Mechanism of plant-soil feedback in a degraded alpine grassland, Tibetan Plateau

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ABSTRACT:

Although biotic and abiotic factors have been confirmed to be critical factors that affect the community dynamics, their interactive effects have yet to be fully considered in grassland degradation. Herein, we tested how soil nutrients and microbes regulated plant-soil feedback (PSF) in a degraded alpine grassland. Our results indicated that, from non-degraded (ND) to severely degraded (SD), significantly ($P<0.05$) decreased soil total carbon (from 17.66 to 12.55 g/kg) and total nitrogen (from 3.16 to 2.74 g/kg) were detected. Despite higher nutrients in ND soil generating significantly ($P<0.05$) positive PSF (0.52) on monocots growth when the soil was sterilized, a high proportion of pathogens (36%) in ND non-sterilized soil resulted in a strong negative PSF on monocots. By contrast, the higher phenotypic plasticity of dicots coupled with a higher abundance of mutualists and saprophytes (70%) strongly promoted their survival and growth in SD with infertile soil. Our findings identified a novel mechanism that there was a functional group shift from monocots with higher vulnerability to soil pathogens in the ND fertile soil to dicots with higher dependence on nutritional mutualists in the degraded infertile soil. And the emerging irreversible eco-evolutionary in PSF after degradation might cause a predicament for the restoration of degraded grassland.

Keywords: Plant-soil feedback, Plant function groups, Degradation, Alpine grassland, Tibetan Plateau
1 Introduction

Plant species can shape soil chemistry, structure and biota, and subsequently alter the survival and growth of other conspecific or heterospecific plants, i.e. plant-soil feedbacks (PSF), which is interpreted as mechanisms for the maintenance of diversity and plant coexistence, species invasion, and community succession (Bever et al. 1997, van der Putten et al. 2013, Baxendale et al. 2014). Such PSF can be negative, which is typically associated with resource depletion or accumulation of natural enemies (Semchenko et al. 2018, Bennett and Klironomos 2019), and can maintain diversity and promote species coexistence (Lekberg et al. 2018, Crawford et al. 2019). They can also be positive, generating species dominance through resource improvement or mutualists dominance (Bennett and Klironomos 2019, Zhang et al. 2019). Among many candidate factors, soil fungal diversity (pathogens, mutualists and saprophytes) has been identified as one of the key drivers to mediate PSF (Semchenko et al. 2018, Wang et al. 2019). However, the effects of soil microbes on plants are closely associated with plant species identity (Semchenko et al. 2017, Klinerova and Dostal 2020), because plant species strongly affect soil nutrients by secreting root exudates, returning litter to soil and interacting with soil microbes (Cornwell et al. 2008, Freschet et al. 2013). Although soil nutrient availability strongly impacts abiotic and biotic interactions and affects plant growth (De Deyn et al. 2004, Chagnon and Bradley 2013, Png et al. 2019), no single factor can be responsible for PSF (Bennett and Klironomos 2019). Taken together, despite the interactions between plant species, soil microbes and nutrients have been proven to provide critical mechanisms for biotic competition (Lekberg et al. 2018), plant diversity (Teste et al. 2017, van der Putten 2017), ecosystem productivity (Kulmatiski et al. 2012, Wang et al. 2019), vegetation succession (van der Putten et al. 2013, Heinen et al. 2020) and
biological invasions (Liao et al. 2008, Suding et al. 2013), few studies have systematically and simultaneously investigated their combined effects on grassland degradation.

