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Habitat Ecology of *Ophiocordyceps sinensis* in Western Nepal

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*As a valuable entomophagus fungus species, caterpillar fungus (*Ophiocordyceps sinensis*) is endemic to the alpine meadows of the Tibetan Plateau and adjoining Himalayas. However, little is known about its ecological niche and habitat. We investigated its associated plant species and habitat across different sites in Dolpa, west Nepal, and explored how associated plant species and soil characteristics affect its density and growth during the months of June and July in 2 consecutive years. Detrended correspondence analysis was used to capture the distribution pattern of plant species. Principal component analysis was applied to visualize the gradients of the soil data, and generalized linear models were employed to test the effects of nutrients and vegetation on the availability and size of caterpillar fungus. A total of 33 plant species were frequently associated with caterpillar fungus across the investigated sites. The abundance of the fungus was significantly affected by vegetation composition, whereas the individual fungal traits were independent of soil nutrients or vegetation composition. Therefore, it is essential to protect associated plant species to better conserve caterpillar fungus at high elevations.*

Keywords: *Alpine region; plant species; soil; caterpillar fungus; detrended correspondence analysis; Nepal.*

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Introduction

Diverse animals, fungi, and plants have always been an integral part of life in the Himalayas (Shackleton and Pandey 2014). At high elevations, many endemic species are of economic and cultural importance and extremely rare with high medicinal potential (Lama et al 2001; Grytnes and Vetaas 2002; Rokaya et al 2010). The species with high medicinal potential have received worldwide attention due to their high potency, low number of side effects, and hefty prices (Winkler 2008; Shrestha and Bawa 2013). Caterpillar fungus is a highly valued fungus species.

Caterpillar fungus is endemic to the alpine meadows of the Tibetan Plateau and adjoining Himalayas (Winkler 2008). It is a combined life form of fungus and caterpillar (with a basal caterpillar part and an upper fungal part); the mature fungus spore infects a caterpillar, often of the ghost moth, and mummifies it (Figure 1). The caterpillar fungus is considered a flagship species for the conservation of fungi and plays a significant role in maintaining healthy alpine ecosystems (Cannon 2011). It has a long history of use for nutritional and medicinal purposes, including as an antioxidant (Li et al 2001), hypoglycemic (Zhang et al 2006), and treatment for sexual dysfunction (Liu et al 1997) and against high lipid and cholesterol in blood (Francia et al 1999). However, less attention has been paid to its conservation and sustainable use (Dahlberg 2001).

Caterpillar fungus grows at elevations from 3500 to 5200 m above sea level (masl) in Nepal (Devkota 2010), Bhutan (Cannon et al 2009), India (Singh et al 2010; Negi et al 2015), and China (Winkler 2010). It is able to survive under harsh climatic conditions including low temperature, high solar radiation, and aridity (Chlebicki 2002; Schmidt et al 2012), but it is threatened by intensive collection, habitat loss and degradation, and climate change (Shrestha and Bawa 2013). It has been widely studied in terms of taxonomy, medicinal properties,

FIGURE 1 Larva with study species, *Ophiocordyceps sinensis*. (A) Uninfected larva; (B) larva infected with fully grown *O. sinensis*. Arrow shows head of larva in (A) and caterpillar fungus in (B). (Photos by Shalik R. Sigdel)

phytochemistry, genetic diversity, and trade (Holliday and Cleaver 2008; Ji et al 2009; Bhandari et al 2010; Shrestha and Bawa 2013; Quan et al 2014). Vegetation composition is considered a key factor to identify fungus species (Chlebicki 2002; Begon et al 2006; Cavieres et al 2014) and could be used to detect the abundance of caterpillar fungus.

The Dolpa region in west Nepal is a representative area for caterpillar fungus distribution. However, little is known about its ecological niche and habitat in that region (Devkota 2006, 2010; Shrestha and Bawa 2013). The objective of this research was to investigate these issues further. We hypothesized that the abundance and growth of caterpillar fungus are associated with plant species composition and soil characteristics.

