2	the Loess Plateau, Shaanxi, China?
3	Lin Hou ¹ , Sijia Hou ²
4	1 College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, China
5	2 College of Transportation, Southeast University, Nanjing, Jiangsu, 211189, China
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How long should the fully hillside-closed forest protection be implemented on

Comment [A1]: It seems that the fully hillside-closed forest protection program is the same as Sloping Land Conservation Program? See Xi et al. 2014. Challenges to sustainable development in China: a review of six large-scale forest restoration and land conservation programs. Journal of Sustainable Forestry, 33:1-19. If they are the same, then change your title to "How long should sloping land conservation program be implemented on the Loess Plateau, Shaanxi, China?" Also, if this change is made, then go through the paper and change all "fully hillside-closed forest protection" to "sloping land conservation program."

18	Corresponding author
19	Lin Hou
20	3 Taicheng Road, Yangling, Shaanxi, 712100, China
21	E-mail: houlin1969@163.com
22	Abstract
23	Background. Restoration of degraded forest ecosystem is crucial for regional sustainable
24	development. To protect the country's fragile and fragmented environment, the Chinese
25	government has initiated an ecological engineering, the Natural Forest Protection Program in
26	seventeen provinces in China since 1998. Fully hillside-closed forest protection (vegetation
27	restoration naturally without any artificial disturbance) was one of vital measures of the Natural
28	Forest Protection Program applied national wide. Whether plant diversity, biomass and age
29	structure of dominant tree species and soil nutrients in protected stands may become better with
30	increase of protected period are still open problems.
31	Methods . We investigated community diversity, biomass of dominant tree species, age structures.
32	and analyzed soil chemical properties of a Pinus tabulaeformis population at protected sites
33	representing different protected ages at Huanglongshan Forest Bureau on the Loess Plateau, Shaanxi,
34	China.
35	Results. Plant species richness of <i>Pinus tabulaeformis</i> community was significantly affected

index of plant species generally increased with protection age. Stands protected for 45 years had the highest tree biomass and considerable natural regeneration capacity. Contents of organic carbon, available phosphorus and available potassium in top soil increased in protected stands less than 45 years, however decreased significantly thereafter. Long-term forest protection also decreased content of mineral nitrogen in top soil.

Discussion. We found that richness of shrubs and herbs was significantly affected by forest protection, and evenness indices of tree, shrub and herb increased inconsistently with protected ages. Forest protection created more complex age structures and tree densities with increasing age of protection. Content of soil mineral nitrogen at 0-20 cm soil depth showed a decreasing trend in stands of up to 30 years. Soil available phosphorus and potassium contents were higher in stands with greater proportions of big and middle trees. Long-term protection (> 45 years) of *Pinus tabulaeformis* stand in southeast Loess Plateau, China, may be associated with decreasing plant species richness, proportion of medium to large trees, dominant biomass of *Pinus tabulaeformis*

(p<0.05) by forest protection and the effect attenuated with protection age. Shannon evenness

Comment [A2]: The word middle designates position and not size unlike big or small. Instead use mid-sized or medium trees. You can say "......proportions of big and mid-sized trees." Try to effect this correction throughout the paper. See lines 47, 196, 197

Keywords: Fully Hillside-closed Forest Protection, Optimal Protection Age, *Pinus tabulaeformis*,

the Loess Plateau, Restoration

and soil nutrients.

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54	INTRODUCTION
55	Ecological restoration is being recognized as an international priority(Aronson & Alexander
56	2013; Wortley et al. 2013) and it plays a crucial role in rebuilding ecological equilibrium and
57	reversing ecosystem degradation (Ma et al. 2013). As a part of ecological engineering (Mitsch
58	2012), the practice is being widely incorporated into natural resource strategies from the local to
59	global level (Wortley et al. 2013).
70	To protect the country's fragile and fragmented environment, the Chinese government has
71	initiated an ecological engineering, the Natural Forest Protection Program (NFPP) since 1998 (Xu

et al. 2006). Logging and harvesting of partial or full timber was prohibited in protected areas from 1998 to 2008(Xu et al. 2006). Fully hillside-closed forest protection (vegetation restoration naturally without any artificial disturbance) was applied in national wide nation-wide. Ecosystems have the capacity to self-organize and the self-design or self-organizational properties of natural systems is an essential component to ecological engineering(Bergen et al. 2001). It is obviously Obviously, fully hillside-closed forest protection is in accord with the ecological engineering principle self-design.