Recent studies have shown that ecosystem degradation generated concurrent impairments in aboveground characteristics of plant communities e.g. biomass, coverage, diversity (Jiang et al. 2014, Zhou et al. 2021a), belowground soil moisture and nutrients (Plaza et al. 2019, Zhou et al. 2021a, Lai et al. 2023), and changes in microbial community composition and function (Wu et al. 2021, Breidenbach et al. 2022), subsequently might regulate PSF. Loss of soil nutrients after degradation may modify plant-plant and plant-soil biota interactions, and drive the eco-evolution of plant and soil microbial traits (Revillini et al. 2016a, Wu et al. 2021, Zhou et al. 2021a). For example, grasslands with a high diversity of graminoids are always dominated by grass-associated fungal pathogens (Kulmatiski et al. 2017, Heinen et al. 2020) and exhibit a stress-tolerator strategy (Zhou et al. 2021a). On the contrary, forbs exhibit more flexible competitors, stress tolerant and ruderal (CSR) strategies (Zhou et al. 2021a) and growth defence traits to adapt to resource-limited environments (Lekberg et al. 2018, Heinen et al. 2020). As degradation intensifies, insufficient soil resource is expected to directly generate negative effects on forb growth (Zhou et al. 2021d). In this scenario, fungal mutualists might be important drivers that induce positive effects on the survival and fitness of forbs in degraded environments with water and nutrient stress (Lekberg et al. 2018, Heinen et al. 2020). Changes in soil nutrients and pathogens/mutualists, as well as their interactions during degradation, will lead to both positive and negative feedback to plant growth (Revillini et al. 2016b, Lekberg et al. 2018), but their net outcome effects remain largely unknown. Generally, eco-evolutionary dynamics in plant traits and soil microbial communities (terHorst and Zee 2016) enable plants to survive, grow and reproduce in limiting or luxury soil resources (Revillini et al. 2016a, Crawford et al. 2019, Govaert et al. 2019). Despite this knowledge, the interdependence effects of biotic and abiotic factors in
determining PSF that drive the community dynamics in grassland degradation remain poorly understood.

Here, to reveal the mechanism of alpine grassland degradation on the Tibetan Plateau, we examined how the plant functional groups (monocots and dicots), soil nutrient availability and soil microbes (fungal diversity of pathogens, mutualists and saprophytes) regulate the outcome of PSF. Due to high diversity of graminoids in the non-degraded (ND) grassland (Semchenko et al. 2018, Zhou et al. 2021a), we hypothesize that there might be more accumulation of pathogens in the ND grassland and thus resulting in strong negative PSF for monocots. Whereas, optimal resource allocation supported that resource limitation can drive ecological adaptations and symbiotic interactions between plants and soil microbes (Ke et al. 2015, Revillini et al. 2016a), which might contribute to the growth of forbs in degraded-stressful environments. Therefore, we further hypothesize that the species in degraded alpine grassland with oligotrophic soil might generate positive interactions with soil biota.

2 Materials and methods

2.1 Identification of degradation

In recent years, overgrazing and climate change have led to severe degradation in alpine grasslands (Dong et al. 2013, Guo et al. 2019). According to the community species and soil nutrients in Damxung alpine meadow station (91° 04’ E, 30° 30’ N, ~4400 m), typically ND grasslands and severely degraded (SD) grasslands were defined in our study (Table 1). As degradation intensifies, the species composition and structure in the community gradually shift from monocots (Stipa capillacea, Festuca ovina L., Kobresia pygmaea, Carex montis-everestii) in the non-degraded communities to dicots (Anaphalis xylorhiza, Artemisia hedini. and Potentilla bifurca L.) in degraded grasslands (Zhou et al. 2021a).
2.2 Plant soil feedback experiment

The conditioning stage in the field

In the field, soils in ND and SD represent two different grassland habitats that are conditioned by monocots and dicots, respectively. Specifically, in 2019, the soil in ND was conditioned by the monocot species of *Festuca ovina* and *Carex montis-everestii* (because we removed the dicots every fifteen days from early June to September), while the soil in SD was conditioned by the dicots of *Artemisia hedinii* and *Potentilla bifurca* (because we removed the monocots every fifteen days from early June to September). Therefore, the soils in ND and SD can be regarded as own-conditioned soil by monocots and dicots, respectively. At last, the conditioned soil by two common monocot species in ND and two common dicot species in SD were used in the mesocosm feedback stage experiment (Fig. 1a), respectively.

After the conditioning stage in the growing season in 2019, the soil nutrients and microorganisms in ND and SD were different. Specifically, from ND to SD grasslands, water content, concentrations of total carbon (STC), total nitrogen (STN) and total phosphorus (STP) in soil decreased by 3.65 %, 5.11 g kg\(^{-1}\), 1.43 g kg\(^{-1}\) and 128.96 mg kg\(^{-1}\), respectively (Table 1). Meanwhile, the composition of bacterial and fungal communities in ND and SD was obviously different (Fig. 2 and Supplement Fig.1). These discrepancies indicate that the soils conditioned by either monocots in ND or dicots in SD are different and valuable for testing the PSF effects in the feedback stage.