Methods

Study species

Ophiocordyceps sinensis (Berk.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatafora (synonym *Cordyceps sinensis*) is commonly known as the caterpillar fungus or *yartsa gunbu*, meaning summer grass and winter worm in the Tibetan language. It is endemic to the Himalayas (Nepal and Bhutan as well as Uttarakhand, Sikkim, Himachal Pradesh, and Arunachal Pradesh in India) and the Tibetan Plateau in China and is well adapted to cold and dry climates. It covers an elevation gradient from 3500 to 5200 masl. Caterpillar fungus has a complex life cycle that depends on the availability of host insects as well as on soil characteristics and precipitation. The fungus spore infects the host insect larva in the soil in August and grows under the snow during the winter, developing fusiform hyphae, which divide by budding and eventually fill the host larva's core. The fungus emerges aboveground as a cylindrical stroma in the early spring (Yang et al 1989; Zeng et al 2006; Stone 2008).

Study area

The study was carried out in different parts of Dolpa region, a pristine and naturally diverse area in Nepal. It lies at 28°24′–29°43′N and 82°24′–83°38′E with an elevational gradient from 1510 to 7707 masl. Annual precipitation ranges from 1000 mm/year at lower elevations to 200 mm/year at higher elevations (Ghimire et al 2005). We selected the Dolpa region as our research area because of the abundance of fungus and their intensive harvest and trading (Shrestha and Bawa 2013). The study sites (Figure 2) were located in alpine meadows in Raha (4558–4632 m), Phoksundo (4249–4832 m), and Sahartara (4286–4535 m). Phoksundo is inside Shey Phoksundo National Park, Raha is in the park's buffer zone, and Sahartara is in a government-managed forest.

Associated plant species and caterpillar measurements

Field sampling of associated plant species was carried out during June and July in 2007 and 2008. A total of 45 permanent plots with a size 10 m × 10 m plots were established in 3 study sites. At each study site, 15 plots were randomly distributed. To cover more area in each study site, we maintained a gap of at least 100 m in between plots. Each plot was marked by a permanent colored tag, and its precise location was noted with a Garmin GPS (Global Positioning System) device. Presence and absence of each plant species within each plot were noted. The abundance of caterpillar fungus was measured in terms of both existing fungi and recently dug collection pits. The length of the fungus and caterpillar was measured in the field, and their fresh weight and dry weight (the latter taken 2–3 weeks after collection, which is a normal time from harvest to sale in the study sites) were taken using a digital scale. To determine the fungal development rate, the caterpillar and fungal parts were measured every day at all 3 study sites until the formation of the sporangium.