The previous studies regarding NFPP have mainly focused upon the introduction of the related policy issues(Grumbine & Xu 2011; Li 2004; Wang et al. 2015), the spatial-temporal succession of regional vegetation (Huang et al. 2014) and ecological restoration programs and payments (Yin & Zhao 2012).

However, a range of questions remain, particularly in relation to stand function and associated environmental parameters following stand protection. We hypothesize that fully hillside-closed forest protection may promote plant diversity, biomass and age structure of dominant tree species and soil nutrients with increase of protected period.

The objectives of this study are to address a few of these key knowledge gaps, including: (i) do the stands exhibit significant differences in plant assemblage; (ii) does soil fertility change with stand age structure; (iii) can a functional relationship be defined regarding length of stand protection and stand quality, i.e., are stands protected for longer timeframes "better" than other stands; and (iv) based on findings of i-iii above, can a preliminary estimate regarding the optimal

- 92 time span for *Pinus tabulaeformis* stands be recommended to the Natural Forest Protection
- 93 Program?

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MATERIALS AND METHODS

Site description

The study was conducted in Huanglong County (35°28'49"-36°02'01"N, 109°38'49"-96 110°12'47"E) on the southeast Loess Plateau ,Shaanxi, China. Stands in this area (a part of NFPP 97 98 area) play key ecological roles in soil erosion abated and sand storm mitigated (Chen et al. 2014). The vegetation type is a northern deciduous broad-leaved forest sub-region. Pinus tabulaeformis is 99 dominant tree species in the currently existing stands. The associated tree species are Quercus 100 liaotungensis, Syringa oblate, Populus davidiana, Prunus davidiana, Betula platyphylla and 101 102 Toxicodendron vernicifluum. Shrubs and herb species in understory are abundant. The altitude 103 ranges from 1100 to 1300 m. It is dominated by a warm temperate and semi-humid continental climate. The annual average precipitation is 612 mm and the mean atmospheric temperature is 104 105 8.6 °C. Cinnamon soil is the main soil type in the forest region. 106 Due to poor communication and a small human population in the past years, stands on some

Due to poor communication and a small <u>human</u> population in the past years, stands on some special sites have not been disturbed since 1950, especially since 1998.

According to data from Huanglongshan Forest Bureau, Yanan, Shaanxi, China, stands with protected age sequence were found in four forest farms (Table 1).

Field methods

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Comment [A3]: play key ecological roles in abatement of soil erosion and mitigation of sand storm (Chen et al. 2014).

The field investigation and sampling was conducted between June 5 to July 15, 2003. Each plot of trees, shrubs and herbs was 20 m×20 m, 2m×2 m and 1 m×1 m respectively. Five sub-plots of shrubs, herbs and regeneration regenerating seedlings were arrayed diagonally in each tree plot respectively. The indices, species, number, Height (H), diameter at breast height (DBH) and canopy density of trees, and species, height, cover ratio, number of shrubs, herbs and regeneration regenerating seedlings were measured. All community data were collected from 27 tree plots spreading among the age cohorts and 270 sub-plots (Table 1).

Comment [A4]: You can use 'advance regeneration' or 'regenerating seedlings' to refer to seedlings growing on forest floor.

Three soil samples were obtained randomly by a special drill in each tree plot. Surface soils (0-20cm depth) at all sites were assessed for soil properties including organic carbon, mineral N, available phosphorous and potassium.