Feedback stage in the greenhouse

Soils used for the feedback experiment were collected from ND and SD in Damxung County on May 2, 2020. The collected soils were homogenized by sieving through a 0.5 cm mesh, before mesocosm pots (diameter = 25 cm, depth = 20 cm) were filled with 1500 g of soil. Seedlings of uniform root length and growth (N=3 for each species, including monocot
species of *Festuca ovina* and *Carex montis everestii*, dicots of *Artemisia hedinii* and *Potentilla bifurca*) were collected in the natural grassland of Damxung County on May 20, 2020, and were carefully washed by 1% NaClO to eliminate the transfer of soil organisms before planting.

Finally, i) we randomly planted disinfected plants in mesocosms pots with ND and SD soil (treatment without sterile, coded as Non-sterile); ii) planting in soil that was sterilized by a high temperature and pressure facility (126 °C, 2 h, treatment coded as Sterile). Five replicates were set for each treatment. In the greenhouse management, pots were watered every two days to ensure 50% water holding capacity in the soil, and plants were harvested after 13 weeks when the planting plants were at the end of growth.

### 2.3 Leaf traits, plant biomass and CSR strategy

For each pot, plant shoots and roots per species were harvested. Meanwhile, for each species, 10 leaves were randomly selected for leaf area measurement. The selected leaves were scanned by Canon LiDE 70 and the leaf area was obtained by ImageJ (https://imagej.nih.gov/ij/). Then, their fresh weights were measured. After that, these leaves were oven dried at 65 °C for 48 hours and weighed to obtain leaf dry weight (Zhou et al. 2021a). Moreover, based on the method in a previous study (Zhou et al. 2021a), we calculated specific leaf area, leaf water content, leaf succulence index, leaf dry matter content and leaf mass per area, the CSR strategy (competitor, stress-tolerator and ruderal) for each species.

After measuring leaf traits, plant shoots and roots of monocots and dicots in each pot were oven-dried at 65 °C for 48 h to measure aboveground biomass (AGB) and belowground biomass (BGB). Next, dried shoot and root samples of monocots and dicots were ground with a ball mill (NM200; Retsch, Haan, Germany), and the N concentrations were measured by a
Vario MACRO cube elemental analyzer (Elementar Analysensysteme GmbH, German), respectively (Zhou et al. 2021d).

2.4 Soil properties

The conditioned soil samples were collected in ND (n=5) and SD (n=5) soil, and sieved through 2 mm mesh in the laboratory. Then, soil water content (SWC) was measured by the oven-dried method. Air-dried soils were used to measure STC and STN by MACRO cube elemental analyzer (Elementar Analysensysteme GmbH, German), and STP by the method of molybdate colorimetric test with the perchloric acid digestion (Zhou et al. 2021d).

2.5 Soil microbial communities

The conditioned ND (n=5) and SD (n=5) fresh soil sample gDNA was purified by Zymo Research Biomics DNA Microprep Kit (Cat#D4301), and the integrity of gDNA was detected by 0.8% agarose electrophoresis, and then the nucleic acid concentration was detected by TECAN F200 (PicoGreen dye method). PCR amplification, the primer sequences of ITS1F/ITS2R (5’-CTTGGTCATTAGAGGAAGTAA-3’, 5’-GCTGCGTTCTTCATCGATGC-3’) and 515F/907R (5’-GTGCCAGCMGCGCCGCGG-3’, 5’-CCGTCAATTCMTTTRAGTTT-3’) were used to amplify the universal sequencing primers in the soil fungal and bacteria community, respectively. The PCR products were mixed with 2% agarose gel electrophoresis and recovered by the Zymoclean Gel Recovery Kit (D4008). At last, the recovered DNA was sequenced by Qubit® 2.0 Fluorometer (Thermo Scientific).

Operational taxonomic units (OTUs) were divided by the UPARSE algorithm with 97% consistency, and we selected representative sequences at the same time. The soil fungal communities were identified by the UNITE database. Microsoft Excel 2016 was used to preliminarily process the data, and Python 3.6 was used to classify the fungal sequencing
OTUs data based on the FUNGuild database (Nguyen et al. 2016). Then, fungal sequencing data were classified into three functional groups: pathotroph, saprotroph and symbiotroph. Finally, the relative abundance of pathotroph, saprotroph and symbiotroph in soil under different treatments was calculated.

