 480 mm, and annual evaporation of 1240 mm; Henan County averages  $-0.14^{\circ}\text{C}$ , 560 mm precipitation, and 1250 mm evaporation [39]. Average wind speed is  $3.8\text{ m}\cdot\text{s}^{-1}$ , with maximum speeds reaching  $23\text{ m}\cdot\text{s}^{-1}$  [40].

Regional vegetation consists primarily of alpine meadow and alpine shrub. Main plant types include *Kobresia* species: *K. pygmaea*, *K. humilis*, *K. capillifolia*, *K. tibetica*, *K. vidua*, and *K. kansuensis*, plus *Nardostachys chinensis*, *Spiraea alpina*, *Polygonum viviparum*, *P. macrophyllum*, *Rumex acetosa*, *Polygonum*, *Gentianaceae*, *Poa*, *Elymus*, *Draba nemorosa*, *Potentilla*, *Saussurea*, *Taraxacum*, *Aster*, *Ligularia*, and *Leontopodium*. Alpine shrub is dominated by *Potentilla fruticosa* and *Hippophae* communities [41–42].

### Field Work

During July–August 2018, we conducted vegetation quadrat surveys and livestock dung sample collection in Henan and Zeku Counties. We randomly collected 5–10 unweathered fecal pellets from the same livestock species within  $100\text{ m}\times 100\text{ m}$  areas, mixing them into one sealed sample. A total of 49 dung samples were collected: 30 yak (*Bos grunniens*), 11 horse (*Equus ferus caballus*), and 8 sheep (*Ovis aries*). Each sampling point was georeferenced with GPS, and vegetation communities and main vegetation types within the sampling range were recorded.

### Laboratory Analysis

Pollen extraction followed conventional acid-alkali treatment methods. Each sample used 10 g dry weight, with one *Lycopodium* tablet ( $27,637 \pm 563$  grains  $\cdot$  tablet $^{-1}$ ) added before processing to calculate pollen concentration. Carbonate cement was removed, samples were sieved through 200  $\mu\text{m}$  mesh to remove large plant residues, treated with 10% HCl to remove carbonates, and heated in a water bath at  $90^{\circ}\text{C}$  to remove organic matter. Appropriate amounts of HF were added to remove silicates, and pollen was finally concentrated using 7  $\mu\text{m}$  nylon mesh in an ultrasonic oscillator, preserved in glycerin, and mounted on slides. Pollen identification and counting were performed under  $400\times$  optical microscopy, primarily referencing published pollen morphological atlases and literature [43–45] and modern pollen standard slides from the Qinghai-Tibetan Plateau collected and preserved in our laboratory. Each sample typically yielded over 300 pollen grains. Tilia software was used to create pollen percentage diagrams [46], and Canoco software performed principal component analysis [47].

## Results

### Pollen Assemblage Characteristics

We identified 45 pollen types in the 49 livestock dung samples, including 5 arboreal, 4 shrub, and herbaceous taxa. Arboreal pollen included *Betula*, *Corylus*, *Pinus*, *Picea*, and Cupressaceae. Shrub and herb pollen included Rosaceae, *Polygonum*, *Hippophae*, Elaeagnaceae, Cyperaceae, Ranunculaceae, Gentianaceae, cereal Poaceae, *Artemisia*, Fabaceae, *Aster*-type, *Saussurea*-type, *Thalictrum*, *Potentilla*, *Taraxacum*-type (<35 m), Chenopodiaceae, Geraniaceae, Liliaceae, Brassicaceae, Lamiaceae, and *Plantago*. Pollen percentage diagrams were prepared for each livestock type (Fig. 2).

**Yak dung** samples showed highest Cyperaceae content (2.6%–77.6%, average 37.7%), with other major types including Gentianaceae (0.6%–38.8%, average 19.8%), Ranunculaceae (1.5%–33.8%, average 18.3%), weed Poaceae (0–43.6%, average 18.7%), cereal Poaceae (0.3%–19.7%, average 10.3%), *Artemisia* (0.4%–14.3%, average 10.9%), *Polygonum* (0–12.7%, average 10.0%), *Saussurea*-type (0–13.8%, average 10.0%), *Thalictrum* (0–5.9%, average 5.3%), and *Potentilla* (0–2.5%, average 1.4%). Minor pollen types including Geraniaceae, Brassicaceae, Chenopodiaceae, Liliaceae, *Plantago*, *Hippophae*, *Humulus*, and Elaeagnaceae averaged below 1%. Arboreal pollen was rare, averaging only 1.5%, with *Betula* (0.2%–10.6%, average 5.3%) and trace amounts of *Corylus*, *Picea*, *Pinus*, and Cupressaceae below 1%.