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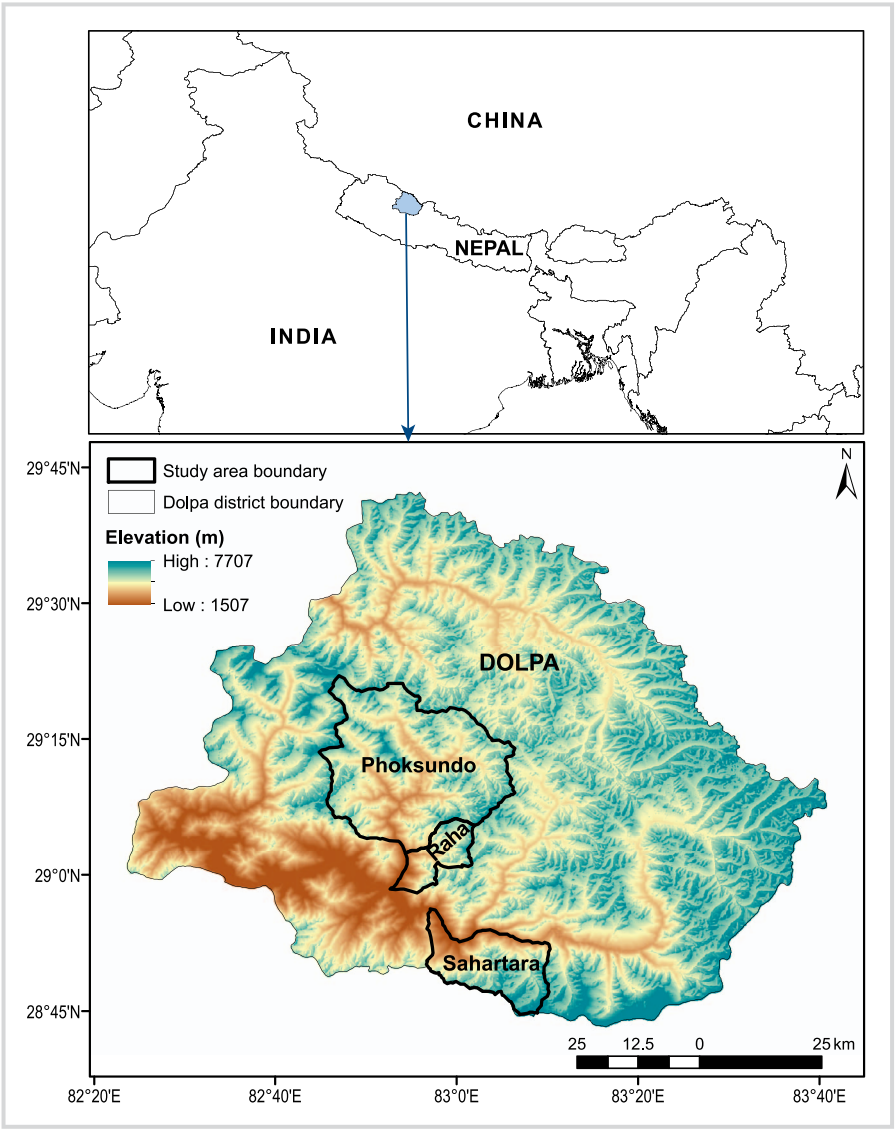
FIGURE 2 Map of the study area. (Map by Suraj Shrestha)