2.6 Data analyses

Based on the field community survey in ND and SD, the plant diversity indices of the Shannon-Wiener index, Simpson index, Margalef index and Pielou index were calculated.

For each alpine grassland species, plant-soil feedback indices were calculated by the average shoot and root biomass in conspecific and heterospecific soils (Bever 1994):

\[ PSF = \ln\left(\frac{Bio_{own}}{Bio_{Foreign}}\right) \]

where \(Bio_{own}\) and \(Bio_{Foreign}\) are the biomass of plants in their own conditioned soil and foreign conditioned soil, respectively.

One-way ANOVA was conducted to test the effect of grassland degradation on biomass, PSF, plant traits and soil properties. All analyses were conducted by using R 4.2.1 (R Core Development Team, R Foundation for Statistical Computing, Vienna, Austria).

3 Results

3.1 The plant and soil feedback in monocots

The effects of soil nutrients and microbes on plant biomass and PSF were explored. There was no significant difference in monocots’ biomass between ND (1.62 ± 0.35 g) and SD (1.20 ± 0.18 g) soil when the soils were not sterilized (Fig. 3a). However, the biomass of monocots was significantly (\(P < 0.05\)) higher in ND soil than that in SD soil when the soils were
sterilized. Specifically, the biomass decreased from $6.11 \pm 0.49$ g in ND to $1.85 \pm 0.09$ g in SD when the soil was sterilized (Fig. 3b). Therefore, significant ($P < 0.05$) positive monocots’ PSF (0.52) was observed in sterile soil (Fig. 4a).

However, neutral PSF of monocots was observed in soil with microbes (Fig. 4a). In other words, significantly ($P < 0.05$) higher soil nutrient availability in ND (STC = 17.66 g/kg, STN = 3.16 g/kg) than that in SD (STC = 12.55 g/kg, STN = 2.74 g/kg, Table 1) resulted in monocots’ significant positive PSF in soil without microbes, but this effect disappeared when monocots grew in soil with microbes (Fig. 4a).

These results not only directly confirmed that higher soil nutrient in ND generated positive effects on monocots’ growth, but also indirectly supported our hypothesis that soil microbes in ND have negative effects on monocots.

### 3.2 The plant and soil feedback in dicots

Dicots’ biomass was significantly lower in SD soil ($2.90 \pm 0.08$ g) than that in ND soil ($4.15 \pm 0.15$ g) when soils were sterilized (Fig. 3d). However, no significant difference in dicots’ biomass in ND and SD soil was detected in soil with microbes which was conditioned by SD species (Fig. 3c). Hence, significant ($P < 0.05$) negative PSF ($-0.16$) calculated by dicots’ biomass was detected in sterile soil, but neutral PSF in soil with microbes (Fig. 4b). That is to say, lower soil nutrient availability in SD than that in ND (Table 1) begot dicots’ significant negative PSF in soil without microbes, but this effect disappeared when dicots grew in soil with microbes. Together, these findings not only demonstrated that low soil nutrient availability in degraded grassland led to negative effects on dicots, but also confirmed our hypothesis that soil microbes in degraded environments could generate positive effects on dicots.
3.3 The soil fungal diversity in non-degraded and severely degraded grassland

After fungal sequencing data classifying with FUNGuild database, a total of 149 OTUs in ND soil and 90 OTUs in SD soil remained. And the OTUs number of pathotroph, saprotroph and symbiotroph in ND soil were 52, 73 and 24, respectively. In SD soil, the OTU numbers of pathotroph, saprotroph and symbiotroph were 31, 46 and 13, respectively. After counting the reads of OTUs, the proportions of pathotroph, saprotroph and symbiotroph in ND soil were 36% (97 reads), 51% (139 reads) and 13% (35 reads), respectively (Fig. 5a). Notably, a decreased proportion of pathotroph was detected after grassland degradation. Specifically, the proportions of pathotroph, saprotroph and symbiotroph in SD soil were 30% (46 reads), 57% (86 reads) and 13% (19 reads), respectively (Fig. 5a).