Community diversity

The importance of species richness and evenness in influencing diversity-associated productivity has been demonstrated in a meta-analysis of 54 studies (Zhang et al. 2012). In this study, we chose indices of richness and evenness to reflect characteristics of community. Species richness index (S) was derived from field survey data. To characterize the diversity of the stand community, the Shannon-Wiener index (H') and evenness index (J') were calculated as the following:

Shannon-Wiener index $H' = -\sum P_i \ln P_i$

Shannon evenness index $J' = \frac{H'}{\ln S}$

where P_i is the relative frequency of the i-th species, and S is total number of species in plots and subplots (Magurran 2004).

Biomass of dominant tree species

Average DBH (cm) and height (m) of *Pinus tabulaeformis* in each plot were calculated and living biomass (Mgha⁻¹) of whole trees (*Pinus tabulaeformis*) were estimated (Chen & Peng 1996;

Pan et al. 2004).

136 Y = 15.525 + 0.6269v

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 $\ln v = 0.99138 \ln(D^2 H) - 10.30211$

where *Y* is the living biomass of trees(Mgha⁻¹) ν is the stand growing stock (m³ ha⁻¹), D(cm) is diameter at breast height and H (m) is height.

Combining the density of dominant tree species (Table 1) with equations, biomass of *Pinus* tabulaeformis in protected stands was determined.

Age structures

DBH of tree species correlate significantly to their ages under the same environmental condition(Parker & Peet 1984). Lacking of analytic wood data, we adopted DBH structures of *Pinus tabulaeformis* population instead of its age structures. Combining DBH to_and_H, age structures of *Pinus tabulaeformis* population were classified as following: Iseedling, H≤0.30 m; Ilyoung tree, 0.30m<H≤2.00m, DBH≤6.00cm; Ill small tree, H>2.0m, 6.0cm<DBH≤12.0cm;

Comment [A5]: Are the equations from Chen and Peng 1996; and Pan et al. 2004? If yes, Lines 133 – 135 should read - Average DBH (cm) and height (m) of *Pinus tabulaeformis* in each plot were calculated and living biomass (Mgha-1) of whole trees (*Pinus tabulaeformis*) were estimated according to Chen and Peng 1996; and Pan et al. 2004.

Wmiddle tree,12.0cm<DBH≤20.0cm; V big tree, DBH>20.0cm. The ratio of seedlings, young trees, small trees, middle trees and big trees in stand with same protecting age was used to illustrate age structures. Probable age of individual was determined by their whorled branches.

Chemical analyses

Analyses were made on air-dry soil material that passed through a 2 mm sieve. Soil organic carbon content (SOC) was determined by dry combustion with a TOC/TON analyzer (TOC-VTH-2000A, Shimadzu Corporation, Japan). Soil mineral nitrogen (ammonium nitrogen, NH⁺₄-N and nitric nitrogen, NO⁻₃-N) content was determined by the colorimetric method with automatic flow injection (AA3, BRAN+LUEBBE,Germany). Available phosphorus content was extracted in 0.5M NaHCO₃ and determined by Mo-Sb colorimetry. Available potassium content was determined by method of flame photometry(Bao 2000).

Data processing and analysis

SPSS 17.0 and Origin8.0 (OriginLab Corporation) software were used for statistical analysis and plotting. A graphic check of the postulates was performed based on the residual distribution. One-way analysis of variance (ANOVA) following by Fisher's least significant difference (LSD) test (p<0.05) was used to compare the protection age effects on diversity of plant community and soil nutrients respectively.

RESULTS

Diversity of plants in protected stands

Richness index of tree, shrub and herb was highest in the stand protected for 30 years (Table 2). A significant difference in the tree species richness index was observed in the 30 year protected stand compared to stands protected for 16 years (n=5, p<0.05) and 45 years (n=8, p<0.05), but not in other stands with different protected ages (Figure 1). The richness index of within stand shrubs differed significantly between stands protected for 30 years compared to stands protected for 16 years (n=25, p<0.05), 45 years (n=40, p<0.05), 60 years (n=20, p<0.05) and 75 years (n=20, p<0.05) (Figure 1). Significant differences in the within-stand shrub richness index were also found in stands protected (i)16 years and 60, 75 years, (ii) 45 years and 60 years, 75 years (Figure 1). The within stand herb richness index in the stands protected for 30 years differentiated significantly to—from stands protected 16 years (n=25, p<0.05), 45 years (n=40, p<0.05), 60 years (n=20, p<0.05) and 75 years (n=20, p<0.05) (Figure 1). Richness of within stand herb at stands protected for 16 years also varied significantly to—from stands protected for 60 years and 75 years (Figure 1).