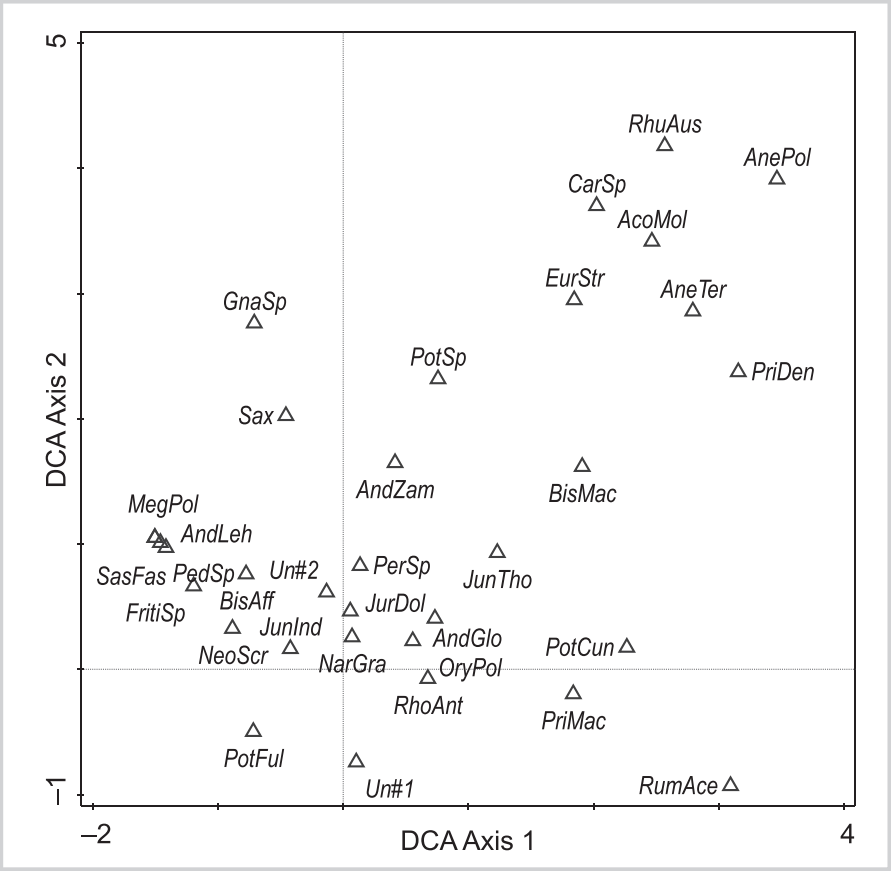


Most of the associated plant species were identified in the field; unidentified plants were collected, pressed, and dried between papers and were later identified with the help of different books (Polunin and Stainton 1984; Stainton 1988; Lama et al 2001). However, we were unable to identify few grasses. The nomenclature of Press et al (2000) was followed.

Soil sampling and analysis

Soil samples were collected from the 4 corners and the center of 18 plots, 6 at each site, at a depth of 10–15 cm. The number of plots with soil samples was limited due to difficulty in transporting the samples. The subsamples were mixed thoroughly, and about 200–300 g of collected soil was air-dried in the shade and stored in airtight bags until laboratory analysis. Soil analysis was carried out by Nepal Environmental and Scientific Services. Soil cation exchange capacity, phosphorus, potassium, pH, organic matter content, texture, and total nitrogen were determined in different soil samples. Soil cation exchange capacity was determined by flame emission spectrophotometry (for K and Na) and atomic absorption spectromephotometry (Ca and Mg) (Jones 2001), soil phosphorus was calculated according to Olsen et al (1954), potassium by calculating ammonium ion exchange using a galvanometer, pH by calibrating the pH meter with buffer solutions of known pH (pH 4 and 7), organic matter by Walkey and Black's rapid titration method (Walkley and Black 1934), soil texture was determined by using a mechanical method (Jones 2001), and nitrogen using the micro-Kjeldahl method (Jacobs 1951).

FIGURE 3 Detrended correspondence analysis of vegetation composition in the study sites. See S1, Supplemental Material, 10.1659/MRD-JOURNAL-D-16-00075.S1, for abbreviations.



Data analysis

Detrended correspondence analysis was performed to identify the distribution pattern of different plant species associated with caterpillar fungus. The position of the samples on the first and second canonical axes in the analysis was then used to describe the vegetation composition of each site. Plant species with fewer than 4 occurrences in the dataset were excluded. Rare species, as defined by ter Braak and Šmilauer (2002), were down-weighted to further reduce the negative effect of their occurrence on the results. A principal component analysis was performed to identify the main gradients of the soil data. The data were then standardized by the dependent variables (chemical soil properties). The analyses were carried out using Canoco 5.01 (ter Braak and Smilauer 2012).

We tested the effect of plant species composition and the most important soil characteristics (pH and content of organic matter, nitrogen, phosphorus, and potassium) on the numbers of caterpillar fungi (per year—2007 and 2008—separately, as well as total number), and their caterpillar length, fungal length, and weight. Generalized linear models were used to test the effect of nutrients and vegetation on caterpillar fungus traits. Specifically, we used models with Poisson distribution and log link function for the number of caterpillar fungi in different years. Caterpillar length, fungal length, and weight were right skewed in distribution, and gamma distributions with inverse function in generalized linear models were used. The significant variables were determined by using the stepwise function. The univariate analyses were performed with S-plus (S-Plus 2000).