4 Discussion

We found that the effect of higher soil fertility on plant growth was offset by the negative impact of the monocot-associated pathogen in ND, whereas the growth of dicots was enhanced in low soil nutrient availability due to an increase of saprotrophic microbes, which supported our hypothesis that positive plant-soil feedback promoted the thriving of dicots in the degraded community. Our experiment provided a mechanism by which integrated roles of plant functional groups, microbial composition and soil fertility affected the direction of plant-soil feedback, and therefore degradation succession.

4.1 Monocots-associated pathogens mediated the negative PSF in non-degraded grassland

Soil microbes determine specific feedback in species directly through its effects on plant-soil biota interactions, which has been supported by many studies (Ke et al. 2015, Semchenko et al. 2018). Our results demonstrated that there was more accumulation of pathogens in ND
(36%, conditioned by monocots) than that in SD (30%, conditioned by dicots) (Fig. 5a). The fine roots of monocots in ND-fertile soil would be expected to attract diverse communities of pathogens, and thus generating negative PSF and promoting the coexistence and significantly ($P<0.05$) higher diversity of species in ND (Shannon-Wiener index of 2.0 in ND vs 1.2 in SD, Supplement Fig. 2). Several lines of evidence show that the accumulation of pathogens in monocotyledonous soil is common (Heinen et al. 2020), which results in strong negative effects on grasses (Smith-Ramesh and Reynolds 2017, Heinen et al. 2020) and overwhelms soil nutrients-mediated PSF (Bennett and Klironomos 2019). Actually, species tend to suffer from more negative PSF due to the faster accumulation of pathogens in wetter and more fertile environments (Comita et al. 2010, Revillini et al. 2016a, Crawford et al. 2019). Our results just indirectly demonstrated that there was more accumulation of pathogens (Fig. 5a) and stronger negative PSF (Fig. 4a) caused by microbes in ND soil with higher soil moisture (13.47% in ND vs. 9.82% in SD, Table 1).

In addition, the traits of plants have been identified as another important driver of PSF (Baxendale et al. 2014, Teste et al. 2017, Lekberg et al. 2018). Specifically, according to the CSR theory, the stronger competitors (15.1% and 14.8% of competitor strategy in ND and SD, respectively) of monocots are expected to experience greater negative PSF than weaker competitors of dicots (competitor strategy was 8.2% in ND and 7.4% in SD, respectively, Supplement Fig. 3), because PSF is expected to counteract competitive exclusion and promote species coexistence in luxury soil resource environments (Bever 2003, Crawford et al. 2019). Contrary to several previous studies on plant traits demonstrating that fast-growing plant species with high specific leaf area and high root N concentration (Orwin et al. 2010, de Vries et al. 2012, Baxendale et al. 2014) tend to suffer from more negative PSF. However, in our study, unlike dicots, the fast growth rate of monocots with exploitative traits (higher specific leaf area, Supplement Fig. 4) and significant ($P<0.05$) low shoot and root N
concentrations (Supplement Fig. 5) suffered more negative impacts from pathogens. Apart from the above reasons, the close phylogenetic distance of grasses than that of forbs (Crawford et al. 2019, Heinen et al. 2020) might explain why stronger negative feedback was driven by the monocots-associated pathogens in our study. Multiple studies have demonstrated that pathogens lead to strong negative effects on grasses because the close phylogenetic distance of graminoids is more likely to share pathogens (Gilbert and Webb 2007, Parker et al. 2015). Consequently, we speculated that the fine roots, high competitiveness and close phylogenetic distance of grasses would result in a high ratio of pathogens, ultimately generating negative effects on monocots and promoting species coexistence in ND grassland with fertile soil (Fig. 6).

4.2 Why can dicots thrive in degraded environments with water and nutrient stress?

Interestingly, why can dicots thrive in degraded grassland with lower soil water and nutrient availability? There may be two main reasons. First, the high phenotypic plasticity of plant traits in dicots can generate advantageous effects on their own via positive interactions with soil microbes. Plant traits (e.g. leaf area, root exudates and litter biomass) are important factors to control plant-mediated soil nutrient cycling (Freschet et al. 2013, Ke et al. 2015). According to the optimal resource allocation model, ecological adaptations and symbiotic interactions of plants and soil microbes might be driven by resource limitations (Klironomos 2002, Revillini et al. 2016a). Actually, previous and present studies both have proved that, unlike monocots, dicots adapt to degraded environments with deficient water and nutrients via various survival strategies (e.g. flexible nutrient utilization (Zhou et al. 2021d), biomass allocation (Zhou et al. 2021c) and CSR strategy (Zhou et al. 2021a), high root length as well as litter decomposability (Chen et al. 2021)). Specifically, dicots (Artemisia nanschanica decomposed 46.7%) produce litters that decompose faster than the litter of monocots after
three years (*Carex moorcroftii* decomposed 32.0%, Fig. 5b) (Chen et al. 2021), providing more resources to microbial symbionts (Cornwell et al. 2008, Prescott and Zukswert 2016) and accelerating plant-microbial nutrient cycling (Ke et al. 2015). In this situation, soil microbes quickly decompose litter and improve plant nutritional status (Kuzyakov and Xu 2013, Capek et al. 2018, Zhou et al. 2021b), thereby generates positive effects on the growth of dicots in degraded conditions.