Shannon-Wiener evenness index of tree, shrub and herb was the highest in stands protected for 30 years, 45 years and 75 years respectively (Figure 2). The index of herb generally increased with protected ages except in stands protected for 16 years to 30 years (Figure 2). However, the index of tree and shrub fluctuated with stand protected years and did not follow a trending relationship (Figure 2). Tree and shrub Shannon-Wiener index increased with stand protection age, with the exception of tree index 30-45 year stand protection and shrub index 45-60 year stand protection (Figure 2).

Comment [A6]: Make sure that all n=? are as shown on the table.

Biomass of *Pinus tabulaeformis* in protected stands

Biomass of *Pinus tabulaeformis* increased in stands until 45 years of forest protection; however, for sites older than this protection age, stand biomass decreased (Figure 3). Peak biomass was $70.60 \pm 8.00 \text{ t} \cdot \text{ha}^{-1}$ in the stand protected for 45 years, while biomass in the stand protected for 75 years $(19.90 \pm 9.2 \text{ 0 t} \cdot \text{ha}^{-1})$ was lower than the stand protected for 16 years $(23.70 \pm 17.10 \text{ t} \cdot \text{ha}^{-1})$ (Figure 3).

Age structure of Pinus tabulaeformis population in protected stands

Although age classes of *Pinus tabulaeformis* occurred in protected stands, they varied greatly (Figure 4). Only young (II) and small trees (III) were found in the stand protected for 16 years, small (III) and middle trees (IV) dominated the stand protected for 30 years (Figure 4). For the stand protected for 45 years, big (V) and middle trees (IV) were main components, but seedlings (I) and young trees were considerable also (Figure 4). In contrast, for stands protected for 60 and 75 years, seedlings (I) were the dominant component, followed by young (II) and small trees (III), with big trees (V) lowest in distribution (Figure 4).

Soil nutrients

Significant differences of soil organic carbon content at 0-20 cm soil depth were observed between the stands, with higher soil organic carbon content observed in stands protected for longer than 30 years (Figure 5 A). Content of mineral nitrogen at 0-20 cm soil depth demonstrated significant differences in stands before and after the protected 30 years (Figure 5 B). No significant

differences were found between stands protected for 16 years and 30 years, and among stands after protected for 30 years (Figure 5 B). Content of available phosphorus at 0-20 cm soil depth increased as protection of stand age increased, with significant differences observed mostly at youngest and oldest stand ages (Figure 5 C). No significant difference in available phosphorus was observed in stands between 45—30 and 30—45 years of protection—age, and between 45 and 60 years of protection—age (Figure 5 C). Content of available potassium at 0-20 cm soil depth decreased in stands younger than 45 years forest protection—and thereafter increased (Figure 5 D). Significant differences were demonstrated among stands with different protection ages, except at ages 45 years—and 60 years (Figure 5 D).

Discussion

Response of plant diversity to forest protection

Species richness is one measure of biodiversity and is very important for ecosystem functioning, stability and integrity_(Coroi et al. 2004). We found that richness of shrubs and herbs was significantly affected by forest protection, although richness (Figure 1) and evenness (Figure 2) indices of tree, shrub and herb increased inconsistently__in an unpredictable manner_with protected age. The richness of plant species increased in stands protected for 16 years to 30 years, decreased in stands protected for 30 years to 45 years and remained fairly stable in stands protected for longer than 45 years (Table 2). Due to adequate sunlight and growing spaces, some pioneer tree species (*Populus davidiana*, *Betula platyphylla*) and drought resistant shrubs (*Rubus corchorifolius*, *Rosa hugonis*, etc.) and herbs (*Artemisia gmelinii*, *Saussurea petrovii*, etc.) were

more prevalent in the younger forest protection sites, increasing plant species richness of these stands (Table 2). With the growth of trees, canopy density increased and some drought resistant plant species disappeared. The naturally regenerating seedlings Advance regeneration in stands protected for 45 years and older occupied made up a large proportion of the species observed, impeding invasive plant species and stabilizing plant diversity of the community assemblage.