Results

The habitat of caterpillar fungus in our study sites ranged from 4249 to 5100 masl in elevation, with rough and inclined terrains that were generally well drained with luxuriant grass vegetation. A total of 33 plant species were frequently associated with caterpillar fungus (Table S1, Supplemental Material, http://dx.doi.org/10.1659/MRD-JOURNAL-D-16-00075.S1). The most frequently occurring plant species were *Bistorta macrophylla, Juncus thomsonii,* and *Saxifraga* species along with other important medicinal plants such as *Nardostachys grandiflora* and *Neopicrorhiza scrophulariiflora*. The detrended correspondence analysis identified strong vegetation gradients (Figure 3). The first ordination axis, which

TABLE 1 Matrix of correlation coefficients of soil attributes. Significant correlations ($p \leq 0.05$, $N = 18$) are marked in bold.

	Calcium	Cation exchange capacity	Potassium	Magnesium	Nitrogen	Organic matter	pH
Cation exchange capacity	**0.80**						
Potassium	0.14	**0.48**					
Magnesium	−0.18	−0.30	−0.14				
Nitrogen	−0.32	−0.41	−0.09	**0.83**			
Organic matter	0.05	0.41	**0.54**	0.32	0.10		
pH	**0.49**	0.04	**−0.60**	−0.02	−0.03	**−0.53**	
Phosphorus	**−0.66**	−0.41	0.06	0.02	−0.05	0.05	**–0.64**

explained 16.16% of the variation, showed a gradient from shorter herbs in shaded habitats (sometimes caused by shading of *Juniperus indica*) to habitats with tall herbs that prefer moderate dry and open habitats. The second axis, which explained 10.02% of the variation, showed a gradient from taller plant species such as *Potentilla fulgens*, *Rhododendron anthopogon*, and *Rumex acetosa* to shorter herbs (*Androsace zambalensis*, *Carex* species, *Aconogonum molle,* and *Anemone polyanthes*) preferring open to moist habitats.

Species composition identified by principal component analysis had a significant effect on the total number of caterpillar fungi. Specifically, the abundance of caterpillar fungus increased with higher plot loading along the canonical axis 1 ($p < 0.04$, $R^2 = 0.040$) and decreased with higher plot loading along the canonical axis 2 ($p < 0.001$, $R^2 = 0.13$). The associated plant species include *B. macrophylla*, *J. thomsonii*, *Oxygraphis polypetala*, *Potentilla cuneata, P. fulgens*, *Primula macrophylla*, *Rheum australe*, *A. molle*, *Euphorbia stracheyi,* and *Carex* species.

The most favorable soil for caterpillar fungus growth was acidic (pH 4.5–6.5) with a high percentage of sand (average 51.11), followed by silt (43.88), clay (3.95), and humus content (1.06). Soil nutrients showed high variations (for example, calcium 0.4–4.4 mg/g, magnesium 0.12–124 mg/g, nitrogen 1.86–22 mg/g, organic matter 4.9–30.2%, phosphorus 1.04–10.66%, potassium 22–154.5 mg/g, and cation exchange capacity 2.6–10.3 meq/100 g). Soil calcium was positively correlated with pH and cation exchange capacity and negatively correlated with phosphorus. Cation exchange was positively correlated with potassium, potassium positively with organic matter and negatively with pH, magnesium positively with nitrogen, organic matter negatively with pH, and pH negatively with phosphorus (Table 1).

The first axis of the principal component analysis explained 38.58%, and the second axis explained 25.47% of the variation in soil data (Figure 4). The first axis showed a gradient from clay-sandy soils rich in magnesium and nitrogen to soils rich in silt, potassium, organic matter, and free cation exchange capacity. The second axis showed a gradient from calcium rich soils with high pH to acidic soils with high phosphorus, organic matter, and potassium content.