Second, a high ratio of symbiotic and saprophytic biota in degraded grassland (70% in SD soil, Fig. 5a) can strongly promote dicots’ survival and growth in low moisture and drought-prone environments (Heinen et al. 2020). Several studies have confirmed that dicots (*Stellera chamaejasme* L (Sun et al. 2009), *Glycine max* (Vink et al. 2017), *Solidago Canadensis* (Ye et al. 2019), *Cytisus scoparius* and *Ulex europaeus* (Drake 2011)) can improve their living conditions via positive interactions with soil microbes, as such, increase the abundance of detritivore and mycorrhizal fungi in the rhizosphere but reduce predator abundance through litter pathway (Zhang et al. 2019), consequently reduce dicots’ water and nutrient stress in drought and other extreme conditions (Revillini et al. 2016a, van der Putten et al. 2016). The mycorrhizal fungi not only can reduce their host plants’ water stress by improving nutrition, increasing access to soil moisture and maintaining plants’ stomatal conductance in hostile environments (Lehto and Zwiazek 2011, Auge et al. 2015), but also can help dicots defense against pathogens by producing distinct organic acids and allelochemicals at the same time (Sikes et al. 2010, Jung et al. 2012). In turn, dicots species with significant (*P* < 0.05) high N in their shoot and root than that of monocots (Supplement Fig. 5) and a fast nutrient cycle support soil microbial communities to enhance their performance and nutrient capacitation (Freschet et al. 2013, van der Putten et al. 2016, Zhou et al. 2021a). Moreover, dicots produce similar quality and quantity of litter than monocots (Prescott and Zukswert 2016, Zhang et al. 2019), then generate home-field advantage of litter decomposition (Freschet et al. 2012, van
der Putten et al. 2016). In this situation, high litter quality and decomposition (Fig. 5b) coupling with a high ratio of symbiotic fungi (Fig. 5a) directly generates a synergistic increase in root-biota interactions (Kuzyakov and Xu 2013, Suding et al. 2013), and ultimately increase the availability of soil nutrients for dicots in degraded environments.

Our results are in accordance with these findings that local limiting resource conditions can drive the adaptation of plants and microbial communities, such as maximum acquisition of limiting resources by nutrient interdependence and symbiotic cooperation relationships between plants and microbes (Fig. 6). When plant-microbial mutualisms dominate, dicots provide their symbiotic fungi with labile C compounds and receive mineral nutrients from microbes in return (Zhou et al. 2021b), which ultimately can alleviate microbial nutrients stress (Lehto and Zwiazek 2011, Ke et al. 2015) and introduce positive effects on dicots growth (Hodge and Fitter 2013, Revillini et al. 2016a). In summary, eco-evolutionary in plant traits and soil microbial communities (terHorst and Zee 2016) enable dicots to survive, grow and reproduce in degradation-stressful conditions (Revillini et al. 2016a, Crawford et al. 2019, Govaert et al. 2019). And the emerging alternative eco-evolutionary in plant and soil microbes' interactive effects in degraded grassland indicates an irreversible course of degradation (Breidenbach et al. 2022), which might result in a predicament for the restoration of alpine grassland (Zhou et al. 2021a).