Inherent spatial variability within the landscape may provide a possible explanation for this pattern observed, since *Pinus tabulaeformis* stands are distributed across variable site conditions within the region. Soil moisture is considered to be the key limiting factor on the Loess Plateau for differences in plant species growth and regeneration (Chen et al. 2014) and it is possible that the differences in soil properties property as observed in this study reflect affected variation in plant-available moisture.

Forest protection in Huanglongshan forest region, Yanan, Shaanxi, China was initiated in 1950. Stand structure within the protection area under the natural restoration condition differed. Stands with diversified age diversity—structure generally were more-richer in species rich than stands with less diversified age structure (Thompson 2012). Findings in this study partly support this notion. Stands protected for great than 16 years had more species with diverse age structures and plant species richness (Figure 1). Age class structure in stands protected for 30 years were generally simpler than stands protected for longer periods (Figure 4). However, stands older than 30 years forest—of protection had lower richness index of tress and understory species (Figure 2).

Our results suggest that sustainable forest protection can potentially contribute to plant

diversity conservation by increasing species richness generally (Table 2) and promoting <u>even</u> distribution of trees and herbs <u>more even</u> (Figure 2).

Response of age structure to forest protection

Forest protection created more complex age structures (Figure 3) and tree densities with increasing age of protection (Table 1). Seedlings, middle__medium_and big trees were absent in younger stands (Figure 4) which indicated tree biomass was low (Figure 3) and lacked natural regeneration capacity. Although plants species were most abundant in stands protected for 30 years (Figure 1), this protection age contained the lowest proportion of big trees (V) among age classes (Figure 4) limiting tree biomass. Both seedlings (Figure 4) and density of trees (Figure 1) in older (> 60 years) protected stands protection—were higher than in younger stands, suggesting that a better natural regeneration capacity. However, more seedlings and small trees without adequate big trees (Figure 4) in the__some_older stands also unveiled their were evidence of insufficient productivity of such stands (Figure 3).

Our results support the widely accepted view that the rate of stand biomass accumulation peaks in the early stage of development, usually at the time of canopy closure, and declines thereafter (Acker et al. 2002; Mcmahon & Schlesinger 2010; Sarah Lesley Taylor 2005; Xu et al. 2012). The stands protected for 45 years had not only the highest canopy density (Table 1), but also the highest proportion of big trees and tree biomass and as well as considerable seedling density (Figure 4), suggesting adequate regeneration capacity at this age.

Comment [A7]: ?

Response of soil nutrients to forest protection

Vegetation plays a key role in maintaining the soils in which they grow (Mishra et al. 2003), by directly influencing soil nutrients accumulation and consequently soil development by via above ground inputs_(Blazejewski et al. 2009; Drouin et al. 2011; Giese et al. 2000). Litter fall and its decomposition is an important mechanism governing soil chemical properties (Mishra et al. 2003), especially the upper soil layer (Ma et al. 2007).

In the present study, *Pinus tabulaeformis* tree growth (Figure 3) and understory plant species richness increased quickly for stands protected less than 30 years (Figure 1), however litterfall input to soil was lower due to the absence of big trees in these stands (Figure 4). Tree and canopy density (Table 1) decreased in stands protected for more than 30 years, with highest values observed in stands protected for 45 years (Figure 3). Increased litter_fall input, decomposition rate and higher soil organic carbon contents were also observed at older forest sites (Figure 5A).

Content of soil mineral nitrogen at 0-20 cm soil depth showed an a decreasing trend in stands of up to 30 years of protection although no significant differences were found among stands (Figure 5B). This trend supports previous studies which have observed that young or developing stands accumulate forest floor nitrogen, tending towards relatively stable conditions in undisturbed mature forests (Johnson & Turner 2014; Miller 1981; Turner 1981).