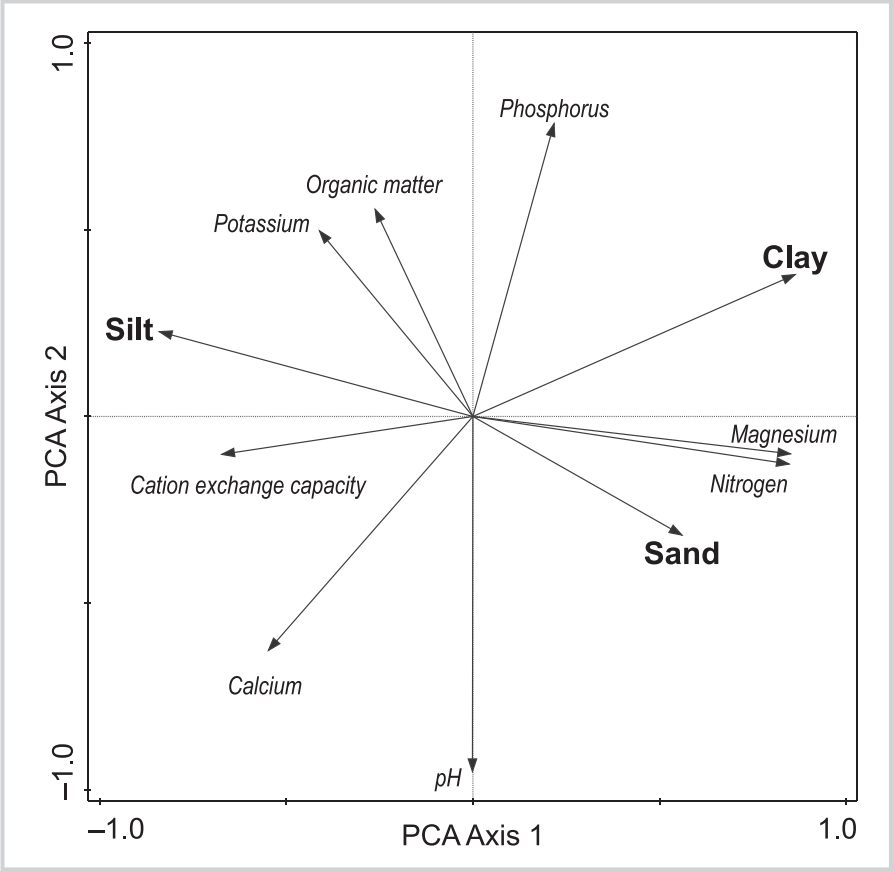
The number of caterpillar fungi increased with increasing pH throughout our study sites ($p < 0.001$, $R^2 = 0.12$) and decreased with increasing phosphorus content ($p < 0.001$, $R^2 = 0.13$). The patterns for a single year were largely similar to the patterns for the whole dataset. In contrast to the number of caterpillar fungi, their fungal length, caterpillar length, and weight were independent of soil nutrients and vegetation composition.

Caterpillars hosting fungi collected during the field study were identified as the larvae of ghost moths (belonging to the genus *Thitarodes* genus). We recorded 1 more species of moth (*Perissandria sikkima*) and 1 species of butterfly (*Parnassius hardwickii*) at the same site, but we did not observe larvae of these 2 species forming caterpillar fungus. They might be potential host species. Larvae infected by a fungus tend to form caterpillar fungi that are golden brown in color with the fungal part developed on the head, light brown to gray (Figure 1). The fresh weight of a whole caterpillar fungus ranged from 0.7 to 1.8 g, and dry weight ranged from 0.2 to 0.5 g. The total length of caterpillar fungus ranged from 4.3 to 11.3 cm.