**Horse dung** samples also showed highest Cyperaceae content (3.9%–83.7%, average 65.4%), with other major types including Ranunculaceae (0.2%–15.6%, average 11.3%), Gentianaceae (3.3%–27.2%, average 14.0%), cereal Poaceae (0–11.3%, average 5.3%), *Aster*-type (0.2%–26.3%, average 10.9%), *Artemisia* (0–17.1%, average 10.0%), *Saussurea*-type (1.0%–6.4%, average 4.1%), *Taraxacum*-type (<35 m) (0–3.7%, average 1.0%), *Thalictrum* (0–12.4%, average 4.8%), *Polygonum* (0.3%–4.8%, average 2.8%), *Potentilla* (0–2.8%, average 1.4%), and *Hippophae* (0–3.7%, average 1.4%). Minor types including Rosaceae, Fabaceae, Chenopodiaceae, Geraniaceae, Brassicaceae, Liliaceae, and *Humulus* averaged below 1%. Arboreal pollen was dominated by *Betula* (0–5.3%, average 2.8%), with trace *Picea* and *Pinus* below 0.5%.

**Sheep dung** samples showed highest Cyperaceae content (1.3%–92.5%, average 68.3%), with other major types including Ranunculaceae (0–34.7%, average 17.5%), weed Poaceae (0.5%–72.5%, average 20.6%), *Polygonum* (0.2%–9.8%, average 5.3%), *Potentilla* (0–14.1%, average 5.3%), cereal Poaceae (0.5%–3.0%, average 1.4%), *Aster*-type (0–7.5%, average 2.8%), *Artemisia* (0–8.0%, average 2.8%), and *Taraxacum*-type (<35 m) (0–2.8%, average 1.4%). Minor types including Rosaceae, Fabaceae, Chenopodiaceae, *Saussurea*-type, Brassicaceae, Geraniaceae, Liliaceae, *Humulus*, *Hippophae*, and *Thalictrum* averaged below 1%. Arboreal pollen was dominated by *Betula* (0–5.3%, average 2.8%), with trace *Picea* and *Pinus* below 0.5%.

## PCA Analysis Results

From the 49 samples, 45 pollen families/genera were identified. After removing taxa with percentages below 0.5%, principal component analysis was performed on the remaining 25 genera (Fig. 3). Axis 1 explained 23.7% of variance, Axis 2 explained 16.3%, and Axis 3 explained 11.4%, with the first three axes cumulatively explaining 40.8% of total variance (Table 1).

The PCA ordination (Fig. 3) shows that Axis 1 primarily separates herbaceous pollen types: *Aster*-type, *Taraxacum*-type, *Saussurea*-type, Chenopodiaceae, Brassicaceae, Fabaceae, Gentianaceae, and cereal Poaceae cluster on the negative side, while *Thalictrum* lies on the positive side. Weed Poaceae, *Polygonum*, Rosaceae, Caryophyllaceae, and *Thalictrum* concentrate near Axis 1. Axis 2 separates arboreal and shrub pollen: *Pinus*, *Picea*, and Cupressaceae occupy the positive side, while *Betula*, *Corylus*, and *Hippophae* occupy the negative side.

The ordination diagram (Fig. 3) shows intermingling of yak and horse dung samples, mostly overlapping and concentrated on the negative sides of Axes 1 and 2, making them difficult to distinguish. Sheep dung samples, except for one, distribute on the positive side of Axis 1, showing some separation from yak and horse samples.

## Discussion

### Comparative Analysis of Dung Pollen Assemblages Among Different Herbivores

Pollen assemblages from yak, horse, and sheep dung are dominated by Cyperaceae, Poaceae, Gentianaceae, and Ranunculaceae, averaging 68.3% of total pollen. Secondary types include *Thalictrum*, cereal Poaceae (average 20.6%), *Polygonum*, *Artemisia*, *Taraxacum*, *Aster*-type, and *Saussurea*-type. Box plots (Fig. 4) reveal significant variation in major pollen types among the three livestock types, likely due to large pasture areas, extensive livestock movement, and diverse, random plant community consumption.

Although we surveyed vegetation within 100 m × 100 m of each dung sample, the pollen assemblage may represent the broader pasture range rather than the immediate sampling area. Vegetation community composition and grazing range directly affect feeding habits, influencing dung pollen assemblages. The dominant pollen types across all samples are Cyperaceae and Poaceae (combined average 37.7%), indicating high grazing pressure on these preferred species.

In alpine meadows of the Qinghai-Tibetan Plateau, selective feeding on palatable Cyperaceae and Poaceae leads to degradation of these dominant species under heavy grazing, while unpalatable toxic weeds such as *Ligularia*, *Leontopodium leontopodioides*, *Potentilla*, and *Stellera* increase in the community. High Gentianaceae and Ranunculaceae percentages in dung pollen reflect both the flowering phenology (our sampling in mid-July coincided with their peak

bloom) and long-term grazing-induced increases in these families [48]. While livestock exhibit selective feeding, when vegetation richness declines, they consume less-preferred species [49], demonstrating that vegetation type influences grazing behavior.