The primary source of phosphorus and potassium in terrestrial ecosystems <u>are derived derived</u> from mineral materials in weathering parent rock (Filippelli 2008; Sheng 2005; Smeck 1985;

Comment [A8]: if no statistical significant differences were found in this study, then the results do not support the finding of previously published information. This is a major contribution and it is important to report it as such. It is necessary to say that soil nitrogen at 0-20 cm in this study did not support previously published information about soil nitrogen in the upper 0-20 cm. This is significant so scientists are aware and so pay special attention to this at their research sites.

Tiessen et al. 1984). A proportion of there leased the released phosphorus and potassium, available in exchangeable and soluble (available) fractions, can be assimilated by plants and soil microorganisms directly (Schachtman et al. 1998; Sheng 2005). Soil phosphorus availability is also enhanced through phosphorus solubilizing and mineralizing microbial biomass (Richardson & Simpson 2011). Many soil microorganisms excrete organic acids to directly dissolve rock potassium to bring the potassium into solution (Bennett 1998; Friedrich et al. 1991; Groudev 2010; Ullman et al. 1996).

In the present study, soil available phosphorus (Figure 5C) and potassium (Figure 5D) contents were higher in stands with greater proportions of big and middle medium trees. We suggest that the stands with greater biomass accumulated more litter and humic mineral in the top soil, which provided a substantial energy source and favorable conditions for microbial activity (Fontaine et al. 2003). In younger stands, more nutrients may be taken up by the vegetation during intense tree growth phase than can be replaced within the soil from mineral weathering and litter decomposition (Brais et al. 1995) which may explain why soil available potassium decreased in stands of up to 45 years of protection in the present this study (Figure 5D).

The optimal age for the fully hillside-closed forest protection

No restoration project is undertaken in a social vacuum (Knight et al. 2010). The goods and services provided by forests are an important source of income for local people in the rural part of China (Ma et al. 2013). Even when the intentions of ecological restoration are good and the restoration strategy suitable for the environmental conditions (Ma et al. 2013), restoration action

will not be sustainable if it does not take into account the profit potential of local people

Our results showed that long-term protection (> 45 years) of *Pinus tabulaeformis* stands in southeast Loess Plateau, China, may be associated with decreasing plant species richness (Table 2), proportion of medium to large trees (Figure 4), dominant tree biomass_(Figure 3) and soil nutrients (Figure 5). We suggest that it is possible, based on the findings above, to couple forest management policy without exacerbating the poverty of local people, through the promotion of measured forest indices as evidence-based support for forest protection and use. For this region, we suggest the optimum forest protection age of 45 years would encourage maximum plant diversity and productivity, while supporting the socio-economic conditions of the local population for sustainable land use.

Conclusions

The present study has reported differences of plant diversity, changes in forest age structure and soil nutrients of *Pinus tabulaeformis* stands restoring restoration in chronosequence on the southeast Loess Plateau, China. The richness of plant species significantly differed with age of forest protection, attenuating towards more even distribution with increasing age of forest protection. Sustainable forest protection not only hindered from increased organic carbon content, available phosphorus and potassium in top soil, but it also abated tree biomass. Our findings have practical implications. By using measured forest indices as evidence-based support for balancing forest management policy, ecological restoration and local economy development including sustainable timber harvesting, we conclude that the preliminary optimal age for forest protection in

this area should be not more than 45 years.

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328 comments.

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ADDITIONAL INFORMATION AND DECLARATIONS

330 Funding

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Competing Interests

The author declares there are no competing interests.

Author Contribution

Lin Hou conceived and designed the experiments, performed the experiments, and wrote the paper. Sijia Hou analyzed the data, prepared figures and tables, and reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability. The summary data on which these analyses are based are available in Table 1 and Table 2.

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Comment [A9]: this should be 'we' since two authors wrote the paper. However, if one of the authors wants to thank some individual unknown to the other author, then the sentence should read "One of us,......(name of the author) wants to thank"

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