5 Conclusions

Through linking biotic and abiotic drivers of PSF, our findings provided novel insights into the mechanism by which soil nutrient availability, soil microbes and plant traits of functional groups medicated the outcome of PSF. Specifically, our experiment demonstrated that monocots in the non-degraded, relatively fertile soil mainly suffered from negative PSF due to the accumulation of pathogens, leading to plant species co-existence. By contrast, dicots
thrived in degraded and stressful environments by promoting soil nutrient cycling, avoiding soil pathogens, and enhancing mutualistic symbiosis with microbes. Our results demonstrated that positive plant-soil feedback may be the mechanism by which dicots (forbs) thrived and aggravated grassland degradation. A comprehensive mechanistic understanding of grassland degradation will help manipulate plant-microbial interactions in the restoration of degraded alpine grasslands. However, more researches should be devoted to establishing whether there are general PSFs in different grassland types and regions, as this subject is critical to promoted the management of degraded grassland.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References


Captions for tables:

**Table 1.** The location, dominant species, soil water content (SWC), soil total carbon (STC), soil total nitrogen (STN) and soil total phosphorus (STP) in non-degraded and severely degraded grasslands. Mean, Min, Max and Std indicate the average, minimum, maximum, and standard deviation values, respectively. Different letters indicate significant differences in means at $P<0.05$.

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<th>Level</th>
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<th>Values</th>
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<td>Non-degradation</td>
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<td>30° 23″</td>
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Captions for figures:

**Figure 1** Experimental design. In the conditioning stage (a), the soil in non-degraded (ND) and severely degraded (SD) grassland are conditioned by monocots and dicots in the field, respectively. In the feedback stage (b), seedlings of four species (monocots of *Festuca ovina* L. and *Carex montis everestii*, dicots of *Artemisia hedinii* and *Potentilla bifurca* L., respectively) were randomized transplanted as single individuals into mesocosms pots: 1) ND and SD soil with microbes; 2) ND and SD soil that was sterilized. The shoot and root biomass collected from ND and SD were used to calculate the plant-soil feedback. These were done in a full-factorial design (n=5).

**Figure 2** After the conditioning stage, the community species analysis of soil bacteria (a) and soil fungus (b) in non-degraded (ND) and severely degraded grassland (SD).

**Figure 3** In the degraded gradients (non-degraded [ND] and severely degraded [SD] grassland), the biomass of monocots (a and b) and dicots (c and d) in non-sterilized soil and sterilized soil, respectively. Significance codes: ** represents significant levels at $P<0.01$.

**Figure 4** Plant-soil feedback based on the biomass of monocots (a) and dicots (b). Significance codes: ** represents significant levels at $P<0.01$.

**Figure 5** After the conditioning stage, the relative abundance of pathogenic fungi, saprotrophic fungi and symbiotrophic fungi in non-degraded (ND) and severely degraded grassland (SD, a). And
the mass remaining of monocots (*Carex moorcroftii*) and dicots (*Artemisia nanschanica*) after three years (b), this data is collected from Chen et al., (2021).

**Figure 6** Conceptual graph of plant-soil feedback (PSF) in response to grassland degradation. The high soil nutrient availability in non-degraded grassland (ND) is likely to generate a positive effect on monocots' growth, but soil microbes (pathogens) as primary factors mediated the negative PSF in monocots. On the contrary, despite the low soil nutrient availability in severely degraded grassland (SD) tends to result in a negative effect on dicots’ growth, the high ratio of mutualists and saprophytes contributes to the positive PSF in dicots. Taken together, our findings confirmed a shift from monocots with higher vulnerability to soil pathogens that grow in ND high nutrients soil to dicots with higher dependence on nutritional mutualists that thrive in degraded infertile soil.
Figure 1

(a) Conditioning stage

Monocots
Festuca ovina
Carex montis everestii

Dicots
Artenisia
Potentilla bifurca

Non-degraded soil: own soil for monocots
Severely degraded soil: own soil for dicots

(b) Feedback stage

Treatments
Non-sterile
Sterile

PSF = Ln(Bio_{i, own} / Bio_{i, foreign})

PSF_{mon} = Ln(Bio_{mon, ND} / Bio_{mon, SD})
PSF_{dic} = Ln(Bio_{dic, SD} / Bio_{dic, ND})

Note: PSF = Plant-soil feedback, mon = monocots, dic = dicots, ND = Non-degraded, SD = Severely degraded, Bio = biomass
Figure 2
Figure 3

(a) Monocots

(b) Monocots

(c) Dicots

(d) Dicots

Figure 3
Figure 4

(a) Monocots

(b) Dicots

Figure 4
Figure 5
Figure 6