Discussion and conclusion

Caterpillar fungus was found on rough terrain and well-drained landscapes at high elevations in Dolpa, Nepal. These habitat conditions are similar to those found for the same fungus in Darchula (Chhetri and Lodhiyal 2008) and Dolpa (Devkota 2010) in Nepal, Bhutan (Cannon et al 2009), India (Singh et al 2010), and Tibet in China (Winkler 2008, 2010, 2012). The habitat was characterized by low temperature, short growing seasons, and heavy snowfall in winter. Devkota (2010) identified 15 major plant species associated with the caterpillar fungus, belonging to 10 families; in this study we recorded 33 plant species belonging to 16 families. The difference in numbers could be due to variations in microenvironmental factors such as slope, aspect, elevation, and soil conditions. Soil at higher elevations is typically low in nutrients (Tanner et al 1998) and less

FIGURE 4 Principal component analysis of soil in the study sites. Red denotes soil type.



fertile with high sand content (Devkota 2010). In our study, we also found a high percentage of sand and a low amount of humus containing different soil nutrients.

No direct significant relationship has been identified between associated plant species and abundance of caterpillar fungus, but some of these species might play a facilitative or competitive role for other species (Berkowitz et al 1995). Even though the feeding habits of *Thitarodes* species are unknown, a previous study by Cannon et al (2009) found that grazing intensity and vegetation height have a direct or indirect effect on spore dispersal and abundance of caterpillar fungus. However, we could not analyze this kind of relationship due to lack of vegetation height and grazing intensity data in our sample. Thus, future studies should investigate how grazing intensity and vegetation height affect the availability of caterpillar fungus; tall vegetation may hinder spore dispersal, and overgrazing might reduce the abundance of caterpillar fungus.

The growth of caterpillar fungus is controlled by soil pH; higher pH levels retard the growth of different entomoparasitic known as *Cordyceps nutans* Pat. by disturbing mycelial growth (Sasaki et al 2005). The optimum pH level for caterpillar fungus growth is around 6.0 (Xu et al 2003). Soil nutrients and vegetation composition have significantly influenced the distribution of caterpillar fungus (Wu et al 2009). The present study showed how different species affect the availability of caterpillar fungus as well as what types of species are associated with it.

This study found that the number of caterpillar fungi was significantly affected by vegetation composition. This may be caused by the fact that different plant species are more suitable for different butterfly and moth species. Fungus length, caterpillar length, and total weight were, however, independent of individual soil nutrients or vegetation composition. This may be because different edaphic and environmental factors have a combined effect on the growth of vegetation and in turn on the number of caterpillar fungi. Studies have shown that more robust organisms are found in areas with higher precipitation levels and temperatures (Körner 2003; Grau et al 2007). Further, variations in precipitation and temperature between years (Bhattarai and Vetaas 2003), aspects (Boesi 2003), and slopes (Winkler 2008) may have an interactive effect on the availability of different plant species, which in turn affects the status of the caterpillar fungus.

Different insect species act as either complete host or partial host of caterpillar fungus in different regions (Wang and Yao 2011). The host caterpillars found in Dolpa (*Thitarodes* species) were also reported from high elevations in the Tibetan Plateau (Winkler 2010). Thus, high elevations are characterized by particular types of butterflies or moths whose larvae could be beneficial for the formation of caterpillar fungus.

The legal trade in the highly priced caterpillar fungus, particularly for medicinal purposes, started in Nepal after 2001 and has made a significant contribution to the local economy. Thousands of people have come to collect it, putting its habitat at risk. The fungus has maintained the livelihoods of many rural people at high elevations in Nepal (Chhetri and Lodhiyal 2008; Devkota 2010) and Tibet (Winkler 2010). A notable amount of revenue is also collected by the government from this trade. Due to overexploitation and human disturbances, however, production has decreased in recent years (Shrestha and Bawa 2013).

Associated plant species, host insects, and soil nutrients all play important roles in maintaining the habitat of the caterpillar fungus; they all must be understood and preserved to ensure its sustainable use and conservation. Due to the complex life cycle and overexploitation of this fungus, more detailed research is needed to assess the impact of biotic and abiotic factors, including grazing intensity and height of vegetation, on its distribution, and to monitor ecological factors and regeneration patterns covering a large area where caterpillar fungus is distributed.

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Supplemental material

TABLE S1 Plant species used in detrended correspondence analysis.

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