### Comparison Between Dung Pollen Assemblages and Regional Topsoil Pollen Assemblages

Li et al. [50] studied topsoil pollen assemblages and source areas in our study region. Comparison shows similar percentages of Cyperaceae, *Polygonum*, Ranunculaceae, *Taraxacum*, and *Artemisia* between dung and topsoil samples. However, Poaceae (average 11.4%) and Gentianaceae (average 16.0%) percentages are significantly higher in dung samples than in topsoil (average 5.3% and 2.8%, respectively), while *Aster*-type pollen is significantly higher in topsoil (average 20.0%) than in dung (average 2.8%). This likely reflects both selective feeding and phenological differences during sampling.

Arboreal pollen including *Betula* (average 5.3%), *Pinus* (average 2.8%), *Picea* (average 1.4%), and *Hippophae* (average 2.8%) are more abundant in topsoil than in dung samples (*Betula* average 2.8%, *Pinus* 0.5%, *Picea* 0.5%, *Hippophae* 0.5%), suggesting long-distance wind transport of these pollen types from surrounding mountains that are rarely ingested during feeding. Thus, topsoil pollen assemblages are more influenced by external transport processes, while dung pollen better reflects local vegetation conditions.

Previous microhistological fecal analysis of ungulates in the Hoh Xil region, Tianshan Mountains, Gannan region, and Tibet consistently show Cyperaceae and Poaceae as preferred forage [51-54], consistent with our dung pollen results. Selective feeding directly impacts vegetation community structure and biodiversity, altering community composition.

### Implications for Paleo-environmental Research

Fossil dung pollen analysis effectively reconstructs past animal feeding habits and paleoenvironmental contexts. Analysis of preserved dung strata can reveal ancient vegetation communities [33-36, 40] and seasonal feeding patterns. Modern grazing livestock dung pollen studies thus provide crucial reference for interpreting fossil dung pollen. Our finding of relatively high cereal Poaceae pollen (average 5.3%) indicates human cultivation activity—local herders grow oats as supplementary livestock feed. Similar findings from the Qinghai Lake basin (cereal Poaceae averaging 4.1%) suggest widespread use of grain crops for livestock feeding [55], demonstrating the potential of dung pollen analysis in Tibetan Plateau environmental archaeology.

Numerous prehistoric archaeological sites across the Qinghai-Tibetan Plateau contain preserved animal feces [56] that encode information about domestication, husbandry, and grazing activities. Cereal crop pollen in coprolites can distinguish between nomadic and agro-pastoral economies. Thus, fossil dung

pollen analysis holds significant value for reconstructing prehistoric human environmental contexts, vegetation characteristics, and subsistence patterns on the plateau. Our modern dung pollen research provides essential comparative data for future archaeological dung pollen studies.

## Conclusions

1. Dung pollen assemblages from yak, horse, and sheep in the eastern Qinghai-Tibetan Plateau alpine meadows are dominated by herbaceous and shrub pollen, primarily Cyperaceae, Poaceae, Ranunculaceae, Gentianaceae, *Polygonum*, Fabaceae, *Artemisia*, *Aster*-type, *Taraxacum*-type, *Saussurea*-type, and *Thalictrum*. The three herbivores show similar plant taxa consumption, mainly Cyperaceae, Poaceae, Ranunculaceae, and Gentianaceae, reflecting regional alpine meadow communities. However, proportional differences exist: yaks and horses prefer Gentianaceae, Compositae, and *Artemisia* significantly more than sheep, while sheep prefer weed Poaceae and *Thalictrum* significantly more than yaks and horses.
2. Cyperaceae, *Polygonum*, Ranunculaceae, *Taraxacum*, and *Artemisia* show similar percentages in both dung and topsoil samples. Poaceae and Gentianaceae percentages are higher in dung samples, while *Aster*-type percentages are higher in topsoil samples. Arboreal pollen (*Betula*, *Pinus*, *Picea*, *Hippophae*) percentages are lower in dung than topsoil samples, demonstrating that dung pollen better reflects local-scale vegetation community characteristics.
3. Herbivore dung pollen assemblages clearly reflect regional vegetation community features. Cereal pollen in these assemblages effectively indicates multiple human production activities including animal husbandry, cultivation, and feeding practices. This provides important comparative data for future dung pollen analysis from archaeological sites on the Qinghai-Tibetan Plateau to investigate paleovegetation communities and ancient human subsistence patterns.

## Acknowledgments

We thank Prof. Li Yuecong of Hebei Normal University and Drs. Yang Xiaolan and Zhang Ruchun of the Hebei Academy of Sciences for guidance during field vegetation surveys. We also appreciate the valuable comments from two anonymous reviewers.

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